Chapter 4

Role of SAA in Promoting Endothelial Activation: Inhibition by High-Density Lipoprotein

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Additional information is available at the end of the chapter

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1. Introduction

Serum amyloid A (SAA) is a multi-gene family consisting of highly conserved protein sequences that are known to cluster on chromosome 11 in humans [1] and chromosome 7 in mice [2]. The acute-phase proteins SAA1 and SAA2 (mass of ~12 kDa, 104 amino acids) share > 93% identity in primary sequence structure, are secreted predominantly by hepatocytes and are induced by a broad spectrum of inflammatory cytokines [3]. Extra-hepatic production of acute-phase SAA occurs in many organs and tissues of body including vascular smooth muscle cells and endothelial cells (EC) that are also capable of secreting SAA [4, 5]. By contrast, SAA4 is a glycosylated form that is constitutively produced in a wide range of (histologically) normal tissues and cells [6]. The final isoform, SAA3, is a pseudogene that is not transcribed in humans [1]. In rodents, SAA3 is a functional protein that is expressed in extra-hepatic cells, such as macrophages and adipocytes in response to prolactin or lipolysaccaride (LPS) stimuli thereby, contributing to local inflammation in adipose tissues [7, 8].

Rapid production of SAA in response to the host inflammatory reaction results in plasma levels increasing up to 1,000-fold under some conditions [9]. This marked increase in circulating SAA is linked to the induction of inflammatory cascades that are characterized by local vascular, systemic and multi-organ responses [10, 11]. A wealth of epidemiological and biological research suggests that SAA is also associated with chronic inflammatory conditions such as cardiovascular diseases (CVD) and atherogenesis [12-14]. For example, significantly elevated levels of SAA are evident at different stages of atherosclerosis [15, 16], which, to some extent, echoes a sustained acute-phase response leading to the chronic production of SAA. In fact, SAA is proposed as a potential regulator of inflammation and endothelial dysfunction, implicating adverse outcomes that complicate CVD [4]. SAA is also synthesized in extra-

hepatic tissues and involved in human carcinoma growth and metastases suggesting SAA could participate in tumor development [14] through stimulating pro-angiogenic factors.

The association of circulating SAA with high-density lipoprotein (HDL) is well described with the majority of SAA incorporated as an HDL apolipoprotein [17]. Recent research has focused on the influence of SAA on HDL structure and function (*i.e.*, anti-inflammatory and antioxidant activities of the lipoprotein), including the impact of SAA on HDL's role in reverse cholesterol transport.

2. General pro-inflammatory/pro-thrombotic responses to SAA

SAA may represent a useful clinical marker of acute and chronic inflammation [18]. Similar to the biomarker C-reactive protein (CRP), SAA increases in the blood of patients with various inflammatory conditions. A growing body of research supports the notion that SAA is a potent and rapid inducer of cytokines, monocyte tissue factor (TF) and tumor necrosis factor-α (TNF-α) in human peripheral blood mononuclear cells (PBMC) and THP-1 monocytoid cells within a short period of exposure [19-22]. Initially, this SAA-stimulated cell activation is limited to the local sites of the inflammation. However, upon activation of macrophages, a range of primary inflammatory mediators are released, the most important of which are members of the IL-1 and TNF families of cytokines. These in turn cause the release of secondary cytokines and chemokines (*e.g*, IL-6, IL-8 and MCP-1) from local stromal cells [4, 23]. The chemotactic activities of these chemokines recruit leukocytes such as neutrophils to the inflammatory site, where they in turn provoke a sustained pro-inflammatory cascade that involves local production and release of other cytokines [10,23].

As indicated, SAA stimulation of PMBCs causes a marked increase in the secretion of cytokines including IL-1B, MCP-1, IL-6, IL-8, IL-10, GM-CSF, TNF and MIP-1α with reports of up to 25,000-fold increase compared to baseline levels measured in isolated monocytes / macrophages and lymphocytes [20]. In addition, SAA strongly induces the potent pro-coagulant protein TF, and this activity manifests as an inflammatory-associated thrombosis that also impairs endothelial function. The release of SAA into the circulation in subjects with established coronary artery disease (CAD) may play a role in promoting cardiovascular events since SAA stimulates the expression of TF and TNF in isolated PBMCs [20, 21]. Given the nature of TNF itself as a mediator of inflammatory and its co-localization to atherosclerotic lesions, and TF as a potent pro-coagulating factor, then the concomitant release of these factors is likely to represent a central feature in the pathogenesis and clinical complications associated with developing CAD.

