Suppressor of Cytokine Signaling 1–Derived Peptide Inhibits Janus Kinase/Signal Transducers and Activators of Transcription Pathway and Improves Inflammation and Atherosclerosis in Diabetic Mice

Carlota Recio, Ainhoa Oguiza, Iolanda Lazaro, Beñat Mallavia, Jesus Egido, Carmen Gomez-Guerrero

Objective—Activation of Janus kinase/signal transducers and activators of transcription (STAT) pathway by hyperglycemia and dislypidemia contributes to the progression of diabetic complications, including atherosclerosis. Suppressor of cytokine signaling (SOCS) proteins negatively regulate Janus kinase/STAT and have emerged as promising target for anti-inflammatory therapies. We investigated whether a cell-permeable lipopeptide corresponding to the kinase inhibitory region of SOCS1 could reduce atherosclerosis in diabetic mice and identified the mechanisms involved.

Approach and Results—Streptozotocin-induced diabetic apolipoprotein E–deficient mice (aged 8 and 22 weeks) were given intraperitoneal injections of vehicle, SOCS1-derived peptide, or control mutant peptide for 6 to 10 weeks. SOCS1 therapy suppressed STAT1/STAT3 activation in atherosclerotic plaques of diabetic mice and significantly reduced lesion size at both early and advanced stages of lesion development compared with vehicle group. Plaque characterization demonstrated that SOCS1 peptide decreased the accumulation of lipids, macrophages, and T lymphocytes, whereas increasing collagen and smooth muscle cell content. This atheroprotective effect was accompanied by systemic (reduced proinflammatory Ly6C^{high} monocytes and splenic cytokine expression) and local (reduced aortic expression of chemokines and cytokines) mechanisms, without impact on metabolic parameters. In vitro, SOCS1 peptide dose dependently inhibited STAT1/STAT3 activation and target gene expression in vascular smooth muscle cells and macrophages and also suppressed cytokine-induced cell migration and adhesion processes.

Conclusions—SOCS1-based targeting Janus kinase /STAT restrains key mechanisms of atherogenesis in diabetic mice, thereby preventing plaque formation and increasing plaque stability. Approaches to mimic native SOCS1 functions may have a therapeutic potential to retard the progression of diabetic complications. **(*Arterioscler Thromb Vasc Biol*. 2014;34:1953-1960.)**

Key Words: atherosclerosis ■ cytokines ■ lipopeptides

Atherosclerosis is a chronic multifactorial disease of the artery wall that underlies several important adverse vascular events and is responsible for most of the cardiovascular morbidity and mortality in Western Countries.[1] Diabetes mellitus has been reported to magnify the risk of cardiovascular disease, mainly atherosclerosis and coronary artery disease.[2] These macrovascular complications cause most of deaths and much of the disability among patients with diabetes mellitus.[3]

Evidence supports the involvement of inflammation at all stages of atherosclerosis, from its initiation to its thrombotic complications.[1] Lipid accumulation in the arterial intima and the expression of cytokines (eg, interferon γ [IFNγ], interleukin-6, and tumor necrosis factor α [TNFα]), adhesion molecules (intercellular adhesion molecule-1), and chemokines (CCL2 [CC-chemokine ligand] and CCL5) all determine endothelial dysfunction, leukocyte infiltration, and vascular inflammation, thereby driving atherosclerotic plaque growth and maturation.[4–6]

Janus kinase/signal transducers and activators of transcription (JAK/STAT) is an essential intracellular pathway of cytokines and inflammatory factors[7,8] that regulates key atherosclerotic processes, including leukocyte recruitment, migration, and proliferation of vascular smooth muscle cells (VSMC), foam cell formation, and apoptosis.[9,10] JAK/STAT family comprises 4 kinases (JAK1–3, TYK2 [tyrosine kinase 2]) and 7 transcription factors (STAT1–4, 5A, 5B, 6), and specific combinations have been described in different cells.[7]

Dysregulated JAK/STAT signaling is implicated in many immune and inflammatory disorders.[8] Furthermore, JAK/

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Nonstandard Abbreviations and Acronyms	
IFNγ	interferon γ
JAK	Janus kinase
SOCS	suppressor of cytokine signaling
STAT	signal transducers and activators of transcription
TNFα	tumor necrosis factor α
VSMC	vascular smooth muscle cells

STAT isoforms have been found in the inflammatory regions of human atherosclerotic plaques and in cardiovascular animal models.[11–16] In mice, total and cell-restricted deficiency in STAT1 and STAT3 genes prevents atherosclerosis,[11–13] whereas pharmacological inhibition of JAK2, STAT1, and STAT3 reduces lesion size and neointimal hyperplasia.[8,17–19] JAK/STAT is also a critical inflammatory mechanism by which hyperglycemia contribute to the pathogenesis of diabetes mellitus and its complications.[20–22] In fact, classical STAT-responsive inflammatory genes comprise a wide array of genes including cytokines, chemokines, enzymes, vasoactive proteins, and proto-oncogenes,[7,9,10] many of them also upregulated by diabetic conditions.

