

**P019** Identification of a novel tyrosine phosphoprotein implicated in inflammatory signalling  
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Engagement of different members of the Interleukin-1- and Toll-like- receptor superfamily (IL1R and TLR) can induce distinct patterns of cytokine production and cell surface receptor expression, while utilising a shared panel of signalling molecules. We hypothesised that uncharacterised signalling components might contribute to the specificity of these responses.

Using an unbiased phosphoproteomic approach, we identified a novel protein, RIPP, which is inducibly tyrosine phosphorylated following challenge of the macrophage cell line RAW 264.7 with the TLR4 ligand, LPS.

LPS-induced TNF $\alpha$  (but not IL-6) production was elevated in RAW cells stably expressing a FLAG-tagged version of RIPP. Increased TNF $\alpha$  production was not observed after engagement of TLR2 by PAM3 nor TLR3 by poly(I:C). The TLR4-selective TNF $\alpha$  upregulation occurred without any distinguishable difference in JNK, p38 or ERK MAPK activation, I $\kappa$ B $\alpha$  degradation or COX2 protein induction.

Similarly, cells of the murine fibroblast line, NIH 3T3, stably transfected with FLAG-tagged RIPP produced increased levels of KC and IL-6 in response to LPS or IL-1 stimulation whereas responses to TNF challenge were not significantly affected. We are currently attempting to knock down RIPP expression using small interfering RNAs and to determine the role of RIPP on transcriptional responses using AP1- and NF $\kappa$ B-driven luciferase reporter constructs.