A. CYTOKINE AND IMMUNE RECEPTOR SIGNALING SECTION
Head: Ioannis Tassiulas, MD

Background

Cytokines: Cytokines are secreted proteins that mediate communication between cells and are critical for the development and regulation of the immune response. Cytokines act by binding with high affinity and specificity to cell surface receptors, triggering signal transduction pathways that ultimately lead to gene activation cascades that regulate cellular activation, differentiation, proliferation and survival. Deregulation of cytokine production or cytokine networks have been implicated in the pathogenesis of a number of human autoimmune/ inflammatory diseases including SLE and rheumatoid arthritis

We have shown that IFNα signaling is regulated by crosstalk with the ITAM-Syk pathway, a signal transduction pathway that is used specifically by immune cells and is key in cell activation. Crosstalk between IFNα and Syk-mediated pathways is not static but depends on the activation state of macrophages and is induced by priming with IFNγ (Figure 1). Thus, macrophage responses to IFNα are determined by integration of signals from two main pathways that regulate immune responses (1).

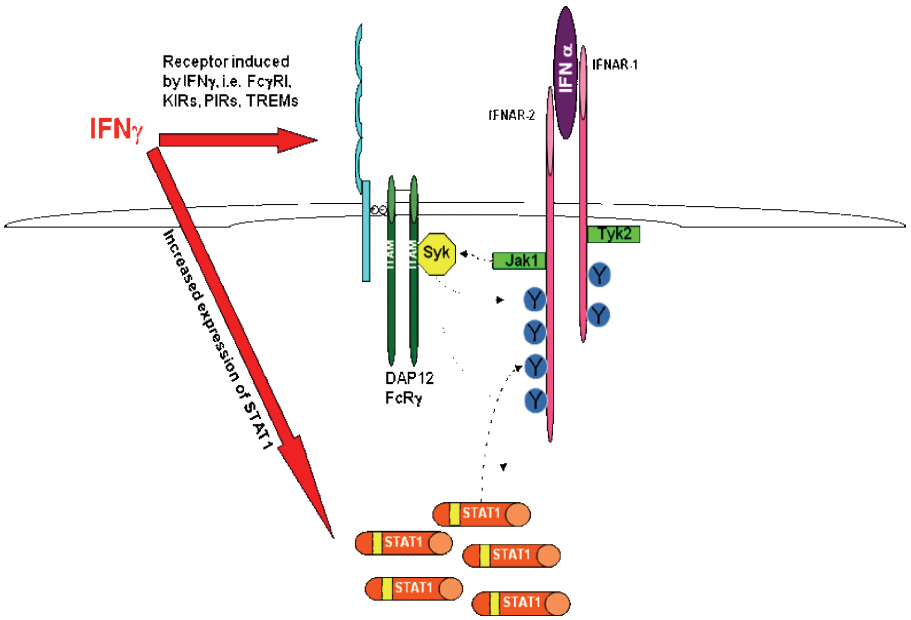

Figure. 1 *Model for enhanced IFNα STAT1 activation mediated by Syk, increased STAT1 expression and FcRγ and DAP12 adaptor proteins. In this model IFNγ priming increases STAT1 expression and induces expression of FcR≥- and DAP12-coupled receptors that interact with IFNAR. IFN± stimulation results in Syk-dependent phosphorylation of STAT1 or of previously unknown docking sites for STAT1 in the IFNAR cytoplasmic domains. Syk itself may be activated by IFNAR-associated Jaks. Thus, the nature of cytokine responses can be altered or reprogrammed by the nature of a particular stimulus or the microenvironment that the cells are exposed.*

Immune cell receptors. A growing number of receptors including the Toll-like receptors (TLRs) and receptors that transmit signals via the ITAM containing adaptor molecules FcRγ and DAP12 play important roles in the activation status of the cells of the immune system. Signaling pathways activated downstream of these receptors include the NF-kB, MAPKs,

PI3K and Ca++-induced pathways that play a central role in the activation, differentiation and survival of the cells. There is experimental evidence of regulation and crosstalk between cytokine and immune receptor signaling in cells of the immune system. A paradigm is the inhibition of interleukin-10 (IL-10) signaling in macrophages after ligation of the FcγRs by immune complexes (2). In this case macrophages become refractory to the anti-inflammatory actions of IL-10, a mechanism that may play important role in the perpetuation of inflammation in diseases characterized by the presence of immune complexes such as inflammatory arthritis and systemic lupus erythematosus.

Tpl2 kinase. Tumor progression locus 2 (Tpl2) encodes a serine/threonine protein kinase that is activated by provirus integration in Moloney murine leukemia virus-induced T cell lymphomas and mouse mammary tumor virus-induced mammary adenocarcinomas. Tpl2 transduces Toll-like and death receptor signals in a variety of cell types and plays an important role in innate immunity and inflammation. Tpl2 knockout (Tpl2-/-) mice develop normally and have no obvious phenotypic defects. However, Tpl2-/- mice are resistant to LPS-induced endotoxin-shock, as well as to TNF±-induced inflammatory bowel disease. Tpl2 plays an obligatory role in the transduction of extracellular signal-regulated kinase (ERK) activation signals induced by LPS and CD40L in macrophages and B cells and by TNF± in macrophages. The failure of ERK activation by LPS in Tpl2-/- macrophages gives rise to defects in the expression of cytokines, chemokines and other molecules involved in the regulation of innate and adaptive immunity. The role of Tpl2 kinase in signaling pathways activated downstream of TLRs other than TLR4, as well as the FcγR has not been elucidated.

