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Review

Inhibition of IL-6 family cytokines by SOCS3

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ABSTRACT

IL-6 a multi-functional cytokine with important effects in both inflammation and haematopoiesis. SOCS3 is the primary inhibitor of IL-6 signalling, interacting with gp130, the common shared chain of the IL-6 family of cytokines, and JAK1, JAK2 and TYK2 to control both the duration of signalling and the biological response. Recent biochemical and structural studies have shown SOCS3 binds to only these three JAKs, all of which are associated with IL-6 signalling, and not JAK3. This specificity is determined by a three residue "GQM" motif in the kinase domain of JAK1, JAK2 and TYK2. SOCS3 binds to JAK and gp130 simultaneously, and inhibits JAK activity in an ATP-independent manner by partially occluding the kinase's substrate binding groove with its kinase inhibitory region. We therefore propose a model in which each of gp130, JAK and SOCS3 are directly bound to the other two, allowing SOCS3 to inhibit IL6 signalling with high potency and specificity.

1. Introduction: IL-6 signalling

IL-6 is a pleiotropic cytokine that exerts both inflammatory and anti-inflammatory effects depending upon its cellular context and is an important differentiation factor during haematopoiesis (reviewed in [1]). IL-6 belongs to a family of cytokines that also include IL-11, IL-27, LIF, OSM, CT-1 and CNTF. These cytokines are structurally similar [2] and signal via association with cell-surface trans-membrane receptors that each consist of a dimer (or higher-order oligomer) of the common shared chain, gp130 and a cytokine-specific alpha chain [3,4].

In classical IL-6 signalling, IL-6 first associates with its specific receptor alpha chain, IL-6Rα, and this dimer then associates with gp130 to form a hexameric signalling competent complex with 2:2:2 stoichiometry [5,6]. Whilst gp130 is expressed on the surface of most cell-types, IL-6Rα expression is more restricted. However, many cells which do not express IL-6Rα still respond to IL-6 by virtue of circulating soluble IL-6Rα (sIL-6Rα). This is termed trans-signalling and is often associated with the pro-inflammatory effects of IL-6 [7,8].

In both classical and trans-signalling, once the gp130 dimer is occupied there is an auto-activation of associated JAKs (Janus Kinases) which are found in an inactive state prior to cytokine exposure [9]. Based on similarity to receptor tyrosine kinase (RTK) signalling (for example insulin signalling) [10], activation is thought to occur by auto-phosphorylation *in trans*. In more detail, according to this model one JAK molecule bound to one cytokine receptor chain is phosphorylated by the JAK molecule bound to the other receptor chain (and vice versa) within the receptor homo- or hetero-dimer. Activation involves phosphorylation of specific tyrosine(s) within the activation loop of the kinase [9] which causes the activation loop to translocate out of the active site in order to allow ATP and substrate to bind [11]. JAK1, JAK2 and TYK2 have all been found associated with gp130 [12] in certain contexts however genetic deletion of these kinases has implicated JAK1 as the most important member of the family for gp130 induced signalling [13]. Upon activation, JAKs then phosphorylate five specific tyrosines on the cytoplasmic domain of gp130. Four of these phosphotyrosines are recruitment sites for STAT1 and/or STAT3 (Signal Transducer and Activator of Transcription-1 and -3) which are then activated by phosphorylation, again through the kinase activity of JAK1, JAK2 or TYK2 [14]. STAT1 and STAT3 are latent transcription factors and once activated, they translocate into the nucleus and induce the transcription of appropriate IL-6-responsive genes. Thus STATs are the primary drivers of the biological response (see Fig. 1, left). However, in addition to the JAK/STAT cascade, the MAP kinase and PI3 kinase pathways are also activated. This is via the fifth tyrosine,

Abbreviations: JAKs, Janus Kinases; SOCS, Suppressor of Cytokine Signalling; STAT, Signal Transducers and Activators of Transcription; IL-6, interleukin-6; IL-10, interleukin-10; IL-11, interleukin-11; IL-27, interleukin-27; gp130, glycoprotein 130; OSM, oncostatin M; LIF, leukemia inhibitory factor; CNTF, cillary neurotrophic factor; CT-1, cardiotrophin 1; IL-6Rα, interleukin-6 receptor alpha-chain; G-CSF, granulocyte colony-stimulating factor; KIR, kinase inhibitory region; SH2, Src homology 2; SHP2, SH2 domain containing phosphatase; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase.

