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EDITORIAL COMMENT

Deletion of *Socs3* Expression in Aortic Smooth Muscle Cells Ameliorates Aortic Dissection*

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Aortic dissection (AD) is the most serious of the aortic syndromes, which include aneurysmal degeneration and mycotic infection. AD is a result of a disruption in the intimal-medial complex of the aortic wall that permits blood flow through the tunica media. The consequence is that perfusion of tissue beds, such as the brain, intestine, kidneys, and extremities, is diverted and/or obstructed by the propagation of the intimal flap created by the dissection, which results in life-threatening ischemic syndromes (1). The clinical presentation of AD is abrupt onset of chest or back pain and is most closely associated with poorly controlled hypertension. AD is categorized by anatomic location: Stanford type A involves the ascending aortic arch and Stanford type B involves the descending aorta. Stanford type A is managed surgically, whereas Stanford type B dissections are managed medically. Despite advances in surgical techniques and medical management, long-term complications are frequent with aneurysmal degeneration being the most common (2).

Recent studies in animal models highlighted the role of a proinflammatory response that promoted destruction of the extra-cellular matrix (ECM), notably collagen and elastin, in the pathogenesis of AD. This was consistent with findings in aortic samples from patients with AD that demonstrated decreased amounts of collagen and elastin compared with healthy human aortic samples. The ECM in the aortic wall is maintained by smooth muscle cells (SMCs) and fibroblasts, but the roles of these cells and their cell–cell interactions in the context of AD and inflammation remain unclear (3). In this issue of *JACC: Basic to Translational Science*, Hirakata et al. (4) aimed to investigate the genetic, molecular, and cellular mechanisms that lead to an imbalance of the ECM and the loss of aortic tissue integrity that leads to AD. Based on the role of SMCs in maintenance of the ECM, they focused on the role of the suppressor of cytokine signaling 3 (*Socs3*) signaling in SMCs. This focus was based on this established group's previous work, which demonstrated that mice carrying a macrophage-specific deletion of *Socs3*, a negative regulator of the Jak/Stat pathway, were more susceptible to AD development (3). Furthermore, this AD-susceptible phenotype suggested that SMCs had a pivotal role in aortic syndromes that demanded further investigation.

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For this study, the investigators created a mouse model with smooth muscle–specific deletion of *Socs3* (smSocs3-KO) and developed a novel tunable model of AD with administration of β-aminopropionitrile (BAPN) in drinking water and angiotensin II (AngII) infusion subcutaneously. The mice developed AD usually by day 7 of treatment, which enabled the evaluation of the molecular events both before and

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after AD onset. These critical analyses allowed determination of the causative factors in a poorly defined disease. To provide clinical relevance to this investigation, human aortic tissue samples from patients with AD and healthy subjects were included in the analyses.

Using immunohistochemical staining of human AD tissue with antibodies for signal transducer and activator of transcription 3 (STAT3), the investigators detected activated phosphorylated STAT3 (pSTAT3) in the adventitial and medial layers of the damaged aortic wall that were localized to SMCs. Correspondingly, with regard to translational significance of this report, immunofluorescence staining revealed Stat3 activation in both SMCs of the media and non-SMCs in the adventitia in the mouse AD model. Aortic sections from mice labeled with antibodies against pStat3 and α-smooth muscle actin (SMA) at baseline (pre-infusion) and after 3 days of BAPN+AngII infusion showed that the baseline proportion of pStat3-positive cells was higher in smSocs3-KO samples compared with wild type (WT) samples. In the smSocs3-KO aortic samples, the pStat3-positive population increased among both SMA-positive SMCs and SMA-negative non-SMCs, which indicated that that *Socs3* deletion in SMCs led to increased non-SMCs in the aorta and caused Stat3 activation in both SMCs and non-SMCs.

