Bone-Marrow Stromal Cells Exert Immunosuppressive Effects on Immune Cells Co-cultivated in 3D Alginate Scaffolds

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Introduction
Stromal cells have been shown to exert a potent immunosuppressive effect on immune cells when co-cultivated on flat two-dimensional (2D) dishes. Yet, 2D cell cultivation may not reliably represent cell function in a physiological setting. In this work we investigated whether stromal cells would exert immunosuppression on bone marrow derived dendritic cells (BMDC) and T cells, when co-cultured within 3D porous alginate scaffolds. Alginate scaffolds herein are replacing the extracellular matrix (ECM), providing both a physical support and biological cues for the seeded cells and closing the gap to in vivo conditions.

Materials and Methods
BMDCs were seeded alone or in a co-culture with stromal cells. BMDC maturation was measured by FACS (CD86 expression) and ELISA (TNF-α secretion). T-cell penetration to the scaffold and their ability to form immune-synapses were assessed by immunofluorescence and live-cell imaging techniques. The effect of stromal cells on T-cell proliferation was evaluated by both [3H] thymidine incorporation and CFSE assays. The effect of stromal cells on T-cell activation was evaluated by ELISA assay of IL-2, INF-γ, IL-17, IL-10, and TGF-β. All antibodies were purchased from BioLegend (San Diego, CA).

Results
Our results demonstrate that 3D co-culture of stromal cells and BMDCs promoted the formation of mixed cell clusters distributed in the scaffold pores (Fig 1. A, B). Lipopolysaccharide (LPS)-induced BMDC maturation was attenuated in the presence of stromal cells as indicated by less dendrites formation (Fig 1. C, D), lower amounts of secreted TNF-α and decreased expression of the co-stimulatory molecule CD86 compared with BMDCs cultured alone. Furthermore, in the presence of stromal cells, BMDCs induced limited T-cell proliferation and activation, secreting lower levels of pro-inflammatory cytokines such as IL-2, INF-γ and IL-17 and higher levels of the anti-inflammatory cytokine IL-10.

Discussion and Conclusions
Our data suggest that 3D cultivated stromal cells can exert immunosuppression presumably via inhibiting BMDC maturation. This suppression effect may have therapeutic potentials in T-cell mediated autoimmune diseases.

References

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Disclosures
None