Suppressor of cytokine signaling (SOCS) family of inducible proteins control the magnitude and duration of JAK/STAT signaling through several mechanisms, including kinase inhibition, STAT binding, and targeting for proteasomal degradation.[23,24] SOCS members (CIS [cytokine inducible SH2-containing protein] and SOCS1-7) contain a variable N-terminal domain, a central SH2 domain, and a conserved C-terminal SOCS box involved in proteasomal targeting. Interestingly, SOCS1 and SOCS3 contain a conserved 12-residue kinase inhibitory region upstream SH2 domain that is involved in direct suppression of JAK tyrosine kinase activity.[7,23]

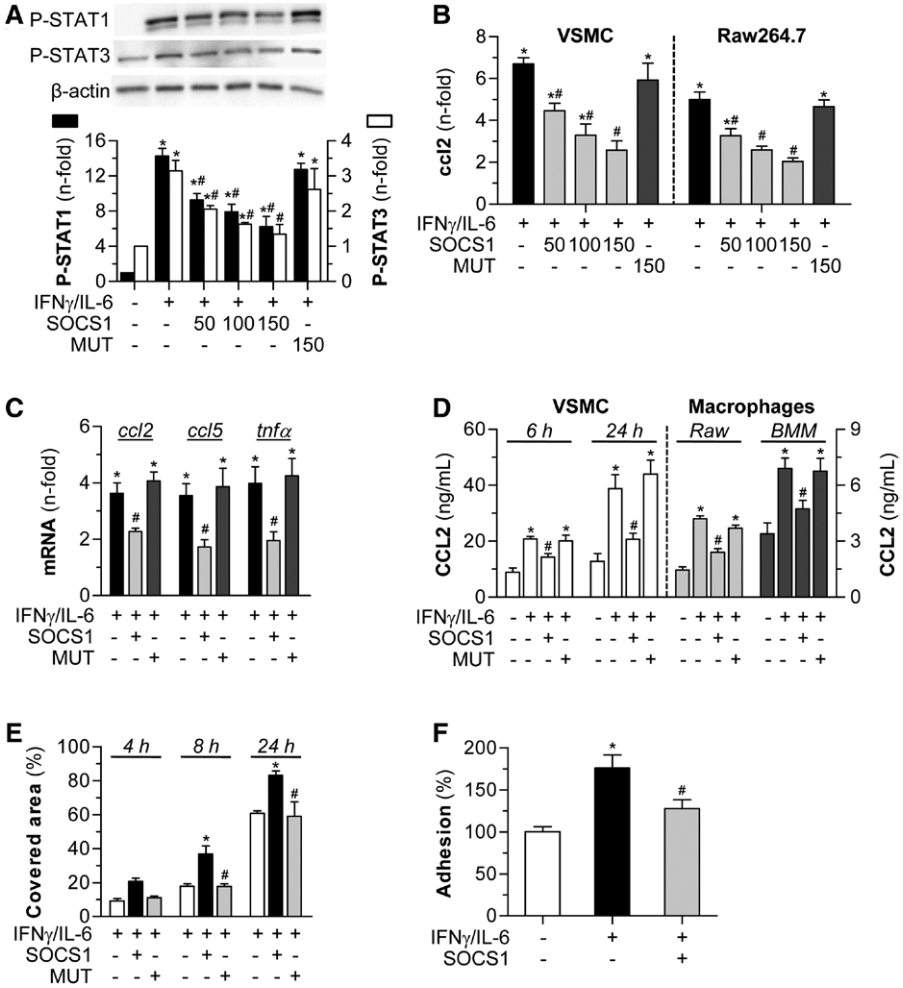

Figure 1. Inhibition of signal transducers and activators of transcription (STAT) activation, proinflammatory gene expression, cell migration, and adhesion processes by suppressor of cytokine signaling (SOCS) 1 peptide. **A**, Raw264.7 macrophages were incubated for 90 minutes with the indicated concentrations of SOCS1 peptide or its structural control (MUT) before stimulation (interferon [IFN] γ plus interleukin-6 [IL-6], 60 minutes). Representative immunoblots and densitometric analysis of P-STAT1 (filled bars) and P-STAT3 (open bars) are shown. Results expressed as n-fold increase over basal conditions. **B**, CCL2 real-time polymerase chain reaction analysis in vascular smooth muscle cells (VSMC) and Raw264.7 macrophages at 6 hours of cytokine stimulation in the presence of the indicated concentrations (μg/mL) of SOCS1 and MUT peptides. **C**, Gene expression analysis in primary macrophages (bone marrow–derived macrophages) at 6 hours of stimulation. **D**, CCL2 chemokine secretion measured by ELISA. **E**, Scratch wound healing assay in VSMC. Quantifications of covered healing areas at the indicated times are expressed as percentage of the initial wound area. **F**, Adhesion assay of calcein-labeled macrophages to cytokine-stimulated VSMC. Values represent the mean±SEM of 4 to 7 independent experiments (*P<0.05 vs basal, #P<0.05 vs IFNγ/IL-6).

Evidence is emerging for the involvement of SOCS proteins in immune and inflammatory diseases.[7,23,24] In particular, SOCS1 and SOCS3, which are recognized as negative regulators of cytokine receptors, have also been linked to a variety of proinflammatory and proatherogenic factors including lipoproteins, lipids, angiotensin II, immune complexes, high glucose, and insulin in cardiovascular and renal cells.[11,14–16,25–27] Therefore, strategies based on the regulatory role of SOCS proteins to impair pathological JAK/STAT activity might be of interest for the treatment of cardiovascular and metabolic diseases. The present work investigates the anti-inflammatory and atheroprotective properties of SOCS1-based JAK/STAT inhibition. To that end, the therapeutic potential of a cell-permeable peptide containing the kinase inhibitory region of SOCS1 was analyzed in a mouse model of diabetes mellitus–accelerated atherosclerosis and in cultured vascular cells.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