To characterize changes in inflammatory cell phenotypes, the investigators used focused quantitative reverse transcription polymerase chain reaction analyses of selective genes, based on wider transcriptomic studies, which demonstrated greater expression of *cd80*, a marker for the proinflammatory and tissue destructive M1 macrophage, in the WT samples only. The M2 macrophage markers *Cd206* and *Cd163* were higher at baseline, and in the presence of BAPN+AngII in the smSocs3-KO strain, they revealed a predominance of the tissue reparative M2 macrophage phenotype with *Socs3* deletion. BAPN+AngII infusion induced more severe tissue destruction in the aortic arch in WT mice than in smSocs3-KO mice. This indicated that *Socs3* deletion in SMCs prevented AD aggravation in the aortic arch, the most vulnerable portion of the aorta. This was associated with an increase in the M2 macrophage phenotype.

The chronological importance of Stat3 expression was highlighted in proteomic analyses of samples from WT and smSocs3-KO aortas isolated before and 3 days after BAPN+AngII infusion (and before AD development). Stat3 activity was significantly higher and was accompanied by Jnk activation in smSocs3-KO aortas than in WT aortas in vivo. Key to the central hypothesis of this investigation, *Socs3* deletion also altered SMC phenotypes. In WT aortas, BAPN+AngII infusion led to increased expression of SMemb, a marker for the synthetic embryonic phenotype of SMCs. In contrast, smSocs3-KO aortas showed higher expression levels of both SM2 (a marker for the contractile phenotype of SMCs) and SMemb at baseline; however, these levels were not changed by BAPN+AngII infusion. Immunostaining showed that approximately 80% of WT aortic wall cells were SMA-positive SMCs, and BAPN+AngII infusion caused a slight but insignificant increase of non-SMCs. In contrast, >30% of aortic wall cells were SMA-negative non-SMCs at baseline. These findings indicated that SMC *Socs3* deletion resulted in a proportionate increase of non-SMCs in the aortic wall. Inspired by this discovery, the investigators sought to identify the cell types within this SMA-negative population by staining aortas with antibodies specific for suspect cell-type markers, notably those associated with connective tissue homeostasis. They discovered that the fibroblast marker, ER-TR7, stained more intensely in the adventitia in smSocs3-KO samples compared with those regions in WT samples. Collagen deposition, specifically in the adventitia, was also more prominent in smSocs3-KO aortas than in WT aortas, both at baseline and after BAPN+AngII. This indicated that *Socs3* depletion in SMCs resulted in increased fibroblasts that promoted adventitial fibrosis in the aortic wall. To assess the physiological significance of this discovery, the investigators measured the tensile strength of aortic rings from the distal aortic arch and found that although the medial tensile strength did not significantly differ between the Socs2 deletion and the WT mouse groups, the tensile strength of the adventitia was higher in smSocs3-KO aortas than in WT aortas. These data implied that increases of fibroblasts and collagen deposition in the adventitia, caused by *Socs3* deletion in SMCs, strengthened the adventitia of the aortic wall. Next, the investigators performed in vitro cell culture experiments using mouse aortic SMCs and fibroblasts to define the mechanism between SMC Stat3 activation and fibroblast activation. Fibroblasts were treated with conditioned media from SMCs cultured with and without the Stat3 activator interleukin-6, and then proteins for tyrosine phosphorylation were analyzed as a marker of growth response. As expected, interleukin-6–stimulated SMC-conditioned media induced increases in tyrosine phosphorylation of multiple proteins in fibroblasts. This suggested that interleukin-6, via Stat 3 activation, induced SMCs to secrete soluble factors that could induce proliferation of fibroblasts.

Collectively, this body of work demonstrated that *Socs3* deletion in SMCs resulted in Stat3 and Jnk

activation in SMCs and non-SMCs, presumably fibroblasts. In addition, *Socs3* deletion resulted in a predominance of the M2 macrophage phenotype that is associated with efferocytosis of apoptotic cells, which promotes wound healing and an increase in fibroblasts, and both of which resulted in decreased severity of AD and improved aortic adventitial strength in the sm-Socs3 mice (5). This was paradoxical to what Hirakata et al. (3) discovered when *Socs3* was specifically deleted in macrophages in a murine AD model in which there was an increase in M1 macrophages and more severe progression of AD. This emphasized that expression of genes and the induction of resultant signaling pathways might promote or mitigate disease severity, depending on the cell type, as demonstrated in the current study. These findings provided critical information for developing novel and effective gene deletion approaches to treating AD because these approaches will also have to target specific cell types.

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