

## Suppressor of cytokine signaling and accelerated atherosclerosis in kidney disease

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The prevalence of cardiovascular disease in patients with renal failure is extremely high and accounts for a large part of the morbidity and mortality. Inflammation participates importantly in host defense against infectious agents and injury, but also contributes to the pathophysiology of many diseases, including cardiovascular atherosclerosis, which is a main problem in patients with renal failure. Recruitment of blood leukocytes to the injured vascular endothelium characterizes the initiation and progression of atherosclerosis and involves many inflammatory mediators, modulated by cells of both innate and adaptive immunity. Excessive inflammatory and immune responses, communicated by these different cell types, are driven by inflammatory cytokines that promote associated tissue damage if cytokine signaling pathways remain unregulated. Thus, pathways capable of suppressing proinflammatory cytokine signaling hold the potential to limit life-threatening cardiovascular events caused by atherogenesis. Suppressors of cytokine signaling (SOCS) are a family of intracellular proteins, several of which have emerged as key physiological regulators of cytokine-mediated homeostasis, including innate and adaptive immunity. Accumulating evidence supports the idea that dysregulation of cytokine signaling by differential SOCS expression is involved in the pathogenesis of various inflammatory, and immunological diseases, including atherosclerosis. Based on recent observations, in which SOCS expression levels are profoundly altered in kidney disease, we discuss the possibilities of SOCS as new intracellular markers of inflammation as well as their potential atherogenic properties in renal failure related cardiovascular disease.

**Keywords:** kidney disease, inflammation, atherosclerosis, SOCS

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### INTRODUCTION

Chronic renal failure (CRF) is a clinical syndrome associated with a slow decline in kidney function over time. CRF may be caused by a number of disorders, which include long-standing hypertension, diabetes, glomerulonephritis and congenital kidney disease. If renal function declines below a certain level (glomerular filtration rate < 15 ml/min per 1.73 m<sup>2</sup>) patients enter the end stage renal disease (ESRD) phase in which renal replacement therapy using dialysis may be necessary. The ultimate cure for these patients is kidney transplantation.

The prevalence of atherosclerotic cardiovascular disease (CVD) in patients with CRF is extremely high and accounts for a large part of the morbidity and mortality in this group (Roberts *et al.*, 2006). In ESRD patients 60% of mortality is caused by atherosclerosis (Levey *et al.*, 1998). In addition to traditional risk factors (e.g. hypertension and dyslipidemia) (Chan, 2005; Roberts *et al.*, 2006), CRF forms an independent risk factor for CVD. Somehow, a decrease in renal clearance and neurohumoral consequences of renal disease aggravate the atherosclerotic process (Mathur *et al.*, 2002; Stenvinkel *et al.*, 2003). Increased oxidative stress and inflammation, both common features of CRF and ESRD, are considered central in the pathogenesis and may contribute to the accelerated atherosclerosis.

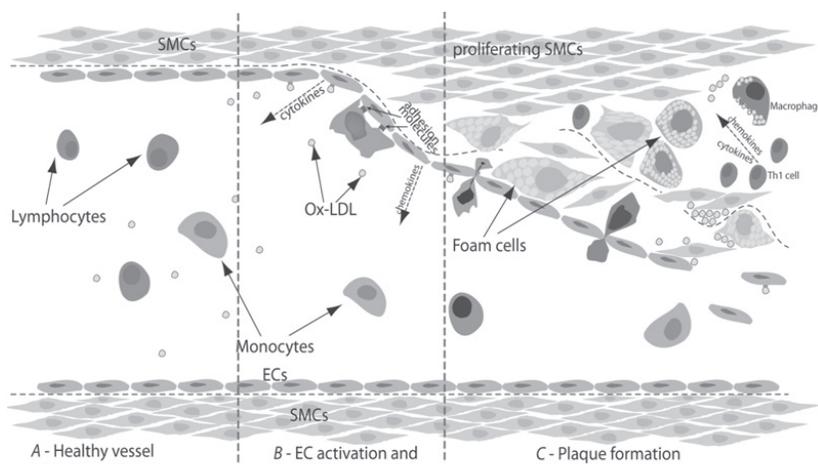
Strong evidence is emerging that management of inflammation could substantially decrease prevalence of CVD (Kwak *et al.*, 2003; Hansson *et al.*, 2006), pointing to the importance of molecules that are able to diminish or inhibit inflammation. Currently no recognized or even proposed treatment exists for renal patients with chronic inflammation (Stenvinkel, 2002), stressing the importance to identify new possibilities to manage inflammation in patients with renal failure. Better understanding of the molecular and clinical mechanisms of inflammation could therefore help to provide new therapeutic strategies to control inflammation and consequently atherosclerotic CVD in renal patients.

### ATHEROSCLEROSIS AND INFLAMMATION

The normal arterial endothelium resists prolonged contact with leukocytes, including monocytes and lymphocytes. Recruitment of blood leukocytes to the injured vascular endothelium characterizes the initiation and progression of atherosclerosis and involves a variety of inflammatory mediators, modulated by cells of both innate and adaptive immunity (Libby,

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**Abbreviations:** CIS, cytokine-induced SH2-containing protein; CRF, chronic renal failure; CRP, C-reactive protein; CVD, cardiovascular disease; EC, endothelial cells; ESRD, end stage renal disease; EPO, erythropoietin; IGF-1, insulin growth factor 1; IFN, interferon; IL, interleukin; IRS, insulin receptor substrate; G-CSF, granulocyte colony-stimulating factor; GH, growth hormone; JAK, Janus kinase; KIR, kinase inhibitory domain; KO, knockout; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; SOCS, suppressor of cytokine signaling; SMC, smooth muscle cell; STAT, signal transducer and activator of transcription; Th, T-helper cell; TNF, tumor necrosis factor; VSMC, vascular smooth muscle cell



**Figure 1. Formation of arteriosclerotic plaque**

(A) A healthy vessel wall is built of a smooth muscle cell layer (SMCs), intima (dashed line) and an innermost layer of endothelial cells (ECs). (B) Activation of scavenger receptors by oxidized LDL particles (Ox-LDL) causes EC activation, which is characterized by secretion of cytokines and chemokines and presentation of adhesion molecules on the surface of ECs as well as increased permeability. Cytokines and chemokines attract lymphocytes and monocytes to the damaged site, adhesion molecules enable these cells to adhere to ECs and then to migrate through the EC layer. (C) Plaque formation involves detachment of ECs and proliferation of SMCs, and endocytosis of Ox-LDL by SMCs and macrophages, which causes these cells to become foam cells. Increased numbers and activation of Th1 cells are a general characteristic of atherosclerosis and involved in the promotion of lesion development and progression. As a consequence, in the progressive stages the lumen of the blood vessel becomes obstructed.

