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Regulation of Anti-Inflammatory Gene Expression in Vascular Endothelial Cells by EPAC1

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Abstract

Suppressor of cytokine signalling 3 (SOCS3) is a potent inhibitor of pro-inflammatory pathways involved in atherogenesis and the development of neo-intimal hyperplasia (NIH), which contributes to the in-stent re-stenosis responsible for the failure of percutaneous coronary intervention (PCI) procedures. We have shown that cyclic AMP sensor EPAC1 triggers induction of the SOCS3 gene in vascular endothelial cells (VECs), thereby attenuating interleukin 6 (IL-6)-mediated pro-inflammatory signalling. We propose that EPAC1 localisation to the nuclear pore controls cyclic AMP-mediated activation of a C/EBPβ/c-Jun transcriptional complex, leading to SOCS3 induction and suppression of pro-inflammatory signalling. Future work in this area will involve an integrated approach to determine the wider significance of the EPAC1-C/EBPβ/c-Jun pathway in controlling human VEC function and identify new therapeutic targets for management of chronic inflammation in vascular settings.

Background

Atherosclerosis is a serious CVD, which arises from chronic localised inflammation at coronary and carotid arterial branch points [1], and remains the principle cause of death in the developed world despite changes in lifestyle and the wide-spread use of anti-hypertensive and lipid- lowering drugs (http://www.who.int/). Atherogenesis involves a diet-induced propagation of pro-inflammatory responses leading to the formation of plaques characterised by cholesterol deposition, fibrosis, remodelling and switching of VECs from an anti-coagulant/anti-inflammatory to a pro-thrombotic/pro-inflammatory phenotype. If untreated, these lesions either occlude vessels or trigger their rupture, resulting in the formation of thrombi that cause myocardial infarction or stroke. Surgical treatment for atherosclerosis typically involves angioplasty, where arterial plaques are removed and a stent is introduced to maintain blood flow. However, in approximately 25-50% of cases, mechanical injury during angioplasty can lead to NIH, characterised by localised inflammation and proliferation of vascular smooth muscle cells (VSMCs), thereby precipitating stent failure and myocardial infarction [2]. The increased inflammatory activity associated with atherosclerosis and NIH is partially brought about by increased levels of pro-inflammatory cytokines in the circulation, particularly IL-6 [3,4]. Sustained IL-6 production is involved in chronic, low-level vascular inflammation that leads to neointimal thickening [5], vascular dysfunction [6], hypertension [7] and increased risk of myocardial infarction [3]. Indeed, IL-6 has been detected in atherosclerotic plaques [8] and increases in IL6 in elderly patients are associated with a two-fold increase in both cardiovascular and other causes of mortality [9]. IL-6 affects vascular endothelial cells (VECs) by triggering counter-productive angiogenesis, through vascular endothelial growth factor (VEGF) production [10], and increasing the secretion of chemokine's, like monocyte chemo-attractive protein 1 (MCP-1) [11], that recruit monocytes to the inflamed endothelium. Given this, there is now a clear need to understand the mechanisms regulating the control of pro-inflammatory IL-6 signalling in VECs if we are to devise new and effective strategies to combat atherosclerosis and NIH.

Signalling by IL-6 occurs through the IL-6 receptor complex, composed of an IL-6-binding α chain (IL-6Rα) and gp130, which interacts with IL-6Rα [12]. IL-6 has been reported to exert both inflammatory and anti-inflammatory actions [13] and a single nucleotide polymorphism (SNP; Asp358Ala) has been identified in the IL-6R, which reduces inflammation and the risk of developing coronary heart disease (CHD) [14,15], although the mechanisms for this remain to be determined [16]. It is IL-6 receptor "trans- signalling" [17] that is thought to underlie the pro-inflammatory actions of IL-6 in a variety of diseases, including atherosclerosis [18]. During trans-signalling, IL-6 binds to soluble forms of IL-6R (sIL-6R) allowing activation of gp130, even in cells that do not normally express IL-6Rα, such as VECs [17]. Since gp130 is present on all cells, trans-signalling therefore dramatically increases the range of cell targets for IL-6 signalling to include VECs [17]. Consequently, binding of the IL-6/sIL-6R complex to gp130 on VECs, leads to receptor clustering and activation of the JAK-STAT3 and ERK, MAPK and PI3K signalling pathways. Of these, it is activated STAT3 that then homodimerises and translocates to the nucleus, where it acts as a transcription factor for the induction of pro-inflammatory IL-6-responsive genes, such as MCP-1 and VEGF [11,19].

Inhibition of Vascular Inflammation by SOCS3

Clearly, regulation of pro-inflammatory IL-6 signalling is vital to prevent chronic inflammation. Understanding the mechanisms controlling IL-6 signalling may therefore pave the way to the development of new strategies to combat vascular inflammation. In this light, one important mechanism for down-regulating JAK-STAT3 signalling is via the suppressor of cytokine signalling (SOCS) family of

proteins [20], which are often induced directly by the same JAK-STAT pathway they inhibit, forming a classical negative feedback loop [21]. For example, SOCS3 binds to JAK-phosphorylated receptors, via the SOCS3 SH2 domain, thereby inhibiting JAK activity and, consequently, activation of STATs 1 and 3 [22]. SOCS3 then also targets multiple SH2-bound proteins for proteasomal degradation [22], with proteolytic targets including JAK2 [23]. Consistent with its role as a negative regulator of inflammatory signalling, SOCS3 expression is localised to atherosclerotic plaques [24,25] and SOCS3 knockdown in apoE-/- mice increases STAT activation and inflammatory gene expression in aorta, leading to enhanced atherogenesis [25]. Moreover, IL-6 has been reported to promote acute and chronic inflammatory disease in the absence of SOCS3 [26] and conditional deletion of the SOCS3 gene in vascular endothelial cells results in pathological angiogenesis [27]. In contrast, overexpression of SOCS3, or introduction of SOCS-derived peptides in to cells, has been shown to suppress JAK/STAT3 signalling, acute inflammation and the development of atherosclerosis and NIH, effectively illustrating the important protective role of SOCS3 [28-30].

