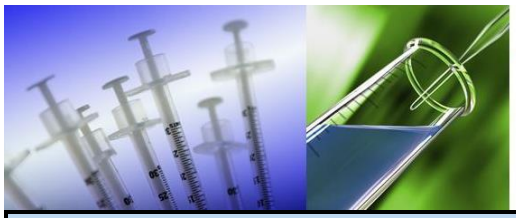
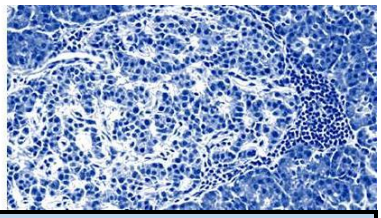

Tolerogenic Effects of Dextran Sulfate in Human Dendritic Cells and T Lymphocytes

AUTHORS

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PURPOSE

Immune dysfunction plays an important role in the pathogenesis of type 1 diabetes (T1D). Low molecular weight dextran sulfate (DS) is a sulfated polysaccharide with immunomodulatory properties and cytoprotective actions. Our lab has recently shown that DS treatment markedly blocks the development and, more importantly, reverses early onset T1D in mice, with decreases in activated T cells (Th1) and increases in regulatory T cells (Tregs). In the current study, we aimed to translate these findings to human immune cells.

METHODS

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy subjects and CD14+-monocytes were isolated and treated with GM-CSF and IL-4 for six days to generate dendritic cells (DCs). DCs were treated with LPS and/or DS for 72h and analyzed for viability, maturation and co-stimulatory markers (CD80, CD83, CD86, CD40, HLA-DR) by flow cytometry. We also analyzed the functional properties of DCs by co-culturing DS±LPS-treated DCs with lymphocytes and measuring lymphocyte activation status and phenotype by flow cytometry. Endocytic capacity of DS±LPS-treated DCs was measured by DS-FITC uptake (30 min, 37°C). To examine the effects of DS on Th1, Th2, Th17, and Treg induction and phenotype, PBMCs were treated with anti-CD3/CD28 for 48 hours or PMA/ionomycin for 24 hours and analyzed for activation markers and cytokine production by flow cytometry.

SUMMARY OF RESULTS

DS decreased the mean fluorescence intensity (MFI) and the number of DCs expressing the co-stimulatory molecule CD80 but did not alter CD83, CD86, CD40 and MHCII expression. Importantly, interaction between DS-treated DCs and autologous lymphocytes led to a significant increase in the proliferation of Tregs (CD4+CD25+FOXP3+CD127low). On the other hand, the incubation of DS-treated DCs with allogenic lymphocytes did not result in proliferation changes. Analysis of the endocytic capacity of DCs showed no difference with DS treatment. Preliminary data also indicates that DS decreased Th1 activation when PBMCs are stimulated with anti-CD3/CD28 but not with PMA/ionomycin.

CONCLUSIONS

Collectively, these results suggest that DS induces a more tolerogenic phenotype in activated human DCs leading to an increase in the proliferation of Tregs. Preliminarily, DS also reduces the number of activated Th1+ cells when activation occurs through external cell signals. Future studies will focus on the impact of DS on DCs as the underlying mechanism decreasing T-cell activation. These studies help to identify targets for intervention in T1D, while providing a useful therapeutic agent with a proven history of safe use in humans.