Inflammatory processes are involved in many vascular diseases and have been linked to a diverse array of mediators. This includes advanced glycation end products (AGEs) that are a collection of chemical entities implicated in inflammation-associated vascular disorders, particularly atherosclerosis. A specific receptor (receptor for advanced glycation end products [RAGE]) has been implicated in mediating the effects of AGEs in promoting inflammation through activation of the nuclear factor κB system. Subsequently, it was recognized that RAGE may promote inflammation through activation by non glycated products. This includes a member of the S100/calgranulin family termed S100A12, calgranulin C, or extracellular newly identified RAGE-binding proteins (EN-RAGE). S100A12 is predominantly secreted from neutrophils that can activate RAGE on endothelial cells, macrophages, and lymphocytes.1

In this issue of Circulation Research, Hofmann Bowman et al provide evidence for a new pathological role of S100A12 in the development of thoracic aortic aneurysms (TAAs). The most common complication of TAAs is dilation and rupture of the ascending aorta. TAAs are frequently associated with a number of well-defined genetic determinants, although there remains many questions regarding the mechanistic basis for the development and propagation of aneurysms in this region.3,4

This study used a combination of mouse models and human tissue. Because S100A12 is not normally expressed in mice, transgenic mice were created to express the human protein under the control of the smooth muscle cell (SMC)-specific promoter SM22α. Convincing evidence was presented demonstrating expression of S100A12 in the aortic media of transgenic mice and in SMCs cultured from aortas of these mice. The accumulation of S100A12 protein in mouse aortas and cultured aortic SMCs was equivalent to extracts of human TAAs and SMCs cultured from human TAAs. This provides some assurance that the effects of S100A12 described in the transgenic mice were not an experimental artifact of excessive overexpression.

Examining several lines of mice expressing S100A12 in SMCs, the authors determined the temporal sequence of changes in biochemical, structural, and cellular characteristics of aortas (Figure). The earliest time point studied was 8 weeks of age. At this young age, SMC phenotype in the transgenic mice had already deviated from normal.5 Aortic extracts of transgenic mice had increased abundance of vascular cell adhesion molecule-1 protein that would facilitate accumulation of specific leukocyte classes. Increased abundance of matrix metalloproteinase-2 was also noted. However, only the latent protein was detected so it is unclear whether this increased abundance was related to vascular changes. Structural changes in aortas were not examined at this interval. At 10 weeks of age, structural changes were evident in aortas of transgenic mice. These included increased aortic thickening, elastin degradation, and fibrosis. Tissue extracts from transgenic mice had increased myeloperoxidase. The source of aortic myeloperoxidase may be either neutrophils or macrophages, although the presence of vascular cell adhesion molecule-1 favors the recruitment of macrophages.6 However, for reasons that are not apparent, no leukocyte antigens could be identified in aortas to determine the cell type delivering myeloperoxidase. The inference of a causal role of leukocyte infiltration would be enhanced by the demonstration that these cells accumulate in the diseased aortic medial layer. In addition to these data derived from ascending aorta, additional insight would have been gleaned from defining whether these changes were restricted to the ascending aorta. This would provide important insight because aortic SMCs are heterogeneous, in part because of the differential developmental origins.7 Therefore, it would be of interest in future studies to determine whether the described changes were throughout the aortic tree.

The most important finding in the SMC-specific expressing S100A12 mice was the dilation of ascending aorta at 16 weeks. At 10 weeks of age, no differences were detected in the dimensions of the ascending aortic lumen by high frequency ultrasound, despite substantial changes in the aortic tissue of transgenic mice at this age. Between 10 and 16 weeks of age, the aortas of SMC-specific S100A12 transgenic mice had a striking increase in maximal diameters compared to their non transgenic littermate controls. It would have been helpful to compliment these ultrasound-defined measurements with ex vivo images and measurements of aortas. Curiously, between 10 and 16 weeks of age was the only interval in which the rate of change of aortic dimensions varied between groups. From 16 to 56 weeks of age, the changes in aortic root diameters between the transgenic and non transgenic mice generally paralleled each other in both males and females. Unfortunately, no characteristics of the aortic tissue were provided at 56 weeks of age. Another indication of the lack of a progressive nature of the defect in transgenic mice is that the dilation was not associated with enhanced rupture. Survival up to 56 weeks did not differ between the transgenic and non transgenic mice. There remains the potential for differential aortic rupture between

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groups at older ages because the study duration was less than half the natural life span of C57BL/6 mice.