An increase in the circulating levels of SAA may enhance TF expression [24]. Studies have demonstrated that TF binds instantly to TF activated factor VII (FVIIa) yielding a complex that serves as a fuse to facilitate blood coagulation by generating thrombin [24]. Furthermore, activated FVIIa stimulates TF provoked factor VII, IX and X, a secondary cascade that ultimately leads to more thrombin formation [24, 25]. In addition to a direct stimulating effect on TF, SAA also acts on vascular EC to modulate TF pathway inhibitors through a mechanism

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involving mitogen-activated protein kinase (MAPK) and the transcription factor, nuclear factor kappa beta (Nfκβ) [25]. Activation of MAPK and Nfκβ) are central to the induction of cytokines by SAA [26, 27].

3. Response of endothelial cells to SAA

A functional endothelium is vital to the maintenance of vascular homeostasis [28]. The primary function of the vascular endothelium is to act as a barrier that regulates vascular permeability to plasma constituents and inhibits platelet and leukocyte adhesion and aggregation as well as infiltration, and finally, regulates thrombosis [29]. Thus the vascular EC is crucial for maintaining vascular tone, fluidity, coagulation, and inflammatory responses [30]. Under normal physiological conditions, vascular homeostasis is controlled by potent mediators such as nitric oxide (NO), prostacyclin-2 and endothelin-1 as well as local angiotensin II activity [31].

Endothelial dysfunction occurs before the appearance of the first morphological signs of atherosclerosis and is a precursor of atherogenesis [32], therefore endothelial dysfunctional can predict the extent of CVD [33]. Redox regulation of intracellular signaling has been implicated as a factor that impacts on endothelial activation [34]. For example, redox modulation of endothelial nitric oxide synthase (eNOS) gene expression, transport of the active dimeric form of eNOS to the cell membrane by lipid rafts and/or eNOS activity can in turn have downstream effects on NO bioavailability and signaling [35]. Decreases in eNOS activity may contribute to endothelial dysfunction by impairing endothelium-dependent vasorelaxation. Alternatively, decreased production of NO can activate other mediators that play important roles in atherogenesis [Reviewed in 36, 37].

Once the balance of vascular homeostasis is compromised the vascular endothelium undergoes a phenotypic change associated with increased expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IFN-γ together with pro-thrombotic factors. Under these conditions the formation of reactive oxygen species (ROS) is increased and this can impact on vascular tone that is susceptible to oxidative stress via a range of mechanisms [29, 31]. For example, the stability of eNOS and production of NO is directly affected by ROS. Indeed, oxidative events have featured in many studies of impaired NO bioactivity [38] and endothelial dysfunction, which in turn impacts on other cardiovascular risk factors such as hypertension [39], diabetes [40] and rheumatic autoimmune diseases [32].

Studies have shown that SAA also promotes both monocyte chemotaxis and adhesion to the vascular endothelium [5, 41], thereby regulating the recruitment of leukocytes to the inflamed endothelium [42]. During this process SAA promotes the production of other pro-inflammatory cytokines and chemotactic molecules, which cause endothelial dysfunction and ultimately lead to atherosclerosis and other related CAD. In support of this idea it is found that SAA co-localizes within microtubules of human coronary artery EC (HCAEC) [43]. Previous work from our group [44] has confirmed that SAA stimulates EC production of TF and Nfκβ gene expression as well as cytokines such as IL-6, IL-8, and MCP-1 that in turn impair NO bioactivity

[44]. For example, exposure of isolated thoracic aortic vessels to SAA (1-25 μg/mL) decreases vascular relaxation in response to the endothelium-dependent vaso-dilator acetylcholine (ACh) (Fig. 1A), whereas endothelium-independent vaso-relaxation to s-nitrosopenicillamine (SNP) remained unaffected by SAA (Fig. 1B). A similar study by Wang and co-workers has reported that clinically relevant concentrations of SAA causes endothelial dysfunction in both porcine coronary arteries and HCAEC by down regulation of eNOS, activation of JNK and ERK1/2 as well as Nfκβ [45]. This mechanism is consistent with a study indicating that inhibitors of MAPK and Nfκβ markedly decreased SAA-stimulated pro-inflammatory cytokines secretion from HEK293 cells [27]. Taken together these combined data demonstrate that SAA enhances stimulates ROS production in cultured EC [47].