SOCS1-Derived Peptide Prevents Cytokine-Induced STAT Activation in Vascular Cells

The in vitro effects of a lipopeptide corresponding to the SOCS1 kinase inhibitory region were investigated in mouse VSMC, bone marrow–derived macrophages, and Raw264.7 macrophage cell line stimulated with cytokines (IFNγ plus interleukin-6). First, structural analysis of SOCS1-derived peptide by circular dichroism spectroscopy revealed a conformational change from random to α-helical conformation on the addition of trifluorethanol (Figure I in the online-only Data Supplement), thus indicating a tendency to acquire an ordered secondary structure in the vicinity of a membrane bilayer. Furthermore, Western blot analysis demonstrated that N-palmitoylated SOCS1 peptide dose dependently inhibited cytokine-induced STAT1/STAT3 phosphorylation in VSMC and macrophages (Figure 1A; Figure IIA in the online-only Data Supplement). The mutated (MUT) peptide sequence showed significant lower inhibitory activity (Figure 1A), whereas the nonpalmitoylated SOCS1 analog was inactive (not shown). Immunofluorescence experiments demonstrated that rhodamine-labeled SOCS1 lipopeptide is homogeneously distributed throughout the cytoplasm of VSMC and inhibited cytokine-induced STAT1/STAT3 activation (Figure IIB and IIC in the online-only Data Supplement).

Anti-Inflammatory Effects of SOCS1 Peptide In Vitro

Pretreatment of VSMC with SOCS1 peptide significantly reduced the mRNA expression of STAT-regulated genes, including monocyte and T-cell chemokines (CCL2 and CCL5), adhesion molecule-1 (intercellular adhesion molecule-1), and proinflammatory cytokine TNFα (Figure 1B; Figure IIIA and IIIB in the online-only Data Supplement). A similar inhibitory effect of SOCS1 peptide was observed on Raw264.7 cell line and primary bone marrow–derived macrophages (Figure 1B and 1C; Figures IIIA and IIIB in the online-only Data Supplement). SOCS1 peptide also prevented CCL2/CCL5 chemokine secretion by cultured cells (Figure 1D; Figure IIIC in the online-only Data Supplement). By contrast, control MUT peptide was ineffective in all the experiments (Figure 1A–1D; Figure IIIA–IIIC in the online-only Data Supplement).

To study the functional consequences of the decreased inflammatory gene expression, we further analyzed the effects of SOCS1 peptide on cell migration and adhesion, 2 important processes involved in plaque formation. In vitro wound-healing assay with VSMC revealed that IFNγ/interleukin-6 promoted a time-dependent increase in directed cell migration and also demonstrated the antimigratory effect of SOCS1 peptide (Figure 1E). Peptide also inhibited the transwell migration of VSMC (Figure IIID in the online-only Data Supplement) and the adhesion of macrophages to cytokine-stimulated VSMC (Figure 1F).

