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# Role of Serum Amyloid A in Atherosclerosis

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## Abstract

**Purpose of Review:** Acute phase serum amyloid A (SAA) is persistently elevated in chronic inflammatory conditions, and elevated levels predict cardiovascular risk in humans. More recently, murine studies have demonstrated that over-expression of SAA increases and deficiency/suppression of SAA attenuates atherosclerosis. Thus, beyond being a biomarker, SAA appears to play a causal role in atherogenesis. The purpose of this review is to summarize the data supporting SAA as a key player in atherosclerosis development.

**Recent findings:** A number of pro-inflammatory and pro-atherogenic activities have been ascribed to SAA. However, the literature is conflicted, as recombinant SAA, and/or lipid-free SAA, used in many of the earlier studies, do not reflect the activity of native human or murine SAA, which exists largely lipid-associated. Recent literatures demonstrate that SAA activates the NLRP3 inflammasome, alters vascular function, affects HDL function, and increases thrombosis. Importantly, SAA activity appears to be regulated by its lipid association, and HDL may serve to sequester and limit SAA activity.

**Summary:** SAA has many pro-inflammatory and pro-atherogenic activities, is clearly demonstrated to affect atherosclerosis development, and may be a candidate target for clinical trials in cardiovascular diseases.

### Keywords

cardiovascular disease; HDL; inflammation; vascular; SAA; atherosclerosis

## Introduction

Serum amyloid A (SAA) is a family of small proteins (103–104 amino acids) that share high levels of sequence homology, encoded by different genes and remarkably conserved throughout vertebrate evolution. In humans, there are 4 SAA genes (SAA1, SAA2, SAA3, and SAA4) of which SAA1 and SAA2 encode acute phase SAA proteins that are highly

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inducible during the acute-phase response. SAA4 is a constitutively expressed SAA protein, and SAA3 is a pseudogene. Mice also have 4 SAA genes. Like humans, SAA1.1 and SAA2.1 encode acute phase proteins and SAA4 codes for a constitutively expressed SAA; however, in mice SAA3 encodes a functional product, [1] unlike human SAA3. Mouse SAA proteins are highly homologous to their human counterparts. SAA is considered to be predominantly produced by the liver, however, not all authors agree with the liver being the major site of SAA synthesis, Sjoholm et al argue that adipose tissue is a major expression site of SAA during chronic inflammatory conditions. [2] A variety of extrahepatic tissues including breast, stomach, small and large intestine, prostate, lung, pancreas, kidney, tonsil, thyroid, pituitary, placenta, skin epidermis, and brain neurons express SAAs.[3] Despite their evolutionary preservation, dynamic synthetic pattern and extensive research in the past 40 years, there are still several unanswered questions concerning the physiological functions of SAA proteins.

## SAA has a causal role in atherosclerosis

A wealth of epidemiological data links SAA with cardiovascular diseases (CVD)[4–8] and increased SAA is associated with CVD mortality.[9] However, although genetic variants in the SAA genes are associated with SAA levels, there were no associations between CVD status and SAA variants in a retrospective cohort study;[10] thus, genetic regulation of SAA likely does not underlie the association with CVD. Several recent animal studies suggest that SAA has a causal relationship with atherosclerosis and is not merely a marker of the disease. SAA mRNA and protein are detected in atherosclerotic lesions of both mice and humans.[11–13] Over-expression of SAA leads to increased atherosclerosis in mice: Dong et al overexpressed murine SAA1 via a lentiviral vector in *apoE*$^{-/-}$ mice and demonstrated that modest but sustained elevation of SAA led to increased atherosclerosis through increased inflammatory cell infiltration.[14] We used repeated injections of an adenoviral vector expressing human SAA1 in immunodeficient *apoE*$^{-/-}$ mice (mice deficient in recombination activating gene-1) and demonstrated increased atherosclerosis in mice with modest but sustained SAA elevations.[15] We proposed that the increase in atherosclerosis was due to SAA mediated induction of transforming growth factor (TGF)-β, which increased vascular biglycan expression and led to increased LDL retention.[15, 16] Proteoglycan-mediated lipoprotein retention is thought to be a critical step in atherosclerosis development.[17] Moreover, we found that even a single injection of the adenoviral vector encoding SAA1 led to increased atherosclerosis, although SAA was only briefly elevated[15] suggesting that even short term inflammation with a concomitant increase in SAA may increase the risk of developing CVD. Using the *LDLR*$^{-/-}$ mouse model for atherosclerosis, Krishack et al showed that deficiency of SAA1 and SAA2 in macrophages decreased atherosclerotic lesion area in the ascending aorta, albeit only in early lesion development.[18] Surprisingly, we found no reduction in atherosclerosis in the absence of endogenous SAA1.1 and 2.1 in *apoE*$^{-/-}$ mice fed either standard chow or western diets.[19] However, our subsequent study using suppression of SAA3 (via anti-sense oligonucleotide [ASO]) in *apoE*$^{-/-}$ mice lacking SAA1.1 and 2.1 demonstrated significantly reduced atherosclerosis compared to mice wild type for all SAAs,[20] implying that all acute phase SAA isoforms have pro-atherogenic properties, and that deficiency/suppression of all 3 acute phase isoforms is necessary to

