Inhibition of pro-inflammatory cytokine receptor signalling by cAMP in vascular endothelial cells

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Abstract
The anti-inflammatory effects of the prototypical second messenger cAMP have been extensively documented in multiple cell types. However, in many instances, the molecular mechanisms by which cAMP elevation disrupts specific pro-inflammatory signalling cascades are unknown. In this review, we will describe the importance of the JAK–STAT (where JAK stands for Janus kinase and STAT for signal transducer and activator of transcription) signalling pathway in vascular endothelial cell function, outline key inhibitory processes that serve to reduce cytokine-stimulated tyrosine phosphorylation and activation of STAT proteins, and discuss possible mechanisms by which intracellular CAMP sensors could interact with these inhibitory processes to diminish cytokine receptor-mediated pro-inflammatory signalling.

The vascular endothelium and disease
Under normal physiological conditions, the endothelium acts as a multifunctional anti-coagulant, anti-inflammatory and anti-thrombotic barrier [1]. However, the ability of vascular ECs (endothelial cells) to dramatically alter their phenotype in response to a variety of stimuli is a characteristic feature of several diseases, including atherosclerosis, sepsis and diabetic retinopathy [2,3]. Therefore the design of new strategies to inhibit the excessive and inappropriately sustained activation of pro-inflammatory/angiogenic signalling pathways activated by cytokines and growth factors in ECs is an important aim. Some of the most important regulators of EC function include class I cytokines, such as the adipocytokine leptin and the ‘IL-6 (interleukin-6) family’ (these include IL-11, oncostatin M and prototypical member IL-6), which predominantly activate the JAK–STAT (where JAK stands for Janus kinase and STAT for signal transducer and activator of transcription) pathway [4,5]. While ECs are unresponsive to IL-6 alone, sIL-6Rα (soluble IL-6 receptor α) chain released from activated monocytes and macrophages at sites of vascular injury is able to bind IL-6 to generate an sIL-6Rα–IL-6 complex that can bind and activate the signal transducer ‘gp130’ [4]. In contrast, leptin meditates its effects by directly binding and activating the leptin receptor ‘Ob-Rb’, of which there are several forms arising from alternative splicing within the stretch of RNA encoding the C-terminus of the protein [5]. However, ECs express the full-length Ob-Rb isofrom exclusively [6].

One potential strategy for limiting endothelial dysfunction is to enhance the activity of endogenous protective signalling pathways. To test this hypothesis, we have been examining the molecular mechanisms mediating the inhibitory effects of the prototypical second messenger cAMP on critical pro-inflammatory signalling pathways in ECs. The protective effects of cAMP elevation in maintaining the barrier function and limiting endothelial dysfunction are well described but the molecular basis for many of these effects remains unclear. In particular, elevation of cAMP in several cell types has been shown to reduce the ability of specific cytokines to activate the JAK–STAT pathway [7–9]. In the present study, we will briefly describe two potential mediators that could account for the ability of cAMP to inhibit signalling from class I cytokine receptors in vascular ECs.

Activation of SH2 (Src homology 2) domain-containing protein tyrosine phosphatase-2 (SHP-2)
SHP-2 occupies a unique dual role in class I cytokine receptor signal, being able to exert both positive and negative effects on separate signalling events. Thus, while SHP-2 is capable of dephosphorylating and inactivating JAKs, STATs and active JAK-phosphorylated gp130 and Ob-Rb, it is also responsible for recruitment of Grb2–Sos complexes to Tyr-phosphorylated gp130 and Ob-Rb, which is essential for receptor activation of the ERK (extracellular-signal-regulated kinase) pathway [4,5]. SHP-2 is constitutively expressed in many cell types, including vascular ECs in which it plays a particularly important role of modulating signals from gp130 [4]. However, its regulation by cAMP in any cell type has not been studied intensively, despite tantalizing evidence that this second messenger can profoundly modulate its activity. For example, elevation of cAMP levels in CTLL-2 T-cells promotes the Tyr phosphorylation and activation of SHP-2, which is thought to be responsible for the observed dephosphorylation of IL-2-stimulated STAT5 phosphorylation in the absence of any effect on JAK phosphorylation [10]. In contrast, cAMP elevation in adrenocortical cells
Figure 1 | Effect of cAMP elevating agents on sIL-6Rα/IL-6-stimulated STAT3 phosphorylation and SOCS-3 induction