2002). Atherosclerosis is initiated when oxidized low density lipoproteins (ox-LDL) accumulate in the intima, causing oxidative stress and activating endothelial cells (EC). This leads to production of cell surface adhesion molecules, chemokines and inflammatory cytokines. These characteristics of EC dysfunction (Hack & Zeerleder, 2001) form an initial step in atherosclerosis development. Subsequent recruitment and translocation of blood borne monocytes and naive lymphocytes from the circulation into the intima is followed by monocyte differentiation into macrophages and later on to foam cells. Lymphocytes have been implicated in the atherosclerotic process in a variety of ways. Subsets of T helper (Th) cells are important producers of pro-inflammatory cytokines; Th1 cells produce  $\text{IFN}\gamma$ ,  $\text{TNF}\alpha$  and IL-12 and IL-18, all of which have been shown to promote atherogenesis (Robertson & Hansson, 2006). Th2 cells secrete IL-4, IL-5, IL-10 and IL-13, and provide help for the recruitment of B-cells and their antibody production (Robertson & Hansson, 2006). Increased numbers and activation of Th1 cells are a general characteristic of atherosclerosis and involved in the promotion of lesion development and progression. Th2 cytokines mostly antagonize pro-atherogenic Th1 effects and thereby should confer athero-protection. Finally, proliferation of smooth muscle cells (SMC) within the lesions is apparent, resulting in vessel occlusion. Excessive inflammatory and immune responses, communicated by these different cell types, are driven by inflammatory cytokines that promote associated tissue damage and contribute to local inflammation and vascular damage (Libby, 2002; Hansson *et al.*, 2006). As such, in patients with CVD plasma levels of several pro-inflammatory factors (e.g. interleukin-6 (IL-6), tumor necrosis factor  $\alpha$  ( $\text{TNF}\alpha$ ) and C-reactive protein (CRP)) are recognized as markers of atherosclerosis.

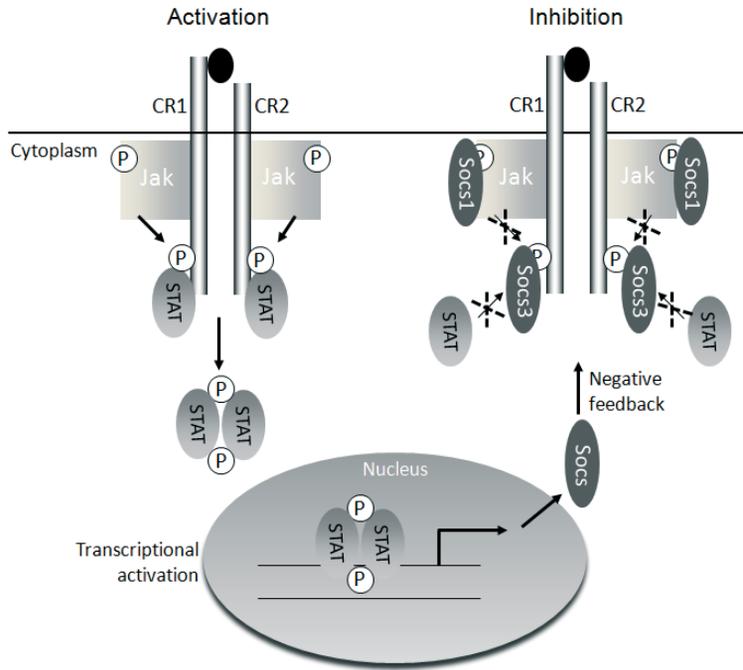
## RENAL DISEASE, INFLAMMATION AND CARDIOVASCULAR DISEASE

The well-known hallmarks of renal disease are formed by decreased filtration of blood components and neurohumoral dysregulation. Among the substances that accumulate are products that increase oxidative stress (AGEs, homocysteine, ox-LDL) (Himmelfarb *et al.*, 2000; van Guldener *et al.*, 2001; Schnackenberg, 2002; Galle *et al.*, 2003; Locatelli *et al.*, 2003). Indeed, increased reactive oxygen species (ROS) have been demonstrated in patients with CRF and ESRD (Schnackenberg, 2002; Locatelli *et al.*, 2003). Increased sympathetic nervous system activity in CRF is well documented and is closely linked to hypertension and to increased activity of the renin-angiotensin system (RAS) that causes oxidative stress. Side effects of treatment may also result in oxidative stress, such as intravenous iron administration (Roob *et al.*, 2000) and/or the passage of leukocytes through tubing and filters during hemodialysis (Menegatti *et al.*, 2002).

Oxidative stress is a major initiator of an inflammatory response. This results in a shift towards the production (and activation) of pro-inflammatory cytokines, such as IL-1 $\beta$ , IL-6 and IL-8 and  $\text{TNF}\alpha$  and  $\text{IFN}\gamma$  (Saadeddin *et al.*, 2002). Indeed, in CRF and ESRD, circulating levels of CRP (Wanner *et al.*, 2002) IL-1 $\beta$ ,  $\text{TNF}\alpha$  and IL-6, have been demonstrated to be predictors of endothelial dysfunction and atherosclerosis (Kato *et al.*, 2002). Endothelial dysfunction is common in patients with moderate renal failure (Anuk *et al.*, 2001) and also in ESRD patients undergoing hemodialysis (Miyazaki *et al.*, 2000) or peritoneal dialysis (van Guldener *et al.*, 1998), characterized by impaired endothelium-dependent vasodilation or increased soluble cell adhesion molecules (Bolton *et al.*, 2001). In addition, renal disease at all stages is associated with activation of peripheral blood mononuclear cells (PBMC) (Sester *et al.*, 2000; Tzirpanlis, 2007).