observe atheroprotection (in mice). Thus, there is a robust literature demonstrating that SAA has a causal role in atherosclerosis, although the mechanisms by which it does so remain unclear.

## Mechanisms by which SAA may affect atherosclerosis: inflammation

Inflammation plays a significant part in the initiation and progression of atherosclerosis and the development of its acute clinical manifestations.[21, 22] Numerous reports demonstrate that SAA has pro-inflammatory properties (for review see [23]). However, a large number of *in vitro* studies utilized a recombinant SAA protein which has two amino acid substitutions (at positions 61 and 71) when compared with native SAA and recent studies indicate that this recombinant form may exert activities not shared by mouse or human SAA.[24, 25] A recent study demonstrated the presence of numerous bacterial proteins in recombinant SAA derived from E.*coli*, which might have contributed to some of the inflammatory properties of the protein.[26] Moreover, most *in vitro* studies examined the effect of lipid-free SAA; an emerging literature suggests that many of these effects are lost when SAA is lipid-associated, the form SAA is believed to exist *in vivo*.[27–29] Thus, some of the purported effects of SAA (based on *in vitro* studies using recombinant SAA and/or lipid-free SAA) are now in question, and *in vivo* studies are needed to clarify the role of SAA in inflammation and atherosclerosis.

The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) was a seminal clinical trial, which found that reducing vascular inflammation using canakinumab, a human monoclonal antibody against IL-1β that has no effect on cholesterol, decreased cardiovascular events.[30] The study found decreased CRP levels in patients randomized to canakinumab vs placebo, suggesting that decreased inflammation was a key mechanism by which the drug exerted its effect.[30] Although the Cardiovascular Inflammation Reduction Trial (CIRT) did not show a benefit on CVD outcomes, the drug used in this study (methotrexate) did not decrease levels of IL-1β, IL-6 or CRP, which may account for the finding of no cardiovascular outcomes benefit.[31] The NLRP3 inflammasome plays a central role in the development of atherosclerosis (for review see[32]). In a recent study, we demonstrated that SAA activates the NLRP3 inflammasome in macrophages and that the deficiency of SAA in mice significantly dampened IL- 1β production in an experimental model of abdominal aortic aneurysm in mice chronically infused with angiotensin II.[33] We have previously demonstrated that SAA deficiency protected mice against angiotensin II-induced aortic aneurysms.[34] A recent study found the presence of a single-nucleotide polymorphism (SNP) within the promoter region of the SAA1 gene (−13C/T) correlated with Adult-onset Still's disease, a rare systemic inflammatory disorder in which inflammasome activation plays a pathophysiological role.[35]

Thus, acute or chronic inflammation may lead to the development and/or progression of atherosclerosis and other vascular diseases, in part via induction of SAA. By rapidly increasing inflammation in the coronary arteries, acute infections may trigger destabilization and possible rupture of vulnerable plaques.[36] Clinical data suggests that acute infections promote the development of acute coronary syndromes (for review see [37]). Kaynar et al demonstrated that cecal ligation and puncture, a model of intra-abdominal sepsis, led to

increased atheroma development,[38] which they postulated was due to prolonged systemic, endothelial and intimal inflammation although there was no evidence of ongoing infection. However, Fuijkschot et al. used intraperitoneal lipopolysaccharide (LPS) in ApoE3*Leiden mice and found no increase in atherosclerotic plaque area or inflammatory cell density despite a profound systemic inflammatory response with striking elevations in SAA.[39] Earlier work by the same group showed that major orthopedic surgery in *apoE*$^{-/-}$ mice, which triggered a systemic inflammatory response and a pronounced elevation of SAA, resulted in an enlarged and necrotic atherosclerotic plaque area.[40] Thus, the literature remains conflicted as to whether an acute inflammatory insult with elevation of SAA can cause initiation or progression of atherosclerosis.

