ImmunoTools *special* Award 2014

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Generation and functional characterization of artificial lymphoid organs with mutipotent stromal progenitors

Secondary lymphoid organs (SLOs) function to drain blood/lymph and to facilitate cell-cell interactions required for generating adaptive immunity. Through the secretion of chemokines and signalling molecules, stromal cells have emerged as crucial organizers of lymphoid compartments and immune cell functions. Stromal cells, including follicular dendritic cells (FDCs) of the B-cell follicles and fibroblastic reticular cells (FRCs) of the T-cell zone are characterized by the expression lymphotoxin (LT) receptors (e.g. TNFR1, LTβR). Upon engagement with their ligands (e.g. TNFα, LTα1β2) expressed by T- and B-cells LT receptors trigger the production of lymphoid chemokines (e.g. CCL21/CXCL13) and signaling molecules that are important to promote attraction, survival and proliferation of lymphoid cells. In addition, stromal cells produce the extracellular matrix (ECM), a network of reticular fibers that facilitate cell adhesion, locomotion and storage of cytokines and growth factors. Given the important role of stromal cells in promoting lymphoid organization and function, it was proposed to exploit stromal-derived factors to promote the formation of immune niches at the site of solid tumours in order to foster local anti-tumour immunity. It was shown the delivery of Ig-LTbR fusion protein was sufficient to promote the formation of ectopic lymphoid cluster in a murine model of melanoma and to enhance anti-tumour immune responses. Furthermore, the use of stromal cells has also been exploited in order to develop artificial lymphoid-like structures for possible therapeutic applications. For instance, it was shown that postnatal stromal progenitors embedded in a biocompatible scaffold and transplanted *in vivo* generated structural and functional artificial lymphoid-like tissues with features of native lymphoid organs (Suematsu et al., Nature Biotechnology 2004; Tan et al., Journal of Immunology 2014; Castagnaro et al. Immunity 2013).

Based on the aforementioned findings, we have started to assess the capacity of different stromal progenitors isolated from lymphoid and non-lymphoid compartments to support the generation of functional lymphoid structures by using a protocol already established

(Suematsu et al. 2004). Specifically, stromal cells will be isolated by cell sorting or immunomagnetic beads and expanded in vitro for 4-6 passages under mesenchymal stem cell culture conditions. Quantitative PCR analysis will be used to measure the expression of stromal-gene signatures of expanded stromal progenitors. Cells will then be harvested and absorbed into a cubic sponge-like collagenous scaffold and transplanted into the renal subcapsular space of wild type mice. Three to four weeks later, scaffolds will be harvested and analysed for the presence of stromal, lymphoid and myeloid cells. Specifically, scaffolds will be harvested, sectioned and immunofluorescence analysis performed to assess the lymphoid architectures, stromal and extra-cellular matrix composition and presence of vascular and lymphatic endothelial cells. In parallel, scaffolds will be harvested and cellular composition analyzed by flow cytometry. We foresee to characterize how different stromal cell subsets support the formation of artificial lymphoid organoids and open to the possibility of exploiting artificial lymphoid organs in pre-clinical setting.