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Molecular Therapy
Commentary

Apoptotic MSCs, COX2/PGE2 and clinical efficacy in Crohn fistula

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The clinical application of mesenchymal stromal cells (MSCs) has been approved for use in pediatric graft-versus-host disease (GvHD) and perianal fistulizing Crohn disease. MSCs have also been investigated in an array of other inflammatory disease indications. Despite promising results in patients who respond to MSC administration, a significant proportion of patients do not respond, and this has significantly dampened enthusiasm for MSC-based cell therapy. A better understanding of the mechanism of action (MOA) involved in the therapeutic effects of MSCs may help to stratify patients who will respond to MSC administration. In this issue of *Molecular Therapy*, Cheung et al. publish their findings on the role of caspase-mediated apoptosis of MSCs, leading to the release of immunosuppressive factors, including prostaglandin E2 (PGE2), which correlated with clinical responsiveness in Crohn's disease patients.[1] The authors build upon on their previous study that identified a correlation between the induction of apoptosis mediated by peripheral blood mononuclear cells (PBMCs) from patients with GvHD who responded to MSC administration.[2] In the present article, Cheung et al. demonstrate once again a correlation between apoptosis induction in MSCs and a role for cyclooxygenase 2 (COX2)/PGE2 in mediating immunosuppressive effects in patients with Crohn's disease who responded to MSC therapy.[1] The significance of this study is that it identifies potential assays that could be used to stratify patients based on the capacity of patient PBMCs to induce MSC apoptosis and/or the use of PGE2 levels produced from PBMC-induced apoptotic MSCs.

This study investigated anti-Fas induction of apoptosis in bone marrow-derived human MSCs. In addition to inducing apoptosis, anti-Fas treatment generated an immunosuppressive secretome consisting of immunomodulatory factors, including interleukin-6 (IL-6), leukemia inhibitory factor (LIF), tumor necrosis factor–inducible gene 6 (TSG-6), and COX2/PGE2. The secretome derived from Fas-induced apoptotic MSCs inhibited $CD3^+$ T cell proliferation and cytokine (IL-2 and interferon- γ [IFN-γ]) production *in vitro*. Blockade of nuclear factor κB (NF-κB) signaling via knockdown of RelA (the p65 subunit of NF-κB) or use of the selective NF-κB inhibitor BAY11-7085 had no impact on Fas-induced apoptosis in MSCs but negatively affected the Fas-induced secretome, significantly reducing the level of COX2 and PGE2. The secretome derived from Fas-induced apoptotic MSCs also contained high levels of chemokine ligand 2 (CCL2), CCL20, and IL-8. Interestingly, CCL2 contained within the apoptotic MSC secretome was shown to mediate the chemotaxis of human monocytes, and CCL2 production by apoptotic MSCs was dependent on COX2. Monocytes exposed to the Fas-induced apoptotic MSC secretome demonstrated enhanced suppression of $CD3^+$ T cell proliferation.

Compared to PBMCs from healthy controls, PBMCs from Crohn's fistula patients enrolled in the ADMIRE-CD trial induced higher levels of apoptosis in adipose-derived human MSCs (the MSC donor used in the ADMIRE-CD trial), and this was associated with increased PGE2 production in a cell contact–dependent manner. Caspase inhibition clearly showed an important role for caspase-mediated apoptosis in patient PBMC-MSC cocultures and subsequent PGE2 production. However, neutralization of PGE2 reduced the T cell immunosuppressive effect only partially, suggesting that other secreted immunosuppressive factors also play a role in T cell suppression. Neutralization of PGE2 had a greater impact on T cell cytokine production.

The real power of this study is in the MOA identified: the induction of apoptosis (mediated by Fas) leading to the production of high levels of PGE2 with downstream effects on T cell proliferation and the induction of immunosuppressive monocytes correlated with clinical patient responsiveness to MSC therapy. However, these findings need to be confirmed in larger patient cohorts. Although caspase-dependent apoptosis and subsequent PGE2 production was also demonstrated in one bone marrow–derived MSC donor, it remains to be determined whether this MOA is common across MSC tissue sources and the level of variability that may exist across donors.

