Mapping the road to peripheral tolerance

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Background

Dendritic cells (DCs) are responsible for eliciting and determining the fate of T cell responses. Manipulating DCs to induce regulatory T cells (Tregs) and re-establish tolerance where there has been a breach in regulation has provided an attractive avenue for cellular therapies. A variety of protocols detailing in-vitro generation of tolerogenic DCs (tolDCs) are now available, however methodological differences have resulted in heterogeneity between tolDC products. It's hypothesised that differences in tolDC products will affect which regulatory pathways are deployed; meaning certain tolDC types would likely be more appropriate for certain pathologies. Further investigation to establish which aspects of tolDC function induce which Treg pathway is required. To this aim, we have compared different methods of tolDC generation; building a model to map specific phenotypical changes to differences in Treg function. Ultimately this will provide a comprehensive referencing tool detailing which tolDC type will likely control which T cell subset.

Methods

Monocyte derived dendritic cells (moDC) from healthy donors were tolerised with the addition of Vitamin D3 alone, Dexamethasone alone or a combination of both. After 7 days culture moDCs were assessed morphologically (light microscopy), harvested and analysed using an optimised flow cytometry panel. Markers of interest were analysed and compared using median fluorescence intensity.

Results

Preliminary data shows changes in the expression of key functional molecules such as PDL-1 and ILT-3 as well as changes in maturation state, differentiation and morphology of differentially generated moDCs. Moreover, novel findings confirming surface expression of CD32 and chemokine receptor ChemR23 on tolDCs generated with dexamethasone provide new markers to distinguish tolDC types.

Conclusion

The phenotypical differences identified highlight substantial heterogeneity between the three tolDC types investigated thus far. This warrants further examination to establish a library of tolDC types and whether their defining characteristics translate to induction of functionally distinct types of Tregs.