Patients with renal disease also display insensitivity to a diversity of humoral factors, including growth hormone (GH) (Lin *et al.*, 1998), insulin (Roelfsema & Clark, 2001), erythropoietin (EPO) (Stenvinkel & Barany, 2002) and insulin growth factor 1 (IGF-1) (Lin *et al.*, 1998). GH resistance leads to severe bone defects in children with chronic renal failure, which has also been related to a decreased sensitivity for IGF-1 (Lin *et al.*, 1998; Roelfsema & Clark, 2001). The anaemia that goes with renal failure is mainly due to a deficiency of EPO. Although decreased EPO levels are restored by administration of human recombinant EPO in severe CRF and ESRD, resistance to erythropoietin therapy is a common complication (Stenvinkel & Barany, 2002). Insensitivity to several of these factors results in metabolic abnormalities and is associated with increased risk of CVD (Stenvinkel & Barany, 2002; Mark *et al.*, 2002; Wheatcroft *et al.*,

INFLAMMATION AND CYTOKINE ACTION

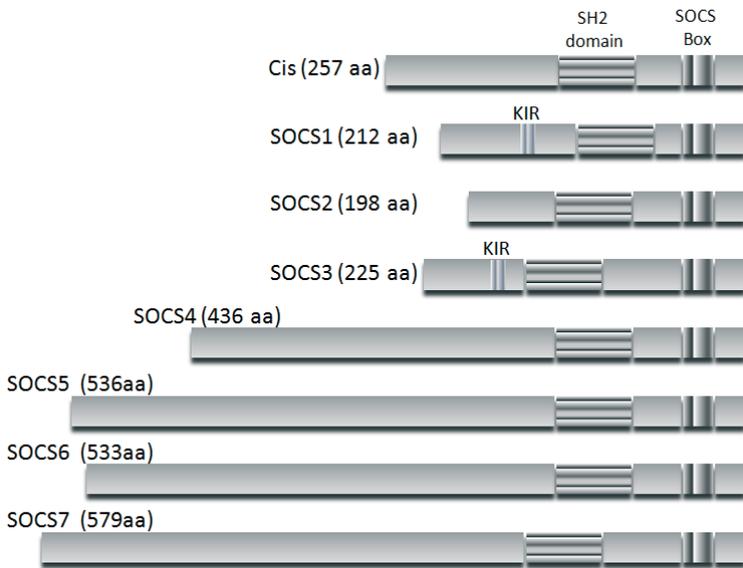


**Figure 2. General paradigm of activation and SOCS-dependent inhibition of cytokine-mediated JAK/STAT signaling**  
 Detailed description is given in the text. CR, cytokine receptor; JAK, Janus kinase; STAT, signal transducer and activator of transcription; P, phosphorylated tyrosine residue; SOCS, suppressor of cytokine signaling.

2003). Insensitivity to humoral factors in patients with renal disease is also mediated by inflammation and inflammatory cytokines. These observations suggest a link between renal failure on the one hand and insensitivity to humoral factors, inflammation and atherosclerosis on the other. Thus, pathways capable of suppressing pro-inflammatory cytokine signaling hold the potential to limit life-threatening cardiovascular events in patients with renal disease.

activation (Fig. 2). The activated JAKs phosphorylate the receptor cytoplasmic domains at specific tyrosine residues, which create docking sites for the members of the signal transducer and activator of transcription (STAT) family: STAT1-6. Upon activation by phosphorylation, STAT molecules dimerize and translocate into the nucleus to activate gene transcription. Crucial immunoregulatory factors include the IL-2 family of cytokines (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21), that generally activate STAT5A and STAT5B, sometimes STAT6 (activated by IL-4). Also IFN $\gamma$ , activating mainly STAT1, but sometimes also STAT3, and members of the IL-6 family of cytokines IL-6, IL-10 and IL-11 using mostly STAT3 for downstream signaling, but sometimes STAT1. IL-12 acts upon STAT4.

The regulation of cellular responsiveness to cytokines determines cytokine activity and the balance between opposing cytokines. Control of the magnitude and duration of cytokine signaling is also essential to prevent pathology. Key regulators of cellular responses to cytokines are members of the suppressors of cytokine signaling protein family (SOCS; also known as cytokine-induced SH2-containing protein (CIS), JAK binding protein, or STAT-induced STAT inhibitor), which plays an important role in feedback inhibition and cytokine cross-regulation (Krebs & Hilton, 2001; Alexander & Hilton, 2004; Yoshimura *et al.*, 2007; Dalpke *et al.*, 2008).



**Figure 3. Schematic representation of the SOCS protein family and their conserved functional domains**  
 Detailed description is given in the text. KIR, kinase inhibitory domain; SH2, Src-homology 2 domain; aa, amino acids.