Nonetheless, this study aligns with other studies showing the importance of apoptosis induction in MSCs following administration *in vivo* in preclinical models of GvHD, allergic airway inflammation, and experimental autoimmune encephalitis.[2,3] It is becoming increasingly evident that the interaction between MSCs and macrophages *in vivo* is central to the protective anti-inflammatory effects mediated by MSCs.[4,5] In terms of the recruitment of monocytes/macrophages, Cheung et al. show an important role for apoptotic MSC-derived CCL2.[1] This aligns with data from preclinical models of allergic airway inflammation showing that CCL2 derived from MSCs and recruitment of $CCR2^+$ monocytes was essential for MSC therapeutic efficacy.[6] Moreover, data from Galipeau's group support a role for the MSC-secreted factors CCL2 and chemokine (C-X-C motif) ligand 12 in the induction of M2 polarized $CCR2^+$ macrophages producing IL-10, which mitigated gut injury in a preclinical colitis model.[7] Although not the focus of the study by Cheung et al., they show that macrophages exposed to the secretome of Fas-induced apoptotic MSCs have enhanced immunosuppressive functions

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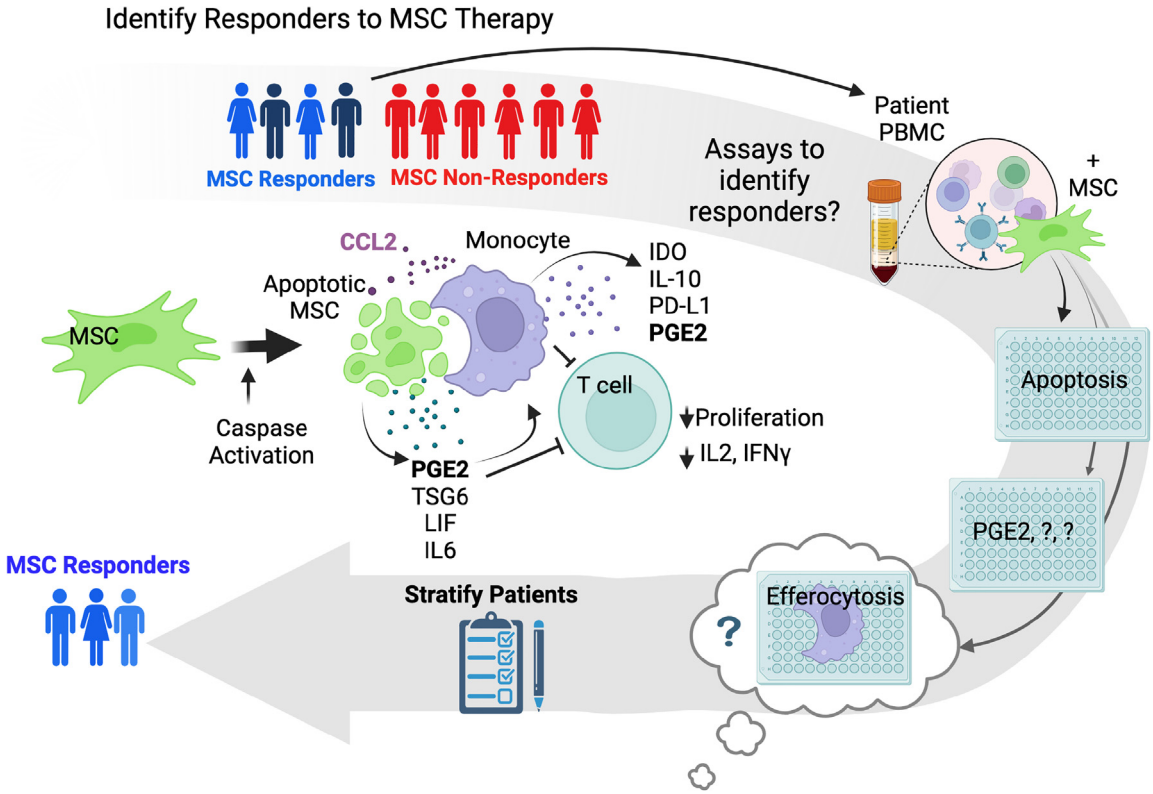

Figure 1. Proposed means to identify responders to MSC therapy

Up to 60% of patients who receive MSC therapy do not respond to it. Evidence now suggests that MSCs undergo apoptosis following administration and that the interaction between apoptotic MSCs and macrophages is essential for the immunomodulatory MOA of MSCs. Caspase-mediated apoptosis induction in MSCs leads to the release of immunomodulatory factors, including PGE2, TSG-6, LIF, and IL-6, as well as the monocyte chemoattractant CCL2. PGE2 is an important factor and can partially reduce $CD3^+$ T proliferation and cytokine (IL-2, IFN-γ) production. Monocytes are attracted to apoptotic MSCs via CCL2 and are known to clear apoptotic MSCs via efferocytosis. Following exposure to the apoptotic MSC secretome, monocytes produce immunomodulatory factors, including PGE2, IDO, IL-10, and PD-L1, and exert immunosuppression on $CD3^+$ T cells. This commentary discusses the evidence for using the capacity of patient PBMCs to induce apoptosis in MSCs as a means to identify patients who may best respond to MSC therapy. In addition to apoptosis, Cheung et al. propose a threshold level of PGE2 released by apoptotic MSCs that correlates with the response to MSC administration in patients enrolled in the ADMIRE-CD trial. Moreover, the combined evidence suggests an important role for the patient-derived monocyte and specifically monocyte efferocytosis. Together, these readouts may help to stratify patients into those most likely to respond to MSC therapy. (Figure created using Biorender.com).