SOCS1 Peptide Reduces Development and Progression of Atherosclerosis in Diabetic Mice

We further studied the efficacy of SOCS1-derived peptide to reduce diabetes mellitus—driven atherosclerosis at both early and advanced stages of lesion development. To this end, apolipoprotein E knockout mice (aged 8 and 22 weeks) were made diabetic by streptozotocin injection[28] and 2 weeks later treated with vehicle, SOCS1-derived peptide, or MUT peptide for a period of 6 to 10 weeks (Figure IV in the online-only Data Supplement). There were no statistically significant differences between vehicle and peptide-treated mice in terms of body weights and hepatic transaminase activities at the end of the study, thus suggesting the safety at

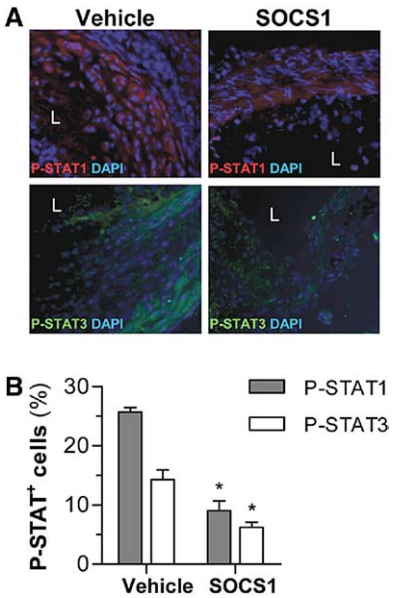

Figure 2. Suppressor of cytokine signaling (SOCS) 1 peptide inhibits signal transducers and activators of transcription (STAT) activation in diabetic mice. **A**, STAT immunodetection in atherosclerotic plaques from diabetic mice at 10 weeks of treatment. Representative micrographs (magnification ×200) showing P-STAT1 (red), P-STAT3 (green), and nuclear staining (diamidino-2-phenylindole [DAPI], blue). **B**, Quantification of positive cells per lesion area. Measurements are expressed as mean±SEM (*P<0.05 vs control).