**Table 1. Biological functions of SOCS proteins**

For references see main text

Gene	Knockout phenotype	Transgenic phenotype	Main affected cytokines
CIS	(?) Increased hematopoiesis disturbed lactation	Reduced weight,	STAT5 signaling (EPO, IL-2, IL-3)
SOCS1	Multiorgan inflammation, neonatal lethality, lymphocyte apoptosis, hematopoietic infiltrations	Disturbed T-lymphocyte development, spontaneous T-cell activation	IFN $\gamma$ , IFN $\alpha$ , IL-4, IL-12
SOCS2	Gigantism	Gigantism	GH, IGF-1
SOCS3	Embryonic lethality, Placenta defects, Disturbed erythropoiesis	Embryonic lethality, increased Th2 differentiation,	gp130, IL-2, IL-6, G-CSF, leptin, EPO
SOCS4	(?)	(?)	(?)
SOCS5	(?) No obvious phenotype (redundancy with SOCS4?)	Disturbed Th2 differentiation	IL-4, EGF
SOCS6	Mild growth retardation (redundancy with SOCS7?)	Improved glucose and insulin tolerance	Insulin (?)
SOCS7	Hydrocephalus, 50% mortality, Hyperinsulinemia	(?)	Insulin

### MODULATION OF CYTOKINE SIGNALING BY SOCS

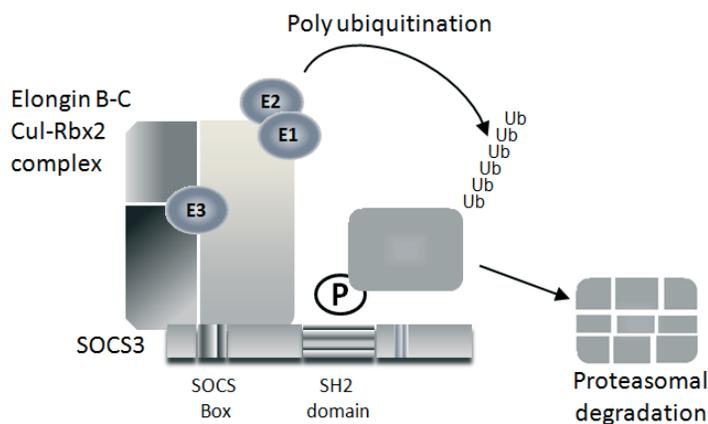
SOCS protein expression is rapidly induced by many cytokines, including those that activate the JAK/STAT pathway. SOCS expression is tightly regulated at the transcriptional level. SOCS mRNAs are induced by cytokines, and the corresponding SOCS proteins can extinguish the signaling pathways that stimulate their production. SOCS proteins therefore act in part of a classical negative feedback loop (Alexander & Hilton, 2004; Yoshimura *et al.*, 2007). Individual SOCS proteins are capable of inhibiting multiple cytokines, but it is not clear how the specificity of inhibition by SOCS is regulated. SOCS are also induced by various other stimuli, such as angiotensin II (AngII), lipopolysaccharide (LPS), growth factors (epidermal growth factor (EGF), platelet-derived growth factor (PDGF)), isoproterenol, statins and cyclic AMP (cAMP), and SOCS1 expression in immature thymocytes is developmentally regulated in the absence

of cytokine signaling. Therefore, SOCS proteins are involved in a wide range of biological processes.

The family of SOCS proteins comprises 8 members: CIS, which was the first identified member (Yoshimura *et al.*, 1995), and SOCS1–SOCS7 (Fig. 3). All members exhibit a similar structure and contain a central Src homology 2 (SH2) domain and a conserved C-terminal 40-residue region termed the SOCS box (Krebs & Hilton, 2001; Yoshimura *et al.*, 2007). SOCS1 and SOCS3 also contain a conserved 12-residue sequence – the kinase inhibitory region (KIR) (Yasukawa *et al.*, 1999). The N-terminal regions have no recognizable motifs (except SOCS7 which contains a possible nuclear localization signal and multiple proline-rich regions).

SOCS proteins control the magnitude and duration of JAK/STAT signaling through at least three possible mechanisms, including receptor interaction, direct JAK inhibition, and targeting receptor complex, and other signaling proteins for proteasomal degradation (Fig. 2 and Fig. 4). The SH2 domain determines in this aspect the target of each SOCS and CIS protein. For example, CIS, SOCS2 and SOCS3 bind to phosphorylated tyrosine residues on cytokine receptors to compete for binding sites that are used to recruit and activate STATs. The KIR domain of SOCS1 and SOCS3 allows them to inhibit JAK tyrosine kinase activity. As such, the KIR domain is proposed to function as a pseudosubstrate and prevent STATs from gaining access to the kinase (Figs. 2 and 3). SOCS1 binds directly to the activation loop of JAKs through its SH2 domain, the SH2 domain of SOCS3 first binds the cytokine receptor before inhibiting JAK through its KIR (Figs. 2 and 3).

The SOCS box serves recruitment of the ubiquitin-transferase system. As such, the SOCS box consists of three  $\alpha$ -helices bound to an E3 ubiquitin ligase complex that together with an E1 ubiquitin-activating enzyme, and an E2 ubiquitin-conjugating enzyme results in the poly-

**Figure 4. Proteasomal degradation of SOCS-targeted proteins**

An E3 ubiquitin-ligase complex bound to the SOCS box motif, ubiquitinates the associated proteins targeting them for proteasomal degradation. Detailed description is given in the text. SOCS, suppressor of cytokine signaling; SH2, Src-homology 2 domain; Ub, ubiquitin; Cul, Cullin; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase complex.

ubiquitination and proteasomal degradation of SOCS binding partners. The active ligase is formed by SOCS interacting with Elongins B and C, Cullin-5 or Cullin-2, and Rbx-1 (Kamura *et al.*, 1998). Thus, SOCS proteins function as potential E3 ubiquitin ligases and mediate the degradation of proteins associated with their SH2-domains (Fig. 4).

## BIOLOGICAL FUNCTIONS OF SOCS PROTEINS

From *in vitro* studies it could be concluded that various SOCS members are able to inhibit a variety of different as well as overlapping cytokines. *In vivo* data, however, argue for their more restricted and specific roles (Table 1). Of interest in this respect is that homology in protein identities is present in pair-wise clusters between SOCS1/SOCS3, CIS1/SOCS2, SOCS4/SOCS5, and SOCS6/SOCS7 (Dalpke *et al.*, 2008).