Clearly, novel treatments based on the regulation of SOCS3 levels in cells could have value in the treatment of diseases like atherosclerosis, where there is hyper-activation of JAK-STAT3 signalling. In this respect, the ubiquitous second messenger, cyclic AMP, which is synthesised in VECs in response to Gsα-coupled G-protein-coupled receptor (GPCR) activation, plays a key role in controlling SOCS3 induction and limiting cytokine action; indeed, we have defined a role for the cyclic AMP-activated guanine nucleotide exchange factor (GEF), exchange protein activated by cyclic AMP (EPAC1) [31,32], as a key mediator of SOCS3 induction [31] and a central controller of anti-inflammatory processes in VECs [33,34]. EPAC1 mediates at least three anti-inflammatory signalling pathways in VECs; namely, down-regulation of IL-6- and STAT3-mediated inflammatory processes [31], which occurs through C/EBP transcription factor-dependent induction of the SOCS3 gene [32], limiting vascular permeability through EPAC1-mediated activation of integrins, involved in cell spreading and adhesion of VECs to the basement membrane [35], and promotion of endothelial barrier function through actin [36-40] and microtubule-dependent [41] cell-cell junction formation through stabilisation of VE-cadherin-mediated adhesion [42]. Overall, the involvement of EPAC1 in multiple anti-inflammatory processes in VECs presents an effective model in which to study how distinct cellular processes may interact to present a co-ordinated program of "protection" against inflammatory stimuli.

Hypothesis

We must now determine the cellular actions of EPAC1 that are linked to SOCS3 induction and inhibition of IL-6 signalling. We propose that EPAC1 may serve as a potential target for future anti-inflammatory drug development, with a reduced risk of side effects, based on the following observations

- EPAC1 promotes SOCS3 induction independently of potentially harmful JNK and ERK 1 and 2, MAP kinase signalling pathways, which are normally triggered by changes in global cyclic AMP (Figure 1) [43,44].
- SOCS3 induction by EPAC1 requires C/EBPβ and c-Jun transcription factors [32,44], which directly interact with the SOCS3 promoter and may therefore serve as a point of integration for EPAC1-regulated signalling to enable effective SOCS3 gene induction in VECs (Figure 2) [44].
- A key AP1 transcription factor binding site is required for EPAC1 to activate the SOCS3 promoter [44].
- C-Jun is constitutively associated with the AP1 site [44], whereas C/EBPβ is recruited to the SOCS3 promoter following EPAC1 activation [32].
- EPAC1 is mainly associated with the nuclear compartment of fractionated HUVECs where it co- localises with the SUMO1 E3 ligase, RanBP2 (results not shown), which was previously identified as an EPAC1 binding protein [45,46].
- The nuclear targeting domain within EPAC1 as being within amino acids 764-838 in the GEF domain [47]. This domain contains two areas that are unique to EPAC1 and not found in EPAC2. Since EPAC2 is not nuclear targeted to the same degree as EPAC1 [45] then we predict that these areas contain the key determinants for nuclear targeting of EPAC1.

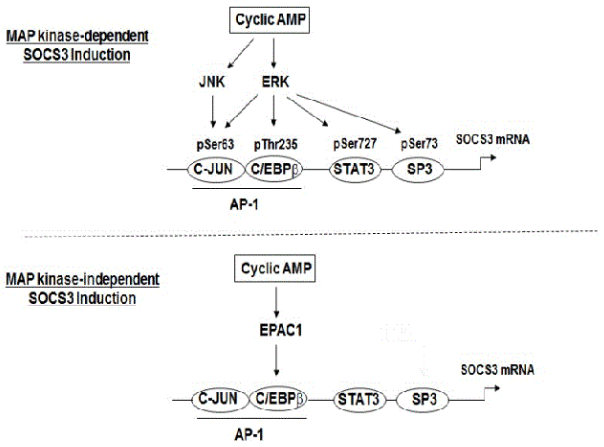

Figure 1: MAP kinase-dependent and -independent control of SOCS3 Gene Induction by cyclic AMP Maximal induction of the SOCS3 gene by cyclic AMP in VECs requires input both EPAC1- (lower panel) and MAP kinase-dependent (upper panel), with no role for PKA [32]. Activation of AP1 and C/EBP transcription factors by EPAC1 is sufficient to induce SOCS3 induction independently of MAP kinase signalling (lower panel) and therefore presents as a potential drug target

Based on these findings our hypothesis is that targeting of EPAC1 to the nuclear pore complex regulates c-Jun and C/EBPβ transcription factors, which control SOCS3 gene induction and suppression of pro-inflammatory cytokine signalling in VECs (Figure 1). Ongoing work is set to test this hypothesis and extend to identify the full range of human genes that are also regulated by this new, protective signalling pathway in VECs. These novel genetic foci will be associated with the protective actions of cyclic AMP and will therefore inform future strategies targeted at combating endothelial inflammation associated with CVD.

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