## Mechanisms by which SAA may affect atherosclerosis: effects of vascular function

The aberrant proliferation of vascular smooth muscle cells (VSMC) promotes plaque formation in atherosclerosis. SAA was shown to induce a phenotype switch in VSMC from quiescent to synthetic forms by reducing the expression of smooth muscle cell markers and enhancing expression of matrix synthesis related markers and increased the proliferative ability of VSMCs through the p38 MAPK signaling pathway.[41] We previously reported that SAA increased vascular biglycan content via induction of TGF-β, and that this led to increased retention of atherogenic lipoproteins.[15, 16, 42]

Endothelial dysfunction is an early and predictive event in the pathology of atherosclerosis, [43] and predicts both the extent of atherosclerosis[44] and long-term disease progression. [45] Several studies have indicated that SAA may promote endothelial dysfunction. SAA stimulates endothelial cell migration and proliferation.[46] SAA promotes angiogenesis *in vitro* by stimulating vascular endothelial growth factor receptor 2 expression resulting in tube formation in endothelial cells cells via the FPRL1/MAPK signaling pathway.[47] In an *in vitro* study, SAA induced expression of adhesion molecules including ICAM1, VCAM1, and E-selectin in human aortic endothelial cells in a TLR2-dependent way.[48] Consistently, Chami et al demonstrated that injection of SAA in *ApoE*$^{-/-}$ mice stimulated pathological changes in the vascular endothelium, including increased VCAM-1 expression, increased leukocyte adhesion, and increased expression of pro-inflammatory cytokines and chemokines.[49] Inhibition of NF-κB activation[50] or addition of HDL[51] protected endothelial function from the pro-inflammatory activities of SAA. Many cell types within the vasculature express SAA,[11–13] and thus either its local expression or deposition from the circulation may lead to changes in vascular cellular composition or function predisposing to atherosclerosis development or progression.

## Mechanisms by which SAA may affect atherosclerosis: changes in lipoprotein association and/or liberation of lipid-free/poor SAA

A number of *in vitro* studies indicate that SAA exhibits pro-inflammatory effects only when lipid-free and not when HDL-bound.[29, 33, 52] Recent structural studies demonstrated that HDL association affects the structural properties of SAA.[53] Consistently, Jayaraman et al

demonstrated that lipoprotein association protects SAA from proteolysis.[54] HDL/lipoprotein association may therefore regulate the physiological functions and related pathological properties of SAA. Transgenic mice with inducible, liver-specific SAA expression do not exhibit increased inflammation despite very high levels of plasma SAA (>1 mg/ml).[55] Thus, under homeostatic conditions, it is unlikely that HDL-bound SAA exerts pro-inflammatory effects. However, there may exist conditions where SAA might be released from HDL; alternately, SAA may be produced and secreted in a lipid-free form in the extracellular milieu to exert pro-inflammatory effects locally. Our recent findings that mice lacking SAA have significantly blunted angiotensin-II-induced increases in plasma IL-1β levels underscores the fact that SAA has pro-inflammatory effects in certain settings.[33] Moreover, we have demonstrated that deficiency of SAA protects against vascular diseases such as experimental abdominal aortic aneurysms and atherosclerosis.[20, 34]