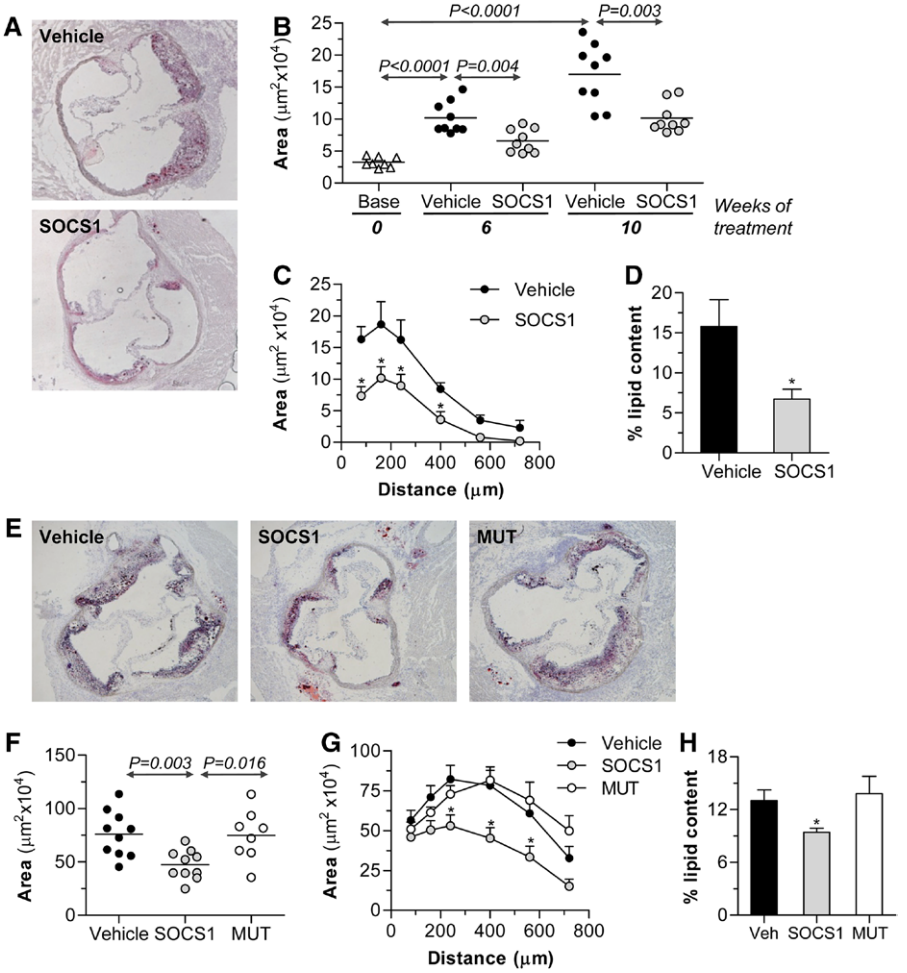

Figure 3. Suppressor of cytokine signaling (SOCS) 1 peptide reduces development and progression of atherosclerosis in diabetic mice. Assessment of atheroma plaques of diabetic mice at early (**A**–**D**) and advanced (**E**–**H**) stages of atherosclerosis. **A** and **E**, Representative photomicrographs (magnification ×40) showing oil-red-O/hematoxylin staining of aortic root sections at 10 weeks of treatment. **B** and **F**, Quantification of individual lesion area in vehicle, SOCS1, and MUT groups. *P* values as indicated. **C** and **G**, Extents of atherosclerosis lesions throughout the aorta in mice at 10 weeks of treatment. **D** and **H**, Quantification of lipid content (% oil-red-O per lesion area). Values are expressed as mean±SEM (*P<0.05 vs vehicle).

the given dose (Table I in the online-only Data Supplement). Fluorescent peptide tracking revealed accumulation of rhodamine-labeled SOCS1 inside the mouse aortic plaques, predominantly in macrophage-rich areas (Figure V in the online-only Data Supplement), thus indicating that vessel cells are indeed targeted by the cell-permeable peptide. Furthermore, SOCS1 peptide effectively inhibited STAT1/STAT3 activation within the atherosclerotic plaques of diabetic mice (Figure 2).

Quantification of early atherosclerotic lesions after oil-red-O/hematoxylin staining (Figure 3A) demonstrated a progressive increase of lesion size in diabetic mice compared with baseline values (2 weeks of diabetes mellitus; Figure 3B). In contrast, SOCS1-treated mice exhibited a 35% to 40% (P<0.03) decrease of lesion area over time compared with age-matched vehicle controls (Figure 3B). SOCS1 peptide diminished not only atherosclerotic lesion size but its extension along the aorta (Figure 3C). Linear regression analysis revealed positive correlation of lesion area with P-STAT1 and P-STAT3 immunostaining (Pearson r values: 0.72 and 0.63, respectively; P<0.02; not shown).

The potential of SOCS1 peptide to retard the progression of already developed atherosclerosis was examined in older diabetic mice. As shown in Figure 3E–3G, aortic lesions of SOCS1-treated mice were 1.5-fold smaller by area and extension than vehicle control mice. Remarkably, no significant differences in mouse atherosclerotic lesions were observed between vehicle and MUT peptide groups, thus excluding any off-target effects.

SOCS1 peptide treatment significantly reduced the neutral lipid area in both early and advanced atherosclerotic plaques of diabetic mice (Figures 3D and 3H) but had no impact on serum lipid levels (Table I in the online-only Data Supplement). Moreover, all groups (SOCS1, MUT, and vehicle) had equivalent hyperglycemia (Table I in the online-only Data Supplement), thus confirming that the treatment did not affect the mouse diabetic metabolism.

SOCS1 Affects Atherosclerotic Plaque Phenotype and Systemic Inflammation in Diabetic Mice

The reduced lesion size by SOCS1 peptide was associated with a decreased accumulation of monocytes/macrophages

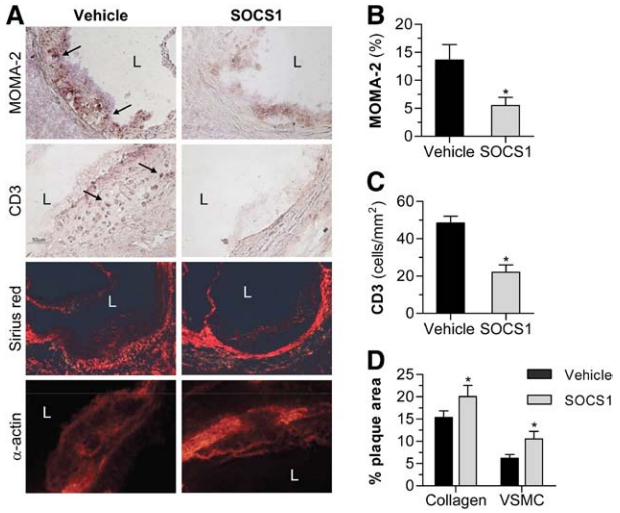

Figure 4. Suppressor of cytokine signaling (SOCS) 1 peptide alters plaque composition and stability. **A**, Histological analysis of macrophages, T cells, collagen, and vascular smooth muscle cells (VSMC; magnification ×200; arrows indicate immunopositive cells; L, lumen) in early atherosclerotic lesions of diabetic mice after 10 weeks of treatment. Quantification of MOMA-2 (**B**), CD3 (**C**), picrosirius red and α-actin (**D**) positive staining per lesion area. Mean±SEM of n=8 to 9 animals per group (*P<0.02 vs vehicle).

