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sequences) and associated with a type-1 IFN response. Induction of these genes by autocrine type-I and type-III IFN signaling was ruled out using both neutralizing antibodies to these IFNs in biological assays and qRT-PCR. Despite the absence of type-I or type-III IFNs, IFN-γ treatment induced ISGF3 formation and ISRE binding, as shown by STAT2 co-immunoprecipitation and ChIP analysis of the PKR promoter. STAT2 and IRF9 knockdown in A549 cells reversed IFN-γ-mediated ISRE induction and antiviral activity – implicating ISGF3 formation as a significant component of the cellular response and biological activity of IFN-γ.

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PS1-65

Investigating the effect of the FHA region of Pellino 3 on TRL4 signaling proteins

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Pellino 3 has been shown to be a negative regulator of TLR4 signalling. The process by which the protein does this has yet to be elucidated. However, the phosphorylation of Pellino 3 by IRAK-1 has been shown to initiate the E3 ligase activity of Pellino 3. This E3 ligase activity in turn leads to the polyubiquitination and subsequent degradation of IRAK-1. The Pellino Family also share a conserved forkheaded associated (FHA) domain that binds to phosphorylated proteins. This FHA region has already been shown to be crucial in Pellino 2, IRAK-1 binding and processing. This study will examine other proteins within the TLR4 signalling cascade that interact with Pellino 3 via the FHA region which then leads to their degradation. This will then provide a mechanism by which the Pellino protein family regulates TLR4 signalling.

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PS1-66

A phosphomimetic substitution of STAT2 serine-287 negatively regulates STAT2 function and type I interferon signaling

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Type I interferons (IFN-α and -β) are cytokines that activate primarily the JAK/STAT pathway to induce an anti-proliferative, pro-apoptotic or antiviral response in cells. We previously identified a motif in the SH2 domain of STAT2 that, when mutated, prolonged nuclear retention of the STAT1/STAT2 heterocomplex during IFN treatment and induced apoptosis in certain tumor cell lines. With the exception of STAT2, other members in the STAT family of transcription factors have been shown to be phosphorylated on tyrosine and serine residues for biological activity. Therefore, we searched for additional phosphorylation sites in STAT2 by a combination of mass spectrometry analysis and prediction software (NetPhos 2.0 and Motif Scan). Serine-287, located in the coiled-coil domain of STAT2, was found to be phosphorylated in untreated STAT2 null U6A cells reconstituted with wild type STAT2 but not in cells treated with IFN-α for 20 minutes. To determine the biological consequence of this putative phosphorylation event, a phosphomimetic mutant (S287D-STAT2) of STAT2 or a phospho inert mutant (S287A–STAT2) was expressed in U6A cells. In response to IFN-α, S287D-STAT2 poorly induced ISG expression and conferred no protection against vesicular stomatitis virus (VSV) infection, whereas the S287A-STAT2 mutant showed prolonged ISG expression and lengthened VSV protection. The molecular mechanism behind these phenotypical changes remains unknown. Yet we observed that STAT2-S287A phosphorylation on Tyrosine-690 was prolonged whereas S287D-STAT2 activation was defective in response to type I IFN, resulting in less STAT2 translocating to the nucleus. Therefore, our study shows that phosphorylation of STAT2 on Serine-287 negatively impacts STAT2 function in the type I IFN signaling pathway and suggests that other signaling pathways modulate STAT2 function and the cellular response to type I IFNs.

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PS1-67

TYK2 is required for IL-17 production by innate immune cells in response to IPS

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The Janus kinase (JAK) family member tyrosine kinase 2 (Tyk2) is an integral part of various cytokine and growth hormone signaling pathways. We have reported previously that macrophages from Tyk2-deficient mice exhibit selective defects in response to lipopolysaccharide (LPS). We demonstrate now, that LPS stimulation induces interleukin-17 (IL-17) production via a Tyk2-dependent pathway in thioglycolate-elicited peritoneal macrophages. IL-17 and IL-17F were upregulated upon LPS treatment with similar kinetics and both mRNAs were considerably reduced in the absence of Tyk2. Interestingly, signal transducer and activator of transcription 3 (STAT3) was not required for LPS-induced IL-17 production in macrophages. In the absence of STAT3, IL-17/IL-17F mRNA and IL-17 protein expression were strongly increased upon LPS treatment and, to a lower extend, produced constitutively. Thus, in contrast to its essential role in the differentiation/maintenance of IL-17 producing T cells (Th17), STAT3 exerts inhibitory rather than stimulatory effects on LPS-induced IL-17 production in macrophages. Of note, we also prove that Tyk2 is indispensable for IL-17 production following LPS challenge *in vivo*. We could exclude mature T cells as main source of IL-17 in spleens following intraperitoneal administration of LPS. Currently, we are investigating the contribution of Tyk2 to innate IL-17 production in specific cell types *in vivo*.

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PS1-68

STAT1α and STAT1β knockin mice: Initial findings and some surprises

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Signal transducer and activator of transcription 1 (Stat1) participates in Jak-Stat signalling pathways which (co-) regulate immunity to infection, inflammatory processes and carcinogenesis. As a result of alternative splicing Stat1 exists in two isoforms, the full length Stat1α and the C-terminal truncated Stat1β isoform. Stat1β lacks 38 amino acids of the transactivation domain including the serine 727 phosphorylation site, which is essential for full transcriptional activation. Previously, it has been reported that *in vitro* only Stat1α is transcriptionally active, therefore Stat1β was considered to act in a dominant negative manner. In order to investigate the Stat1 isoform functions *in vivo* we generated mice, which are deficient for either Stat1α or Stat1β (i.e. Stat1$^{\Delta\alpha/\Delta\alpha}$ and Stat1$^{\Delta\beta/\Delta\beta}$ mice, respectively). Protein expression levels of each isoform are similar to wildtype Stat1α/β expression levels in primary embryonic fibroblasts (PEFs), bone marrow derived macrophages (BMMΦs) and organs. Upon interferon (IFN) stimulation cells derived from Stat1$^{\Delta\beta/\Delta\beta}$fnmice show phosphorylation at tyrosine 701 and serine 727. As expected, in Stat1$^{\Delta\alpha/\Delta\alpha}$ cells only tyrosine 701 of the Stat1β isoform is phosphorylated. Analysis of respective cells show that either Stat1 isoform is able to translocate to the nucleus and to bind to DNA response elements. IFN type I and II induced gene expression of selected target genes in Stat1$^{\Delta\beta/\Delta\beta}$fncells is similar to wildtype cells. Unexpectedly, Stat1$^{\Delta\alpha/\Delta\alpha}$ cells also show gene induction upon IFN type II stimulation. Thus, in contrast to what has been shown before in cell lines, these data demonstrate that Stat1β alone is transcriptionally active. Further analysis will define detailed function of Stat1α and Stat1β *in vivo*.

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PS1-69

Inducible STAT1 protein in mice

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Signal transducer and activator of transcription 1 (Stat1) is an integral constituent of the Jak-Stat signaling network mediating cellular responses including pro-