

**P004** Investigation into a possible role for Btk in SHIP-1 regulation.

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Btk has been described as an important kinase in the regulation of LPS signaling through TLR-4. Recently, the inositol phosphatase, SHIP-1 has been described as a negative regulator of TLR-4 signaling, with a mutant lacking phosphatase activity enhancing NF $\kappa$ B response to LPS in luciferase assays. Looking at signaling intermediates downstream of TLR-4, it has been shown that NF $\kappa$ B response to direct stimulus with MyD88 and Mal is decreased in the presence of SHIP-1. However no interaction can be seen between MyD88 and SHIP-1 or Mal and SHIP-1. Looking further downstream, we found that there is an interaction between SHIP-1 and Btk in both over-expression studies in HEK-293T cells and endogenous co-immunoprecipitation experiments in THP1s and this interaction is inducible upon LPS stimulation. A possible functional significance of this interaction was investigated using phosphospecific antibodies to SHIP-1 and phosphotyrosine antibodies. LPS stimulation of THP1 cells demonstrated a rapid phosphorylation of SHIP-1 on tyrosine 1020. In the presence of a Btk-specific inhibitor, LFM-A13, this phosphorylation of SHIP-1 was delayed suggesting that Btk is involved in the phosphorylation of SHIP-1. Confocal experiments suggest a colocalisation of Btk and SHIP1 and indicate that on LPS stimulation of bone marrow-derived macrophages(BMDMs), Btk trafficks to endosomal vesicles in cells. We are currently examining the functional significance of these responses using shRNA to SHIP-1 to determine the effect of SHIP-1 knockdown on Btk-dependent pathways.