(monocyte macrophage-2 [MOMA-2] staining) and T lymphocytes (CD3 staining) within the early atherosclerotic plaques of diabetic mice (Figure 4A–4C), and regression analysis showed correlation with lesion area (Pearson r values: 0.67 and 0.57, respectively; P<0.03; not shown). Furthermore, atherosclerotic plaques from SOCS1-treated mice had a more stable phenotype, with significant increases in collagen (picrosirius red) and VSMC (α-actin) content compared with vehicle control group (Figure 4A and 4D).

Immunohistochemistry (Figure 5A) and quantitative real-time polymerase chain reaction analysis (Figure 5B) in aortic samples from SOCS1-treated mice demonstrated a reduced local expression of CCL2/CCL5 chemokines and TNFα cytokine compared with controls. SOCS1 therapy also significantly decreased the splenic expression of T helper 1 (Th1) cytokines (IFNγ, interleukin-12, and TNFα) but not Th2 cytokines (interleukin-4 and interleukin-10) in diabetic mice (Figure 5C). Flow cytometry analysis of peripheral blood revealed no differences in number of B cells (CD19), T cells (CD3 and CD4/CD8 subgroups), and monocytes (CD115) between vehicle and SOCS1 groups (Figure 5D). Interestingly, SOCS1-treated mice exhibited lower levels of $CD115^{+}Ly6C^{high}$ monocytes in comparison with vehicle control mice, whereas the frequency of $CD115^{+}Ly6C^{low}$ monocytes was increased (Figure 5E). These results indicate a local and systemic anti-inflammatory effect of SOCS1 peptide in diabetic mice.

Discussion

This study reveals that JAK/STAT/SOCS pathway is a key molecular mechanism by which diabetic conditions affect vascular cell biology to promote atherosclerotic plaque formation and suggests SOCS1 endogenous protein as a feasible approach against diabetes mellitus inflammatory complications.

SOCS family, which is at the crossroad of multiple immunologic and inflammatory pathways, has recently emerged as a promising therapeutic target with tumor suppressor and anti-inflammatory functions. Dysregulated expression of SOCS family members contributes to the pathogenic mechanisms of several inflammatory diseases.[7,23,24,29,30] In patients with chronic kidney disease, SOCS1 and SOCS3 expression levels

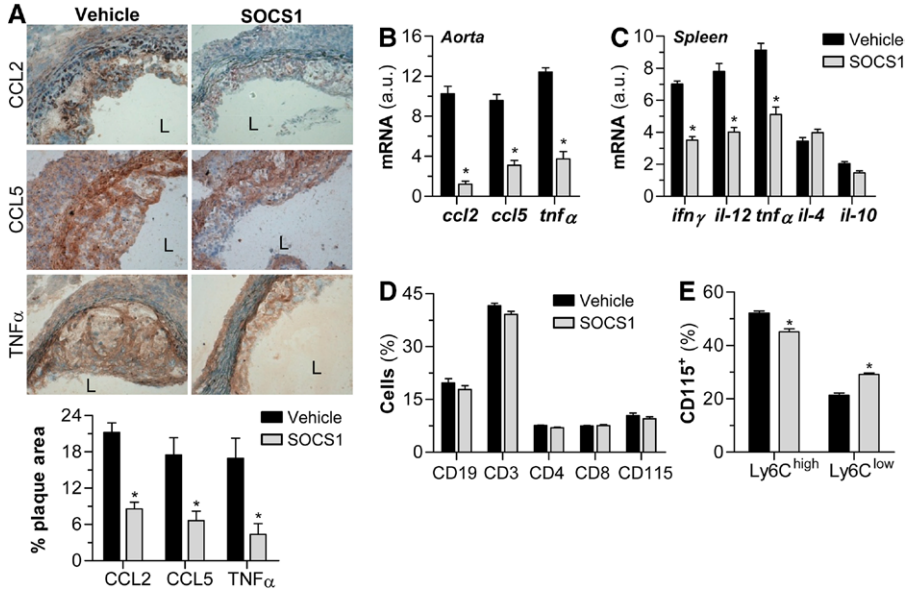

Figure 5. Suppressor of cytokine signaling (SOCS) 1 peptide attenuates plaque and systemic inflammation in diabetic mice. **A**, CCL2, CCL5, and tumor necrosis factor α (TNFα) immunodetection in aortic sections from diabetic mice at 10 weeks of treatment. Representative micrographs (magnification ×200; L, indicates lumen) and summary of morphometric analysis are shown. Real-time polymerase chain reaction analysis in aortas (**B**) and spleens (**C**) of diabetic mice. Values are normalized to 18S and expressed in arbitrary units (a.u.). Flow cytometry analysis of circulating leukocytes (**D**) and relative population of $CD115^{+}$ monocytes (**E**) in total blood from diabetic mice at 10 weeks of treatment. Mean±SEM of 8 to 9 animals per group (*P<0.05 vs vehicle).