in vitro.[1] The data presented here may point toward the use of the apoptotic MSC secretome, but the process of efferocytosis of apoptotic MSCs by macrophages *in vivo* may be required for longer term immunosuppressive effects. Indeed, this has been shown by Pang et al., whereby efferocytic clearance of apoptotic MSCs is an important part of the mechanism of protection.[3] Dazzi and colleagues also demonstrated an important role for the efferocytosis of apoptotic MSCs leading to indolamine 2,3-dioxygenase (IDO)-producing phagocytes, which were essential for MSC immunosuppression in a preclinical model of GvHD[2] and show the importance of monocyte efferocytosis of apoptotic MSCs leading to significantly increased levels of PGE2, IL-10, IDO, and programmed death-ligand 1 (PD-L1) in human monocytes *in vitro*.[8] More importantly, they also showed that blockade of COX2 using the selective inhibitor NS-398 prevents the monocyte induction of IDO, PD-L1, IL-10, and PGE2 without interfering with efferocytosis.[8]

Despite the progress the field has made in understanding the MOA of MSC therapy, we still do not understand why some patients respond to MSC administration and others do not. However, based on the findings discussed, it is plausible that the answer lies in the patients' monocytes. In addition, with better understanding of the role of efferocytosis in dictating the response to MSC therapy alongside assays such as those proposed by Cheung et al.[1] (apoptosis and PGE2 levels), we may be able to stratify patients based on those most likely to respond to MSC therapy (Figure 1). The findings discussed here suggest that the assays that may help to predict MSC responders may vary depending on the disease indication and associated disease pathogenesis, as well as the MOA of apoptotic MSCs in those different settings. To date, this area of research has certainly provided our most convincing data and robust means to

understand the MOA of MSC therapy[9] and deserves more focus.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Cheung, T.S., Giacomini, C., Cereda, M., Avivar-Valderas, A., Capece, D., Bertolino, G.M., delaRosa, O., Hicks, R., Ciccocioppo, R., Franzoso, G., et al. (2023). Apoptosis in mesenchymal stromal cells activates an immunosuppressive secretome predicting clinical response in Crohn's disease. Mol. Ther. https://doi.org/10.1016/j.ymthe.2023.10.004.

2. Galleu, A., Riffo-Vasquez, Y., Trento, C., Lomas, C., Dolcetti, L., Cheung, T.S., von Bonin, M., Barbieri, L., Halai, K., Ward, S., et al. (2017). Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci. Transl. Med. *9*, eaam7828.

3. Pang, S.H.M., D'Rozario, J., Mendonca, S., Bhuvan, T., Payne, N.L., Zheng, D., Hisana, A., Wallis, G., Barugahare, A., Powell, D., et al. (2021). Mesenchymal stromal cell apoptosis is required for their therapeutic function. Nat. Commun. *12*, 6495.

4. Carty, F., Mahon, B.P., and English, K. (2017). The influence of macrophages on mesenchymal stromal cell therapy: passive or aggressive agents? Clin. Exp. Immunol. *188*, 1–11.

5. Weiss, D.J., English, K., Krasnodembskaya, A., Isaza-Correa, J.M., Hawthorne, I.J., and Mahon, B.P. (2019). The Necrobiology of Mesenchymal Stromal Cells Affects Therapeutic Efficacy. Front. Immunol. *10*, 1228.

6. Takeda, K., Webb, T.L., Ning, F., Shiraishi, Y., Regan, D.P., Chow, L., Smith, M.J., Ashino, S., Guth, A.M., Hopkins, S., et al. (2018). Mesenchymal Stem Cells Recruit CCR2(+) Monocytes To Suppress Allergic Airway Inflammation. J. Immunol. *200*, 1261–1269.

7. Giri, J., Das, R., Nylen, E., Chinnadurai, R., and Galipeau, J. (2020). CCL2 and CXCL12 Derived from Mesenchymal Stromal Cells Cooperatively Polarize IL-10+ Tissue Macrophages to Mitigate Gut Injury. Cell Rep. *30*, 1923–1934.e4.

8. Cheung, T.S., Galleu, A., von Bonin, M., Bornhäuser, M., and Dazzi, F. (2019). Apoptotic mesenchymal stromal cells induce prostaglandin E2 in monocytes: implications for the monitoring of mesenchymal stromal cell activity. Haematologica *104*, e438–e441.

9. Galipeau, J. (2023). Macrophages, MIFs, and MSCs: Defining an MOA in murine experimental asthma. Mol. Ther. *31*, 3117–3118.