CIS serves as a negative regulator of EPO, IL-2, IL-3, prolactin and GH action (Yoshimura *et al.*, 1995) by inhibiting STAT5. Transgenic mice overexpressing CIS1 are strikingly similar to STAT5 knockout mice, displaying growth retardation, defects in mammary gland development, and severe defects in natural killer cell, natural killer T cell, and T-cell development; in addition, their helper T cells are biased toward Th2 differentiation (Matsumoto *et al.*, 1999). On the other hand, CIS1<sup>-/-</sup> mice have no phenotype (Marine *et al.*, 1999), although T-cells and hematopoietic cells of these mice are hyper-responsive to EPO (Sasaki *et al.*, 2000).

SOCS1 emerged as a potential negative regulator of many cytokines, including IL-2, IL-4, IL-6, IL-12. In addition, SOCS1 interacts directly with the type I IFN receptor and the IFN $\gamma$  receptor, implicating a very efficient suppressive effect of SOCS1 on IFN signaling. Moreover, SOCS1 is highly induced by LPS, implying that SOCS1 not only inhibits the JAK/STAT pathway, but also the toll-like receptor (TLR)-NF- $\kappa$ B pathway (Mansell *et al.*, 2006). SOCS1<sup>-/-</sup> mice are normal at birth, but die within 3 weeks of rapidly developed pathological manifestations such as severe lymphopenia, activation of peripheral T cells, fatty degeneration and necrosis of the liver, and macrophage infiltration of major organs (Naka *et al.*, 1998; Starr *et al.*, 1998). Defects in SOCS1<sup>-/-</sup> mice appear as a result of uncontrolled IFN $\gamma$  signaling (Starr *et al.*, 1998). In fact the specific role of SOCS-1 in inhibition of IFN $\gamma$ -STAT1-signaling has been confirmed in other studies as well (Yasukawa *et al.*, 1999). Moreover, data from SOCS1<sup>-/-</sup> mice suggest that SOCS1 could potentially modulate cell responses to other cytokines as well. For instance, SOCS1<sup>-/-</sup> mice demonstrate enhanced proliferation of thymocytes and prolonged duration of STAT6 activation upon IL-4 treatment (Naka *et al.*, 1998), indicating a state of hyper-responsiveness in hematopoietic cells to IL-4 (Alexander *et al.*, 1999b; Losman *et al.*, 1999). Furthermore, SOCS1 inhibits IL-6-induced macrophage differentiation (Novak *et al.*, 1999). Even more interesting data regarding the anti-inflammatory effects of SOCS1 were obtained from the SOCS1 transgenic mice that specifically over-express SOCS1 in T-cells. In this mouse model the activation of STATs in response to stimulation with IFN $\gamma$ , IL-4, IL-6 and IL-7 is significantly reduced. This suggests that SOCS1 can attenuate signaling from a wide range of cytokines.

SOCS2 has been shown to be a negative regulator of GH-STAT5 and IGF-1 (Greenhalgh *et al.*, 2002; 2005).

SOCS2-deficient mice develop gigantism, supporting the importance of SOCS2 in the regulation of growth. Interestingly, transgenic SOCS2 mice also have gigantism (Greenhalgh *et al.*, 2002), illustrating the complexity of the role of SOCS2 action *in vivo*.

SOCS3 mainly binds to gp130-related cytokine receptors (Nicholson *et al.*, 2000) as a central regulator of for example IL-6 signaling, but also has been shown to interact with receptors for leptin, EPO and granulocyte colony-stimulating factor (G-CSF) (Takahashi *et al.*, 2003; Kimura *et al.*, 2004). Moreover, SOCS3 suppresses LPS-sensitivity in mice and macrophages probably through inhibition of the JAK/STAT-independent MyD88-dependent pathway (Nakagawa *et al.*, 2002). SOCS3 knockout mice die during embryonic development either by dysregulated fetal liver erythropoiesis or defects of placenta functions (Marine *et al.*, 1999; Roberts *et al.*, 2001). Conditional KO mice studies of SOCS3 in macrophages have proven that SOCS3 is an important negative regulator of IL-6 (Croker *et al.*, 2003; Lang *et al.*, 2003; Yasukawa *et al.*, 2003). By use of other conditional KO strategies, further roles of SOCS3 in hematopoiesis and the endocrine system have been substantiated for G-CSF, leptin and EPO (Croker *et al.*, 2004; Mori *et al.*, 2004).

Within the SOCS subfamily, SOCS4, SOCS5, SOCS6 and SOCS7 remain poorly understood. Little is known about SOCS4 function in cytokine signaling, except that SOCS4 levels are upregulated on EGF stimulation (Kario *et al.*, 2005).

SOCS5 can bind to the IL-4R and suppress STAT6 phosphorylation in Th1 cells. In addition, SOCS5 protein is selectively expressed in Th1 cells, and SOCS5 transgenic mice have disrupted Th2-cell responses and attenuated IL-4 signaling (Seki *et al.*, 2002). SOCS5<sup>-/-</sup> mice, however, have normal T-cell development (Brender *et al.*, 2004).

*In vitro*, SOCS6 interacts with the insulin receptor, insulin receptor substrate (IRS)-4, and inhibits IRS-1 phosphorylation (Krebs *et al.*, 2002). Over-expressed SOCS6 can inhibit insulin signaling. Although SOCS6 transgenic mice display improved glucose and insulin tolerance, SOCS6<sup>-/-</sup> mice do not appear to be more insulin-responsive than wild-type mice. Except for reduced weight by 8% to 10%, these mice seem otherwise normal (Krebs *et al.*, 2002). Thus, *in vivo* functions of SOCS4-6 are not well defined.

SOCS7<sup>-/-</sup> mice similarly show mild growth retardation. In addition, however, about 50% of the mice develop hydrocephalus and suffer neonatal death (Krebs *et al.*, 2004). SOCS7 can interact with STAT3 and STAT5 after prolactin or leptin-induced stimulation (Martens *et al.*, 2005). SOCS7 mRNA levels are induced by insulin stimulation. *In vitro*, SOCS7 interacts with the insulin receptor and IRS-1 (Banks *et al.*, 2005). SOCS7 KO mice are hypersensitive to insulin, suggesting an essential role for SOCS7 in the regulation of insulin signaling *in vivo*.