SMCs cultured from S100A12 transgenic mice exhibited functional changes compared to SMCs from control mice. Cultured SMCs from transgenic mice secreted more interleukin (IL)-6 in response to incubation with lipopolysaccharide. Lipopolysaccharide incubation led to detection of S100A12 in the culture media. However, the increased IL-6 was only partially attenuated by coincubation with a soluble form of RAGE that would inhibit engagement of the ligand with the cellular receptor. This infers that the effect of lipopolysaccharide in augmenting IL-6 secretion in S100A12 expressing cells was predominantly independent of RAGE. Previously, IL-6 has not been mechanistically linked to ascending aortic aneurysmal development. It would be of interest to determine whether aneurysmal formation is changed in S100A12 transgenic mice that are either wild type or deficient in IL-6. Cultured aortic SMCs from S100A12 transgenic mice also inferentially had increased expression of transforming growth factor (TGF)-β, as defined by increased immunostaining and nuclear translocation of phosphorylated Smad2. The attribution of increased pSmad2 being caused by TGF-β secretion was demonstrated by coincubation with a neutralizing antibody. As mentioned previously, aortic SMCs have varying biological responses attributable to cell heterogeneity. Responses to TGF-β epitomize these differences because this cytokine leads to either promotion or inhibition of aortic SMC growth depending on the region used for isolation.8

Cultured SMCs from S100A12 transgenic mice displayed some surprises. One was a reduced rate of proliferation. This was defined by immunostaining of 5-bromodeoxyuridine rather than direct cell counting. The reduced 5-bromodeoxyuridine immunostaining was matched by an enhanced activity of SMCs from S100A12 transgenic mice to reduce the tetrazolium compound, MTS. Increased MTS conversion is usually considered to be indicative of increased cellular proliferation. The reduced staining with MitoSox was not mimicked by the exogenous addition of S100A12 to cultured SMCs from either wild type or transgenic mice. This again is consistent with its intracellular effects that are independent of the cell surface expression of RAGE. The intracellular action was also cleverly described by coculturing SMCs from wild type and transgenic mice and demonstrating increased MitoSox staining only in cells positive for S100A12. Again, it needs to be defined whether the aortic region used to isolate SMCs has an effect on these responses to facilitate the interpretation of mechanisms of aortic root dilation. These studies in cultured SMCs demonstrated a myriad of changes induced by the expression of S100A12. The challenge will be to define the contribution of these changes to the aortic pathology that develops in vivo in S100A12 transgenic mice.

To define whether the findings in the S100A12 transgenic mice extrapolated to human disease, ascending aortic tissue was acquired from a patient carrying a novel mutation in SMC-specific β-myosin (MYH11). Consistent with observations in transgenic mice, S100A12 was only detected in this human aneurysmal tissue, but not in ascending aortic tissue from a donor without TAA. Also in agreement with the mouse studies, immunostaining of S100A12 was mainly colocalized with SMCs. Acquisition of human aortic tissues from patients with normal or ascending aortic aneurysms is fraught with practical difficulties. Unfortunately, the characterization of tissues from a single control and disease patient renders the observations as anecdotal. It also has the shortcoming of being purely observational. Although the information of such translational studies is inherently constrained, the inclusion of complimentary data from clinical samples should be encouraged.

Overall, the findings of Hofmann Bowman et al2 provide a new insight for the role of S10012A in the development of the devastating disease of TAAs. These studies would be complemented by further experiments that attempt to define the mechanistic basis for the localization of the response to the ascending aorta. This will provide a valuable foundation to determine the contribution of this pathway to a disease that progresses in a highly aortic region-specific manner.

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Disclosures
None.
References


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