

P047 Alteration of heparan sulphate composition during inflammation

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Heparan sulphate (HS) plays a crucial role in the regulation of diverse cellular functions, including inflammation. N-deacetylase/ N-sulphotransferase (NDST) enzymes catalyse the first sulphation and direct subsequent modifications of the HS chain. Dependent on the extent and distribution of the sulphate substitutions, HS can bind to a range of proteins, including cytokines and chemokines. Real-Time PCR was employed to examine NDST1 expression levels across cell lines (HepG-2, Jurkat, Molt-16 and THP-1). Basal expression of NDST1 was found to vary between cell types tested and following 16h of stimulation with IFN- γ and TNF- α levels of the NDST1 transcript were reduced in HepG-2 ($p < 0.05$), Jurkat and Molt-16 cells, although the converse was observed in cytokine-stimulated THP-1 monocytes ($p < 0.05$). The importance of HS sulphation was investigated using shRNA to downregulate NDST1; HEK293 cells were generated with 64% and 58% NDST1 reduction on mRNA level and a concurrent reduction in NDST1 protein was verified by Western blotting. A reduction in N-sulphation was observed by the application of specific antibodies which also revealed differences in HS composition. To understand the physiological importance further experiments are currently using these cells to analyse differences in chemokine presentation and their ability to support cell migration.