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The role of indoleamine 2,3-dioxygenase in inhibition of virus-specific CD8$^+$ T cell proliferation by human mesenchymal stromal cells

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Mesenchymal stromal cells (MSCs) exert a strong anti-proliferative effect on allo-antigen-specific T cells, whereas the suppression of virus-specific T cell proliferation by MSCs is still under debate. In this study, we showed that human MSCs suppress the proliferation of both allo-antigen- and virus-specific CD8$^+$ T cells in a dose-dependent manner. In addition, 1-methyl-DL-tryptophan (1-MT, an inhibitor of indoleamine 2,3-dioxygenase (IDO)) can partially restore the proliferation of all-antigen- and virus-specific T cells inhibited by MSCs, thus demonstrating the critical role of IDO.

To investigate the expression of IDO in MSCs, flow cytometry was performed and results showed that MSCs do not constitutively express IDO, whereas interferon-γ (IFN-γ) can induce the IDO expression in a dose-dependent manner. Moreover, the ability to express IDO in response to IFN-γ varies greatly among MSC populations. MSCs with an impaired IDO expression in response to IFN-γ exhibit lower anti-proliferative ability on allo-antigen- and virus-specific CD8$^+$ T cells, indicating that the ability of MSCs to express IDO in response to IFN-γ can reflect the immunosuppressive ability of MSCs.

In vitro expansion is considered to potentially influence the immunomodulatory function of MSCs. Therefore, the number of passages and the seeding density of MSCs during *in vitro* expansion were investigated regarding their influence of

IDO-expressing ability and T-cell inhibitory ability of MSCs. The results showed that the number of passages does not significantly affect IDO-expressing ability and T-cell inhibitory ability of MSCs, whereas low seeding density (1,000 cells/cm^2), when compared to high seeding density (5,000 cells/cm^2), might slightly impair T-cell inhibitory ability of MSCs independent of IDO-expressing ability. This indicates that other factors other than IDO-expressing ability may exist to influence T-cell inhibitory effect of MSCs.

IFN-γ was reported to be crucial for MSCs to exert an immunosuppressive function. In this study, we also found that inhibition of CMV-specific $CD8^+$ T cell proliferation by MSCs can be enhanced by IFN-γ pretreatment but abrogated by IFN-γ blocking antibody in a dose-dependent manner. In our MSC/T cell co-culture system, $CD8^+$ T cells are the main source of IFN-γ. Therefore, we reduced the number of $CD8^+$ T cells in the co-culture and observed a significant decrease in the inhibitory effect of MSCs on CMV-specific $CD8^+$ T cells, which could be fully restored by exogenous IFN-γ added at the beginning of co-culture. This indicates that current divergent opinions on anti-proliferative effect of MSCs on virus-specific T cells might be caused by various IFN-γ levels in the culture systems employed by different studies.

In conclusion, our study demonstrated that MSCs suppress virus-specific $CD8^+$ T cell proliferation through IFN-γ-induced IDO, indicating a potential influence of MSC therapy on immune responses of patients to viruses. Moreover, our study revealed a correlation of T-cell inhibitory ability of MSCs with their IDO-expressing ability, and also investigated the influence of the number of passages and the seeding density on IDO-expressing ability and T-cell inhibitory ability of MSCs. All results give us a better understanding in the immunosuppressive ability of MSCs, which might be helpful for the development of potency assays for Good Manufacturing Practice (GMP)-compliant MSC production.