In conclusion, cytokines can induce negative feedback in their own signaling pathways as well as inhibit action of other cytokines through cross-inhibition, *via* SOCS proteins. In this way SOCS proteins are able to dampen the response to both harmful and to beneficial signals. Furthermore, beyond this classic point of view, increased SOCS expression reflects the activation of intracellular inflammatory pathways that could indicate whether cells experience inflammation. The latter suggests that SOCS expression could be used as a new intracellular marker related to inflammation and inflammatory-related diseases.

**Table 2. SOCS1 and SOCS3 in inflammatory diseases**

For references see main text

Disease	SOCS expression	SOCS therapy in experimental model
<i>Rheumatoid Arthritis</i>	Increased SOCS3 mRNA in synovial tissue	Overexpression-Protective
<i>Asthma</i>	Increased SOCS3 mRNA and protein in Th2 lymphocytes with severity	Overexpression-Damaging
<i>Atherosclerosis</i>	Increased SOCS1 and SOCS3 mRNA in lesional SMC and macrophages	Overexpression-Protective
<i>Ulcerative Colitis</i>	Increased SOCS3 mRNA and protein in colon tissue	Overexpression-Protective Deletion-Damaging
<i>Crohn Disease</i>	Increased SOCS3 mRNA and protein in mucosal sample	Overexpression-Protective Mutation-Damaging
<i>Dermatitis</i>	Increased SOCS1, SOCS2, and SOCS3 protein in keratinocytes and infiltrating leukocytes	nd
<i>Renal Failure</i>		
CRF	Increased SOCS3 mRNA in monocytes and SOCS1 in lymphocytes	nd
ESRD	Increased SOCS1 in monocytes and SOCS1 and CIS in lymphocytes	nd

### SOCS AS A BIOMARKER OF CARDIOVASCULAR DISEASE IN CRF AND ESRD

Accumulating evidence shows that dysregulation of cytokine signaling by differential SOCS expression is involved in the pathogenesis of various inflammatory, immune and infectious diseases (see Table 2). Indeed, evidence is emerging for the involvement of especially SOCS1 and SOCS3 in inflammatory diseases, such as rheumatoid arthritis, and inflammatory bowel disease (IBD) as well as dermatitis. In general, SOCS1 and SOCS3 expression inhibits inflammatory diseases (Chen *et al.*, 2004; Fujimoto *et al.*, 2004). SOCS3 expression is increased in the colon of mice in an experimental model of colitis, and in intestinal T cells from Crohn's disease patients (Suzuki *et al.*, 2001; Niemand *et al.*, 2003). Inhibition of SOCS3 activity, using a dominant-negative transgene, induced hyperactivation of STAT3, and increased the severity of colitis in mice (Niemand *et al.*, 2003). In contrast, in asthma the degree of SOCS3 expression correlates with the severity of the disease (Tang & Raines, 2005), indicating a dual role of SOCS3 in inhibition as well as promotion of inflammatory diseases.

SOCS proteins have also been implicated as important modulators of cell activation during renal inflammation. For example, in experimental models of immune complex glomerulonephritis, the renal expression of SOCS3 significantly increased, in parallel with proteinuria and renal lesions, and the proteins were localized in glomeruli and tubulointerstitium (Gomez-Guerrero *et al.*, 2004). On the other hand, SOCS2 was found to be upregulated in skeletal muscle of ESRD patients during hemodialysis (Raj *et al.*, 2005). Others provided evidence to suggest that SOCS proteins may act as negative regulators of AngII signaling in renal cells and implicated SOCS as important modulators of renal damage (Hernandez-Vargas *et al.*, 2005).

Recently, we demonstrated for the first time increased expression of SOCS3 in monocytes and of SOCS1 in lymphocytes of CRF patients, accompanied by increased plasma levels of the inflammatory cytokines IL-6 and TNF $\alpha$  (Rastmanesh *et al.*, 2008). Interestingly, increased monocyte SOCS3 significantly correlated with progressive loss of renal function, measured by estimated GFR and urea. Moreover, lymphocyte SOCS1 correlated with other known markers and risk factors of CVD such as TNF $\alpha$ , systolic blood pressure (SBP) and pulse wave velocity (PWV), of which the latter have been related to

arterial stiffness (Briet *et al.*, 2006) and to outcome in renal disease (Blacher *et al.*, 1999).

Our additional study in ESRD patients (Rastmanesh *et al.*, 2009) revealed increased monocyte SOCS1 and lymphocyte SOCS1 and CIS1, accompanied by increased plasma levels of IL-6, TNF $\alpha$ , and CRP. Interestingly, monocyte SOCS1 correlated with plasma IL-6 levels, linking monocyte SOCS1 to enhanced activity of this known marker of inflammation and cardiovascular disease. CIS1 was significantly increased in lymphocytes of non-dialysis and peritoneal dialysis patients but not in hemodialysis, potentially pointing to different inflammatory conditions or cell responsiveness in hemodialysis patients (Dhondt *et al.*, 2000) in relation to the hemodialysis procedure (Martin-Malo *et al.*, 2000). Indeed CRP was only significantly increased in hemodialysis patients, but not in the other subgroups. We also found a significant correlation between lymphocyte CIS1 and TNF $\alpha$ , again confirming a link between systemic inflammation and SOCS expression in mononuclear cells in ESRD.

Together, these data are in agreement with other inflammatory diseases, and reveal that SOCS expression levels are profoundly altered in kidney disease, and the profile of SOCS expression is dependent on both the cell type as well as severity of the disease and dialysis modality. More important, we propose to suggest that SOCS could be a new intracellular marker of inflammation and CVD in renal patients, which has to be confirmed in larger CRF and ESRD cohorts.