The SAA isoforms that are produced by hepatocytes during an acute-phase response are released into blood circulation and associate predominantly with HDL.[56] Kisilevsky & Manley reported that essentially all circulating SAA is found with HDL.[57] However, we and others have also found SAA on apoB-containing lipoproteins.[6, 58–60] Though preferentially associated with HDL, we recently reported that SAA can be exchanged between HDL and VLDL/LDL via action of cholesterol ester transfer protein (CETP).[60] Moreover, we found that the presence of SAA on apoB-lipoproteins increased their proteoglycan binding affinity,[60] a key step in the initiation of atherosclerosis.[15] SAA is found on apoB-containing lipoproteins post-prandially,[60] and on LDL in samples from people with diabetes.[58, 61, 62] It remains controversial whether lipid-free SAA exists *in vivo*. We recently reported that all three isoforms of acute phase mouse SAAs - SAA1.1, SAA2.1 and SAA3 are detected in the plasma of LPS-injected mice. However, we demonstrated that SAA3 levels were only ~20% of SAA1.1/2.1 levels, and SAA3 was more loosely associated with HDL compared to SAA1.1 and SAA2.1, implying that SAA3 may exist in a lipid-poor/free state.[1] HDL may serve to sequester SAA and thus limit its pro-atherogenic activities, and liberation of lipid-poor SAA and/or exchange of SAA from HDL to other lipoproteins may be key steps in regulating SAA's biological effects. Further studies are required to understand whether activities of SAA depend on its lipoprotein association.

## Mechanisms by which SAA may affect atherosclerosis: altered HDL function

In an acute phase reaction, increases in SAA are coupled with a decrease in apolipoprotein A-I, the major HDL-associated apolipoprotein, thus SAA comprises up to 87% of the total HDL-protein content. [63] This modulates the metabolic properties of HDL[64–67] but does not change HDL cholesterol measurement.[68] The presence of SAA on HDL is believed to convert atheroprotective HDL to dysfunctional HDL.[65, 69–71] Han et al demonstrated that increased HDL-SAA in inflammation caused the HDL to bind to cell surface proteoglycans on adipocytes, which prevented HDL from accessing the plasma membrane and limited the anti-inflammatory effects of HDL.[69] Although the consensus of previously published literature suggests that SAA imparts an adverse effect on the antioxidant properties of HDL, few papers show SAA reduces the rate of lipoprotein oxidation. Jayaraman et al

demonstrated that SAA added to HDL or LDL at physiologically relevant ratios delayed lipoprotein oxidation by several oxidizing agents including $Cu^{2+}$ and myeloperoxidase. Furthermore they report that mild oxidation of SAA-enriched HDL led to the release of SAA (and apoA-I) from the HDL, and that the lipid-poor SAA mediated the anti-oxidant effects. However, SAA was less efficient than the proteins it displaced from HDL, such as apo A-I.[72] A population study reports that HDL from patients with higher SAA levels has better anti-oxidant activity than controls supports the protective role of SAA.[73] Thus, the impact of SAA on HDL function is unclear, though consensus is that the presence of SAA makes HDL dysfunctional. Deficiency of scavenger receptor class B type 1 (SR-B1), a protein which plays a key role in the metabolism of HDL, results in abnormally large and dysfunctional HDL, interestingly with an increased SAA content.[74] SR-B1 deficiency results in increased susceptibility to atherosclerosis in mice.[75]

## Mechanisms by which SAA may affect atherosclerosis: SAA and thrombosis

SAA levels are strongly associated with blood coagulability and venous thromboembolism.[76] SAA is a potent and rapid inducer of human monocyte tissue factor activity, mRNA, and protein on normal human monocytes,[77] and appears to play a direct role in coagulation via induction of red blood cell agglutination and platelet activation and clumping.[78]

## Conclusion

In summary, there is robust evidence that increased levels of SAA are associated with CVD,[4–8] and murine studies demonstrate a direct link between SAA and atherosclerosis.[14, 15, 20]. Thus, SAA appears to have a direct role in atherogenesis and is not simply a biomarker of disease burden. SAA can initiate and develop atherosclerosis by a number of its pro-inflammatory and pro-atherogenic activities. The CANTOS study was the first demonstration that targeting inflammation can improve CVD outcomes in humans.[30] The lack of benefit in CVD outcomes of the CIRT study does not necessarily refute the role of inflammation in CVD (IL-1β, IL-6 and CRP levels were not altered by methotrexate),[31] but instead points to the need for specific anti-inflammatory therapies. Based on the evidence summarized here, SAA is a logical therapeutic target to consider. ASOs to SAA have been developed[20] and ASOs to other targets have proven effective in clinical trials.[79] Thus, the next step may be a clinical trial of SAA suppression, perhaps via ASO technology, in humans at high risk for CVD.

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**Key points**

- Increased SAA causes atherosclerosis and deficiency/suppression of SAA attenuates atherosclerosis in mice
- SAA activates the NLRP3 inflammasome which may underlie its range of effects
- The lipoprotein association of SAA appears to regulate its activity