(A) HUVECs (human umbilical vein ECs) were pre-treated for 5 h with or without 10 µM forskolin (Fsk) and 10 µM rolipram (Roli) before the addition of vehicle or sIL-6Rα/IL-6 for a further 30 min as indicated. Soluble cell extracts equalized for protein content were then fractionated by SDS/PAGE for immunoblotting with antibodies against Tyr701 phospho-STAT1, Tyr705 phospho-STAT3, total STAT1 and total STAT3. (B) HUVECs were treated as above before preparation of soluble extracts for analysis by SDS/PAGE and immunoblotting with anti-SOCS-3 and anti-tubulin antibodies.

SOCS (suppressor of cytokine signalling) proteins

SOCS proteins constitute a family of eight related proteins (CIS and SOCS-1–7), of which SOCS-1 and SOCS-3 have been characterized most intensively, that function as endpoints in a classical negative feedback loop whereby activation of STATs triggers their induction. Induced SOCS proteins can then bind to and terminate signalling from activated cytokine receptors [11]. A role for SOCS-3 in specifically terminating gp130 and Ob-Rb signalling has been demonstrated by several observations, including the unrestricted agonist-stimulated activation of STAT3 seen in macrophages, hepatocytes and neurons from cell-specific conditional SOCS-3-deficient mice [12–14]. SOCS-3 terminates signalling by binding to JAK-phosphorylated receptors via its SH2 domain, interacting with and inhibiting adjacent JAKs via its ‘kinase inhibitory region’, and thereby preventing the recruitment and Tyr phosphorylation of STATs [11]. SOCS-3 can also potentially competitively block receptor recruitment of SHP-2 to Tyr759 of gp130, thus inhibiting ERK activation [11]. In addition, the C-terminal ‘SOCS box’ domain may target SH2 domain-bound partners for proteosomal degradation by directing interaction with an elongin B–C E3 ubiquitin ligase complex [11].

However, in addition to their well-characterized STAT-mediated classical negative feedback role, it is now clear that both SOCS-1 and SOCS-3 can also be induced via stimuli that do not activate the JAK–STAT pathway, thereby providing a mechanism by which otherwise distinct signalling pathways can negatively cross-regulate cytokine responsiveness. For example, one recently identified signal for SOCS induction is elevation of intracellular cAMP [7,8]. The ability of this prototypical second messenger to suppress activation of the NF-κB (nuclear factor κB) pathway at several levels in many cell types has been the most intensively studied aspect of its anti-inflammatory effects. However, the induction of SOCS-1 and SOCS-3 observed in leucocytes and FRTL-5 thyroid cells suggests a potential mechanism by which cAMP could block pro-inflammatory signalling from multiple JAK–STAT-mobilizing cytokine receptors [7,8]. Classically, cAMP is thought to mediate the vast majority of its intracellular effects by binding and activating cAMP-dependent PKA, which controls the phosphorylation status and activity of multiple intracellular substrates [15]. However, another family of intracellular cAMP sensors termed ‘Epacs (exchange proteins directly activated by cAMP)’ have been recently identified [16]. Epac1 and Epac2 function as cAMP-activated GEFs (guanine nucleotide-exchange factors) specific for the Rap family of small G-proteins, and thus promote the accumulation of active GTP-bound Rap1 and Rap2 [16]. Interestingly, a role for Epac in EC function was recently revealed by the finding that Epac-mediated activation of Rap1 and subsequent formation of adherens junctions contributes towards the ability of cAMP to enhance endothelial barrier function [17]. However, the separate contributions of PKA and Epac towards SOCS-1 and SOCS-3 induction in different cell types, and the promoter elements that confer sensitivity to cAMP, remain to be determined.

Summary

Cumulatively, our observations (Figure 1) support the presence of a novel anti-inflammatory effect of cAMP elevation in vascular ECs that leads to the up-regulation of SOCS proteins, which then down-regulates receptor activation...
of the JAK–STAT pathway. Further investigations will be required to identify the steps linking cAMP elevation to the accumulation of SOCS transcript, and determine the significance of the cAMP/SOCS pathway on regulating the activity of binding partners, such as the insulin receptor and IRS1 (insulin receptor substrate 1), that play critical roles in EC function in disease.

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References

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