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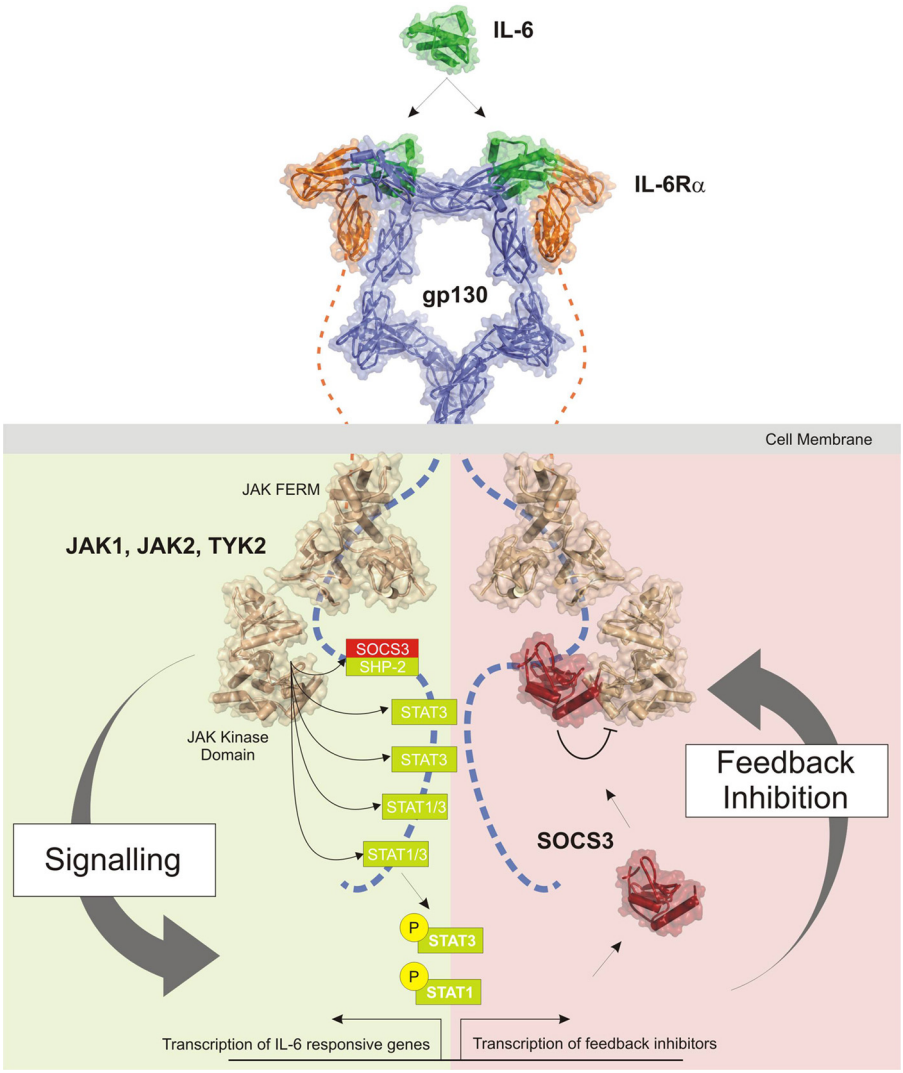

Fig. 1. IL-6 signalling and its inhibition. Schematic diagram illustrating activation (left) of the JAK/STAT signalling cascade in response to IL-6 and the termination of signalling (right) catalysed by SOCS3. IL-6 signals via a cell-surface receptor that consists of a 2:2 gp130 (blue):IL-6Rα (orange) tetramer. Interaction between the cytokine and its receptor induces the autoactivation (*in trans*) of Janus Kinases (JAKs,: JAK1, JAK2, TYK2; shown in beige) bound to the cytoplasmic domain of gp130. Activated JAK then phosphorylates five tyrosines within $gp130^{cyt}$. Four of these phosphotyrosines recruit STAT3 or STAT1/STAT3 which are then themselves phosphorylated, and thereby activated, by JAK, translocate to the nucleus and begin inducing the transcription of IL-6-responsive genes. STATs also upregulate the transcription of SOCS3 (red) which binds to the fifth phosphotyrosine in $gp130^{cyt}$ (pY^{759}) and shuts down the JAK/STAT signalling cascade by binding to JAK and directly inhibiting its catalytic activity, forming a negative feedback loop. This phosphotyrosine also recruits SHP-2, which leads to activation of the MAPK/ERK and PI3K pathways (not shown here) and therefore SOCS3, which competes for this site, is also capable of inhibiting those signalling cascades. Signalling and inhibition is symmetric with respect to both gp130 chains and is shown here divided into left and right for ease of illustration. The structures shown are those solved and/or modelled for components of the signalling cascade, note that the pseudokinase and SH2-like domains of JAK are omitted for clarity in this figure.

Y^{759}, which, once phosphorylated, is a docking site for SHP2. SHP2 is activated by phosphorylation after binding and this leads to stimulation of both the MAPK/ERK and PI3 kinase pathways [15].

In addition to driving the biological response, activated STAT3 also induces expression of SOCS3 (Suppressor of Cytokine Signalling-3). SOCS3 in turn terminates the JAK/STAT signalling cascade, forming a negative feedback loop that allows the cell to return to its basal (unstimulated) state (Fig. 1, right). This action of SOCS3 appears to be the primary mechanism by which IL-6 signalling is regulated within the organism. This review will focus on the mechanism by which SOCS3 inhibits IL-6 (and IL-6 family) signalling.

2. Discovery of the SOCS proteins

In 1997 the SOCS family of proteins were discovered concurrently by the groups of Hilton (Walter and Eliza Hall Institute, Australia), Yoshimura (Kurume University, Japan) and Kishimoto (Osaka University, Japan) [16–18]. Each group used a different approach. Hilton et al., used an expression cloning methodology to identify proteins capable of inhibiting the IL-6-induced differentiation of the mouse M1 myelomonocytic cell-line and discovered, and named, SOCS1 (Suppressor of Cytokine Signalling 1). Yoshimura's group discovered the same entity via a yeast two-hybrid screen aimed at identifying proteins that bind to JAK and termed the protein JAB (JAK-binding protein). Finally, Kishimoto et al. isolated a protein (SSI-1) on the basis of a short region of sequence similarity with STAT3. SSI-1 was found to be related to the SH2 domain-containing protein CIS (cytokine inducible SH2 domain containing protein) and identical in sequence to SOCS1/JAB identified by the other two groups. Collectively, these three manuscripts described the major attributes of the SOCS1 protein: (A) that its expression is induced by a variety of cytokines; (B) it then inhibits the signalling cascade initiated by those same cytokines, forming a negative