correlate with progressive loss of renal function and cardiovascular risk factors.[31] Recently, we demonstrated high levels of SOCS1 and SOCS3 in human atheroma plaques[14] and also in renal biopsies from patients with diabetes mellitus[26] and proposed SOCS expression as a compensatory, but not sufficient mechanism, to suppress tissue damage. Animal studies demonstrate that SOCS gene deficiency, leading to sustained STAT activation, aggravates immune and inflammatory respo nses,[14–16,24,27,32,33] whereas enforced SOCS expression reduces inflammation and cardiovascular disease.[15,16,34] Likewise, our previous study revealed attenuated inflammation and renal function improvement in diabetic rats by adenovirus-mediated SOCS gene delivery.[26] In line with this, the present work demonstrates that a cell-permeable lipopeptide mimicking SOCS1 regulatory protein attenuates pathological JAK/STAT activation, suppresses inflammation, and retards development and progression of atherosclerosis in diabetic mice.

The beneficial effect of SOCS1-derived peptide was independent of any appreciable influence on the metabolic severity of diabetes mellitus, as evidenced by no changes in hyperglycemia, lipid profile, or body weight. Several studies have reported intriguing properties of SOCS proteins in relation to the pathogenesis of diabetes mellitus.[21,22,24] In fact, SOCS1 knockout mice exhibit a low blood glucose level and increased insulin signaling,[21,35] thus suggesting SOCS as a link between elevated levels of cytokines and insulin resistance. However, SOCS1 induction protects β-cells in vitro,[36] with target expression of SOCS1 preventing diabetes mellitus in the nonobese diabetic mouse.[37] Based on these findings and on the fact that both treated and untreated groups have equivalent hyperglycemia, our study proposes SOCS1-derived peptide as a potential therapy to halt the progression of diabetic complications. Future studies on the involvement of JAK/STAT/SOCS axis in the pathological processes leading to both type 1 and type 2 diabetes mellitus would help to elucidate whether targeting SOCS expression or function may improve glucose metabolism in diabetic conditions and confer protection against metabolic stress.

Studies based on mimicking of SOCS proteins have been reported in different experimental conditions. The 12-mer tyrosine kinase inhibitor peptide suppressed cytokine receptor activities by binding to the autophosphorylation site of JAK2, thus preventing STAT1/STAT3 phosphorylation and further downstream signal transduction events in vitro.[38,39] Similarly, peptides containing SOCS1 inhibitory sequence suppress STAT activation by Th1 and Th17 cytokines in leukocytes, splenocytes, and keratinocytes.[40–42] To date, the in vivo effects of SOCS1 mimetic sequences have investigated in mouse models of multiple sclerosis,[38,41] peripheral nerve injury,[43] and viral infection[39] but not in cardiovascular and metabolic diseases. Our study provides first evidence that a small peptide corresponding to the SOCS1 kinase inhibitory region has atheroprotective effects.

Consistent with the predicted model for SOCS1 kinase inhibitory region (2 α-helices at R57–R69 and L74–A77 linked by a small coil segment),[44] circular dichroism spectroscopy studies demonstrated a clear tendency of SOCS1 peptide to adopt α-helical conformation, thus confirming a role for the secondary structure of inhibitory SOCS1-derived peptide. We also observed that a small structural change (lipid modification) conferred cell-permeable properties to SOCS1-derived sequence, assessed by rapid endocytosis and cytoplasmic localization of fluorescence-labeled peptide. This is consistent with previous studies describing that N-palmitoylation is sufficient to allow a lipopeptide to cross cellular (but not nuclear) membranes and protect against endosomal enzyme degradation.[45] In fact, SOCS1 lipopeptide was efficiently taken up in vitro and in vivo by vascular cells in a time- and dose-dependent manner and inhibited STAT1/STAT3 activation, whereas mutated (substitution of Phe for Ala) and nonlipidated analogs were inactive. Importantly, the effect of SOCS1 peptide was accompanied in vivo by a decrease in atheroma size at different stages of atherosclerosis, thus suggesting the potential of SOCS1 peptide to impair early-stage atherosclerosis development and also to retard the progression of already developed plaques. Peptide treatment resulted in the development of a lipid-poor collagen-rich atherosclerotic lesion characterized by higher collagen and VSMC content and lower lipids and macrophages, thereby indicating a less inflamed, more stable plaque phenotype. Because most acute complications of atherosclerosis are caused by the rupture of an unstable plaque,[46] we propose that targeting JAK/STAT/SOCS1-dependent axis to impair proatherogenic activation may have a beneficial role in slowing lesion progression.