Reactive species such as superoxide radical anion ($O_2^{\bullet -}$), lipid (per)oxidation products and the potent oxidizing agent peroxynitrite are all implicated in endothelial activation and impaired NO signaling. Therefore, SAA-stimulation of ROS production is a possible mechanism to explain impaired endothelial function [44, 48]. In agreement with this hypothesis, exposure of cultured EC to SAA (added at a final concentration of 10 μg/mL) reduces NO accumulation in HCAEC stimulated with ACh, whereas human serum albumin (HSA), that is not known to affect EC production of NO, has no effect (Fig. 2). Interestingly, pre-incubating HCAEC with HDL (50 - 200 μg/mL) before addition of SAA restores NO accumulation in response to ACh, and this is dependent on the dose of HDL (Fig. 2) [44]. Moreover, these data indicate that the ratio of SAA-to-HDL might be critical to assessing SAA's effect on the vascular endothelium.

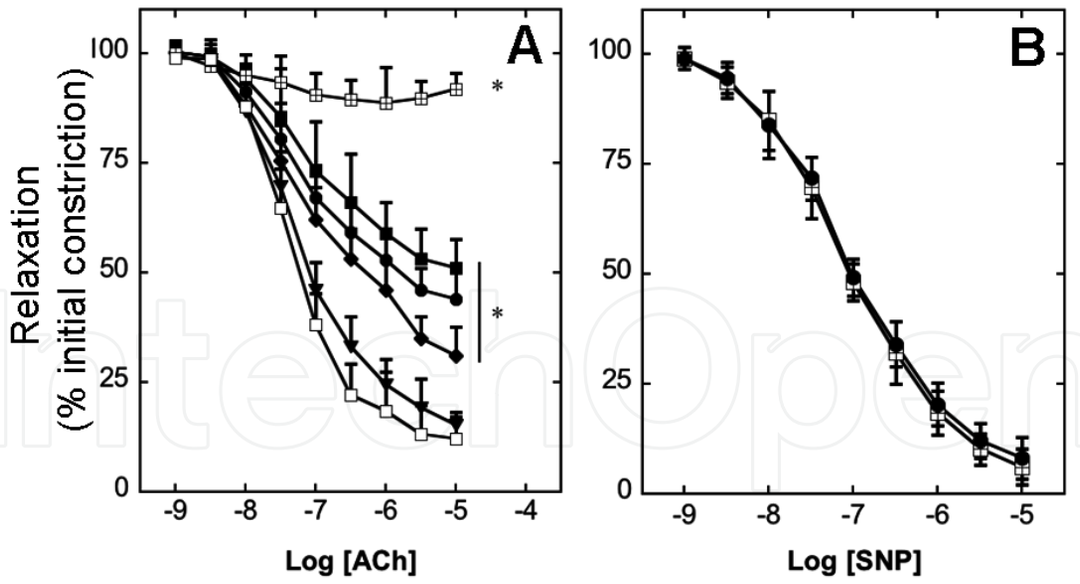

Figure 1. SAA inhibits endothelium-dependent, but not endothelium-independent, relaxation. Aortic rings were incubated with SAA at 1 (inverted triangles), 5 (diamonds), 10 (circles), or 25 μg/mL (solid squares); the soluble guanylate cyclase inhibitor ODQ [46] (used as a positive control, hatched square); or vehicle (control, open squares) for 4 h at 37 °C. Rings were washed and constricted with phenylephrine and then dilated by adding (A) ACh or (B) SNP at the concentrations indicated. Data represent means ± SD (n=6 rings from independent animals except for vessels exposed to 25 μg/mL SAA, n=5 rings from independent animals). *Different to the control in the absence of SAA; $P < 0.05$. The figure was reprinted from Ref [44] with permission from the Publisher.