RESEARCH

1. Role of Tpl2 kinase in FcγR signaling and effector functions. Cross-linking of the FcγR induces phagocytosis and the activation of a gene induction program in macrophages, leading to the production of cytokines and chemokines that shape the ensuing immune response. In preliminary experiments we have found that Tpl2 deficiency regulates the production of a limited number of cytokines and chemokines by macrophages after ligation of their FcγR without affecting phagocytosis. Analysis of the signalling cascades activated downstream of FcγR showed that Tpl2 specifically regulates the activation of ERK1/2 MAPKs and the mechanism of this regulation is under investigation.

2. Role of Tpl2 kinase in TLR signalling. TLR activation leads to the activation of a gene induction program that plays central role in the development of the adaptive immune response and inflammatory reaction. We have found that Tpl2 regulates the production of TNF, IL-10, IL-12 and the chemokine IP-10 from macrophages after stimulation with TLR2, TLR3, TLR4 and TLR9 ligands. Tpl2 deficiency selectively regulates the activation of ERK1/2 MAPKs downstream of TLR activation. We have also found that paracrine/autocrine activation of STAT1 is also inhibited in Tpl2 deficient macrophages indicating that Tpl2 may be essential for the production of type I IFNs downstream of TLR activation.

3. Role of Tpl2 kinase in in vivo models of autoimmune and inflammatory diseases. Numerous experimental animal models have shown the essential role of FcγRs and TLRs in the pathogenesis of chronic inflammatory/autoimmune diseases such as arthritis and systemic lupus erythematosus (SLE). To define the role of Tpl2 kinase in such diseases we are introducing the Tpl2 deficiency in the NZBxNZW F1 model of murine SLE. The effects of Tpl2 deficiency in a number of immunologic parameters and on the histopathology and mortality will be analyzed in these mice providing a definitive answer on the role of this kinase in the development and severity on systemic autoimmunity.

4. Structural/functional studies of Tpl2 kinase: We take an integrated approach using both defined in vitro systems as well as in vivo models of systemic autoimmune and inflammatory diseases. Molecular biology and biochemical approaches are complemented by genetic approaches that utilize gene knockout mice. The long term goal is to identify important signalling mediators of inflammation that may be used as targets of therapeutic intervention in autoimmune/inflammatory diseases. A working structural model has been created (Figure 3) in an attempt to elucidate the functional importance of assembling domains of Tpl2 in conjunction with an analysis of mutations leading to signalling defects.

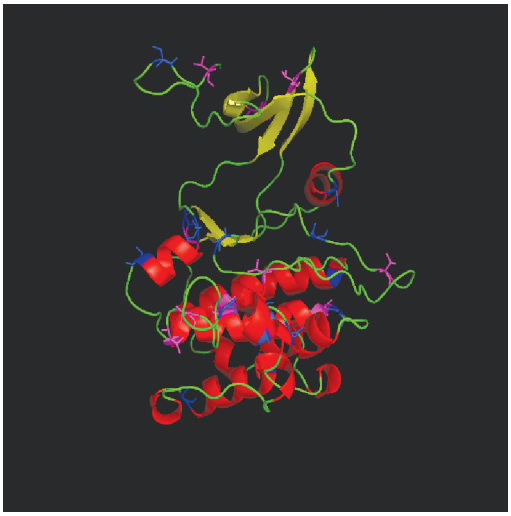

Figure 3. *Structural model of the middle domain of Tpl2, showing the secondary structure arrangement (α helices in red, β-strands in yellow) and the putative phosphorylation sites (Ser, Thr positions in blue and magenta respectively).*

Representative Publications

Tassiulas I, Park-Min KH, Hu Y, Kellerman L, Mevorach D, Ivashkiv LB Apoptotic cells inhibit LPS-induced cytokine and chemokine production and IFN responses in macrophages.Hum Immunol. 2007 Mar;68(3):156-64.

Wang L*, Tassiulas I*, Gil-Henn H, Schlessinger J, Baron R, Zhang J Ivashkiv LB.Regulation of Macrophage Jak Activation and STAT1 Tyrosine Phosphorylation by ITAM-associated Receptors is Mediated by Calcium-dependent CaMK and Pyk2. Nature Immunology 2007(In press) (*equal contribution)

Group members

Ioannis Tassiulas, MD (Head)
Irene Kirmizi, (PhD)
Marianna Ioannou, MSc (cand)
George Goulielmos, (PhD)

Collaborators.

Elias Eliopoulos (PhD)

CONTACT

Ioannis TASSIULAS, MD
University of Crete, Medical School, 1 Voutes str., 710 03, Heraklion, Greece
Tel: +30(281)0392024,
Mobile: + 30 6973880976
Fax: +30(281)0392024
e-mail: tassiulas@gmail.com