### SOCS1 AND SOCS3 IN ATHEROSCLEROSIS

Increased SOCS expression has recently also been recognized in atherosclerosis. For example, SOCS1 and SOCS3 were increased in aortic lesions from ApoE $^{-/-}$  mice (Tang *et al.*, 2005) and both co-localized with Mac-2 positive lesion macrophages. Recently, Yamamoto and colleagues revealed that the absence of SOCS3 in macrophages of ApoE $^{-/-}$  mice decreases atherosclerosis (Yamamoto *et al.*, 2007), indicating a causal link between SOCS3 and atherosclerosis. In addition, in human plaques high expression of both SOCS1 and SOCS3 was revealed in VSMCs and macrophages in the inflammatory region of the shoulders, when compared to the fibrous area (Ortiz-Munoz *et al.*, 2009). *In vivo*, antisense oligodeoxynucleotides targeting SOCS3 exacerbated the atherosclerotic process in ApoE $^{-/-}$  mice by increasing the size, leuko-

cyte content, and chemokine expression in the lesions (Ortiz-Munoz *et al.*, 2009). Finally, Taleb *et al.* (2009) showed that loss of SOCS3 in T-cells increases both IL-17 and IL-10 production, induces an anti-inflammatory macrophage phenotype, and leads to unexpected IL-17-dependent reduction in lesion development and vascular inflammation.

SOCS1 and SOCS3 are important modulators of lymphocyte development, differentiation, cytokine production and activation (Alexander *et al.*, 1999a; Li *et al.*, 2000; Zhang *et al.*, 2001). SOCS1 is specifically expressed in Th1 cells and SOCS3 in Th2. Monocyte survival, activation and differentiation depend on the action of different inflammatory cytokines, including IFN $\gamma$  and IL-6, and the action of SOCS1 and SOCS3. SOCS expression can also be increased in cells from the vasculature. For example, in cultured VSMCs and ECs SOCS1 and SOCS3 were shown to be transiently induced by pro-inflammatory cytokines, proatherogenic lipoproteins, and immune molecules (Ortiz-Munoz *et al.*, 2009). Furthermore, over-expression of SOCS in these cell models suppressed STAT activation and reduced inflammatory gene expression and cell growth, whereas SOCS knock-down increased these cell responses. Our laboratory provides novel evidence to suggest that in ECs and VSMCs *in vitro* increased SOCS3 expression, induced by pro-inflammatory factors like IFN $\gamma$  and LPS, specifically inhibits the anti-inflammatory and proliferative effects of IL-6 through STAT3 and shifts IL-6 signaling in favor of a pro-inflammatory phenotype (Bluyssen *et al.*, 2010). This could represent a novel mechanism involved in endothelial dysfunction and in the initiation and progression of atherosclerosis.

Thus, SOCS1 and SOCS3 expressed in atherosclerotic lesions are key regulators of vascular and immune cell responses, strongly implying that manipulation of these endogenous inhibitors might be of interest in the treatment of atherosclerosis.

#### SOCS1 AND SOCS3 AND ATHEROGENIC PROPERTIES IN RENAL FAILURE RELATED CVD

The increased SOCS1 and SOCS3 expression in PBMCs of CRF and ESRD patients could therefore probably reflect initiation or development of cardiovascular disease.

In case of SOCS3, it has recently been demonstrated that it predominantly plays a negative regulatory role of STAT3 activation and in biologic responses to IL-6 (Crocker *et al.*, 2003; Lang *et al.*, 2003; Yasukawa *et al.*, 2003). Moreover, in SOCS3<sup>-/-</sup> cells IL-6 acts like IL-10 and has anti-inflammatory effect by inhibiting LPS-induced production of TNF $\alpha$  and IL-12. This anti-inflammatory effect of IL-6 is mediated by STAT3 activation, which is prolonged in SOCS3<sup>-/-</sup> macrophages. According to these findings, it could be suggested that SOCS3 has pro-inflammatory effects in macrophages, which is related to inhibition of STAT3-dependent anti-inflammatory effects of IL-6. It is tempting to speculate that increased expression of SOCS3 in monocytes of CRF patients causes (partial) resistance to IL-6 (induced STAT3 activation) or other cytokines. SOCS3 expression in monocytes seems to have pro-atherogenic properties (Yamamoto, 2007; Ortiz-Munoz *et al.*, 2009). Therefore, increased monocyte SOCS3 in chronic kidney disease patients could be related to higher prevalence of cardiovascular disease. In fact, the chance of cardiovascular

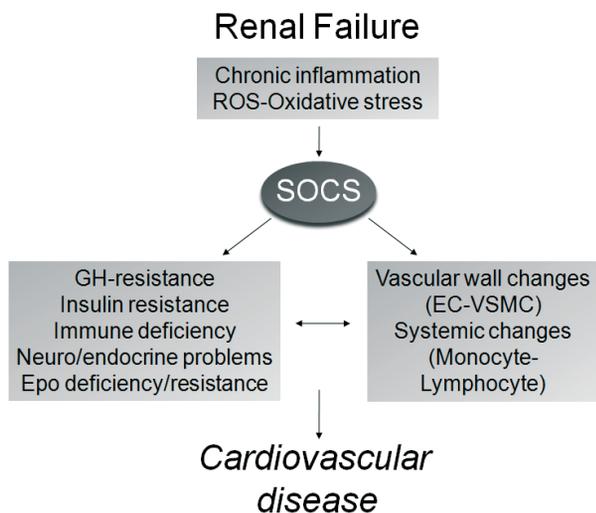
death in chronic kidney disease patients is 5–10 times more than the chance of reaching the end stage stadium (Collins *et al.*, 2003).