The present study characterized in vitro the atheroprotective effects of SOCS1-derived peptide on VSMC and macrophages, key cellular constituents of the atherosclerotic lesion that participate actively in plaque development. Besides inhibiting STAT1/STAT3 phosphorylation and nuclear translocation, SOCS1 peptide prevented STAT-mediated responses induced by IFNγ and interleukin-6, 2 proatherogenic cytokines involved in plaque development, progression, and destabilization.[5,47] It has been proposed that proinflammatory cytokines interplay with hyperglycemia to promote atherogenesis and plaque destabilization in patients with diabetes mellitus via a variety of mechanisms including leukocyte recruitment, monocyte–VSMC adhesive interactions, extracellular matrix remodeling, and proliferation and migration of VSMC.[48,49] In line with this, our results in VSMC and macrophages show that SOCS1-derived peptide suppresses the expression of STAT-regulated inflammatory genes, in particular monocyte and T-cell chemokines (CCL2 and CCL5), adhesion molecule intercellular adhesion molecule-1, and the proinflammatory cytokine TNFα, all of which cooperating in recruitment, migration, and paracrine activation of vessel cells in the injured artery during vascular remodeling.[4–6] Consistently, cell migration processes and monocyte–VSMC interactions were also prevented by SOCS1 peptide without affecting cell viability. These SOCS1 anti-inflammatory actions were also observed in atherosclerotic plaques of diabetic mice in close association with the reduced accumulation of macrophages and T lymphocytes, thus supporting the essential role of JAK/STAT/SOCS pathway in regulating leukocyte infiltration and VSMC migration during atherosclerosis.

Besides this local anti-inflammatory effect, we observed an indirect effect of SOCS1 peptide on systemic inflammation. Previous studies have established that proinflammatory Th1 cytokines promote atherosclerosis, whereas anti-inflammatory Th2 cytokines exert atheroprotective activities.[6] Furthermore, SOCS1 controls the polarization of CD4+ T cells into Th1 and Th2 lineages and also affects CD8+ T-cell maturation and function.[50] Our data of reduced Th1 cytokine expression in diabetic mice suggest that SOCS1 peptide affects the outcome of the adaptive immune response at this stage of the disease. Furthermore, SOCS1 therapy reduced the relative number of circulating $CD115^{+}Ly6C^{high}$ monocytes, the classical inflammatory subset predominant in hypercholesterolemic mice that is preferentially adhered to activated endothelium, accumulated in lesions, and locally differentiated into macrophages.[51] Therefore, our observations suggest that attenuated Th1 response and reduced monocyte activation state are both involved in the atheroprotective effect of SOCS peptide in diabetic mice.

In summary, this is the first description that a SOCS1-derived peptide targeting JAK/STAT activation restrains key mechanisms of atherogenesis, such as proinflammatory gene expression, leukocyte infiltration, and vascular cell activation and migration, thereby preventing development and progression of atherosclerosis and increasing plaque stability in diabetic mice. Thus, peptide-based approaches to mimic native SOCS1 functions may provide insights into developing novel therapies to retard the progression of diabetic complications.

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Disclosures

None.

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Significance

Diabetes mellitus is associated with significantly increased rates of atherosclerosis. Besides hyperglycemia and hyperlipidemia, an excessive production of cytokines participates in the pathogenesis of atherosclerosis in patients with diabetes mellitus. Numerous cellular and molecular inflammatory components are involved in atherogenesis, and uncontrolled activation of proinflammatory transcription factors, such as Janus kinase/signal transducers and activators of transcription pathway, plays a significant role. Suppressor of cytokine signaling (SOCS) family has recently emerged as a Janus kinase/signal transducers and activators of transcription regulatory mechanism with promising therapeutic implications. In this work, we investigated the anti-inflammatory and atheroprotective effects of a SOCS1-derived cell-permeable peptide containing the kinase inhibitory region. In vitro, SOCS1 peptide effectively reduced signal transducers and activators of transcription activation and target gene expression and suppressed cell migration and adhesion processes in vascular cells. In vivo, treatment with SOCS1-derived peptide limited development and progression of atherosclerosis in diabetic mice and also altered plaque composition and inflammation without affecting metabolic parameters. We propose SOCS1 peptide as a therapeutic compound to modulate the progression of diabetes mellitus complications.