With respect to SOCS1, *in vivo* and *in vitro* studies indicate a specific role for this protein in inhibition of IFN $\gamma$  signaling, which has a central role in atherosclerosis (Leon & Zuckerman, 2005). Therefore, increased SOCS1 in monocytes of ESRD patients, could delay monocyte responses to pro-inflammatory cytokines and delay atherosclerosis. In fact, SOCS1 inhibits IL-6-induced macrophage differentiation (Novak *et al.*, 1999) and IFN $\gamma$ -induced CD40 production that is involved in initiation of atherosclerosis (Wesemann *et al.*, 2002). Previous studies, however, indicate an increased number of activated monocytes with a pro-inflammatory phenotype in ESRD (Brauner *et al.*, 1998; Heine *et al.*, 2008), which might suggest that increased monocyte SOCS1 is insufficient to dampen increased inflammatory pressure on monocytes. A similar phenomenon has been shown for example in synoviocytes of rheumatoid arthritis patients, which are hyper-responsive to IL-6 because of inadequate levels of SOCS3 (Shouda *et al.*, 2001). Whether increased SOCS1 in monocytes of ESRD patients is in proportion with increased pro-inflammatory cytokines and whether it could efficiently dampen pro-inflammatory signals remains unclear.

Since SOCS1 expression in Th1 cells specifically inhibits the growth inhibitory effects of IFN $\gamma$  on these cells, but not on Th2 cells (Alexander *et al.*, 1999a), it is possible that increased lymphocyte SOCS1 in CRF and ESRD skews the immune response to the Th1 type in these patients, which is in agreement with previous studies (Egwuagu *et al.*, 2002), and the promotion of atherogenesis. On the other hand, insufficient SOCS1 expression in lymphocytes causes hyper-responsiveness of lymphocytes to different inflammatory cytokines (Fujimoto *et al.*, 2004). Moreover, the absence of SOCS1 results in increased accumulation of macrophages and activated T cells and increased levels of IFN $\gamma$  and other cytokines, and is associated with greater tissue damage in arthritis (Egan *et al.*, 2003) which could also promote atherosclerosis.

#### SOCS IN LOCAL AND SYSTEMIC COMPLICATIONS OF RENAL FAILURE

As implicated above, inflammation is induced in CRF and ESRD by overproduction of pro-inflammatory cytokines, such as TNF $\alpha$  and IL-6 by chronically active mononuclear cells or decreased clearance of inflammatory cytokines. Several inflammatory cytokines, including IL-6, IFN $\gamma$  and TNF $\alpha$  can induce SOCS proteins *in vitro* and *in vivo*. Over-expression of these cytokines could induce a state of resistance to GH and EPO. Both factors have protective effects, since GH resistance causes endothelial dysfunction and EPO therapy could limit cytokine production by activated mononuclear cells. Recently it was shown that dysregulation of JAK/STAT signaling, by increased levels of the intracellular proteins SOCS2 and SOCS3, contribute to the GH resistance in rats with experimental CRF, leading to decreased IGF-1 levels (Schaefer *et al.*, 2001; Wang *et al.*, 2002). Similarly, CIS1 and SOCS3 might be involved in EPO resistance (see above). Impaired JAK/STAT signaling by increased SOCS expression has also been correlated with other systemic implications, including immune deficiencies (Losman *et al.*, 1999; Banerjee *et al.*, 2002), insulin



**Figure 5. Hypothetical convergence of SOCS in renal failure related cardiovascular disease**

Detailed description is given in the text. ROS, reactive oxygen species; SOCS, suppressor of cytokine signaling; EC, endothelial cells; VSMC, vascular smooth muscle cell; EPO, erythropoietin; GH, growth hormone.

resistance (Grimble, 2002; Rui *et al.*, 2002), and neuro-endocrine (LIF signaling) problems (Auernhammer & Melmed, 2001; Chesnokova & Melmed, 2002), which are also associated with renal disease.

On the other hand, insufficient induction of SOCS could allow harmful signals (that pass through JAK/STAT) to act without being interrupted that results in further inflammation. For instance, IFN $\gamma$ , that has obvious pro-inflammatory and pro-atherogenic effects, is specifically modulated by SOCS1. The absence or insufficient induction of SOCS1 will result in prolonged and increased activation of IFN $\gamma$ -induced STAT1 activation. That will result in increased macrophage activation, increased production of adhesion molecules, and increased production of cytokines by both mononuclear cells and endothelial cells. Therefore, insufficient SOCS expression is also involved in dysregulation of JAK/STAT pathway and consequently in its detrimental effects.

Together this could implicate that systemic inflammation in CRF and ESRD results in JAK-STAT impairment and increased action of damaging factors and inhibited transmission of beneficial factors such as EPO, insulin and GH because of increased SOCS expression (Fig. 5). As such, increased SOCS expression in mononuclear as well as vascular cells could possibly indicate the state of cell responsiveness to GH and Epo therapy. In addition, increased SOCS expression could be a reflection and/or mediator of vascular wall and circulating cellular changes, which are responsible for the increased risk of CVD and contribute to endothelial dysfunction and the progression of atherosclerosis in renal patients. Our finding that SOCS indeed are up regulated in circulating leukocytes of patients with CRF and ESRD provides the first clue that this disturbance actually exists in these patients.

## CONCLUSION

SOCS1 and SOCS3 are frequently increased in inflammatory diseases (Wong *et al.*, 2006). A recent study has demonstrated increased monocytes SOCS3 and lym-

phocyte SOCS1 expression in rheumatoid arthritis patients (Isomaki *et al.*, 2007) and patients with renal failure (Rastmanesh, 2008; 2009). SOCS1 and SOCS3 are also critical modulators of inflammatory processes within different cell types of the atherosclerotic vascular wall. The question remains how changes in SOCS1 and SOCS3 expression in renal failure affect monocyte and lymphocyte function in relation to immune responses and CVD and whether these changes also occur in ECs and VSMCs in the vascular wall. Characterizing the role of SOCS1 and SOCS3 in more detail in the development of CVD in CRF and ESRD patients may well provide new therapeutic targets for CVD. Finally, the value of monocyte and lymphocyte SOCS1 and SOCS3 as a new marker of CVD in chronic kidney disease needs to be confirmed in larger CRF and ESRD cohorts. Once confirmed, SOCS could become a marker of subclinical CVD in renal patients without clinical manifestations of CVD. Systemic or local manipulation of these endogenous anti-inflammatory proteins might be a therapeutic strategy for treating cardiovascular diseases.

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