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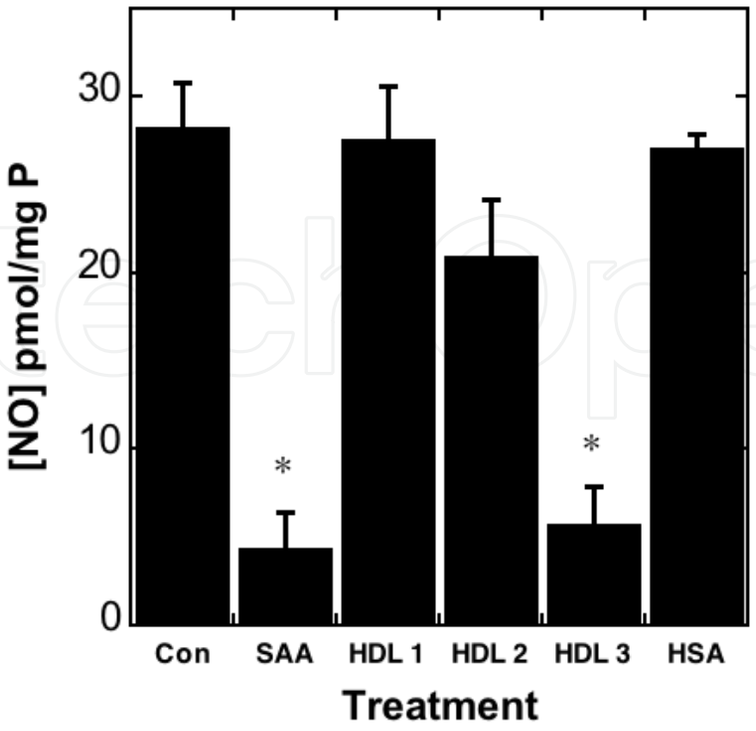

Figure 2. SAA decreased acetylcholine-induced NO accumulation in HCAEC. HCAECs were pretreated in serum-free-medium containing vehicle (control), SAA (10 μg/mL), or human serum albumin (HSA; 1 mg/mL). After 4 h cells were harvested, resuspended in HPSS (~4×106 cells/mL) containing 100 μM Arginine and stimulated with 1 μM ACh. Changes in NO evolution were monitored with an NO electrode. Freshly isolated HDL (50 - 200 μg/mL) added 30 min before incubation with SAA inhibited the action of the acute phase protein. Total nitrite was determined in the medium after incubation with nitrate reductase / NADPH. HDL1, 50; HDL2, 100; and HDL3, 200 μg/mL in protein, respectively. Data represent n=3 HCAEC preparations. Figure reprinted from Ref [44] with permission from the publisher.

Previous studies have identified NADPH-oxidase as a significant source of $O_2^{\bullet-}$ in various cell types, in addition to other potential sources such as uncoupled eNOS, xanthine oxidase, mitochondria and cytochrome p450 [38, 47, 49, 50]. The data shown in Fig 3 demonstrate an enhanced yield of $O_2^{\bullet-}$ after stimulation of cultured HCAEC with added SAA. This increase is inhibited by the pharmacological agents diphenyliodonium (DPI) and apocynin (that target NADPH oxidase) or polyethylene glycated SOD-1-conjugate (PEG-SOD) that binds to the cells and promotes $O_2^{\bullet-}$ dismutation [51]. Pre-incubation of cells with HDL (final concentrations 200 and 100, but not 50 μg/mL) reversed SAA-induced responses, again indicating that the SAA-to-HDL ratio is a determinant of SAA-mediated endothelial dysfunction [44].

Other ROS derived from the uncontrolled production of $O_2^{\bullet-}$, such as hydrogen peroxide (H_2O_2) and hydroxyl radical (.OH) can also affect endothelium-dependent contractile responses [52]. For example H_2O_2 can promote vascular constriction [53] and its ability to readily cross cell membranes underlies its ability to stimulate matrix metalloproteases (MMP) in the vascular wall [54]. Another example of $O_2^{\bullet-}$-derived ROS is.OH that is implicated in endothelial dysfunction associated with diabetes [55]. Therefore, exposure of the vascular endothelium to SAA can lead to uncontrolled production of multiple ROS that impact on EC function.

Mounting evidence suggests that ROS are key mediators of vascular inflammation and atherosclerosis [45, 56]. The documented ability of SAA to initiate the production and release of pro-inflammatory cytokines is further supplemented by studies that show SAA can propagate ROS production in rabbit aortic EC [48].

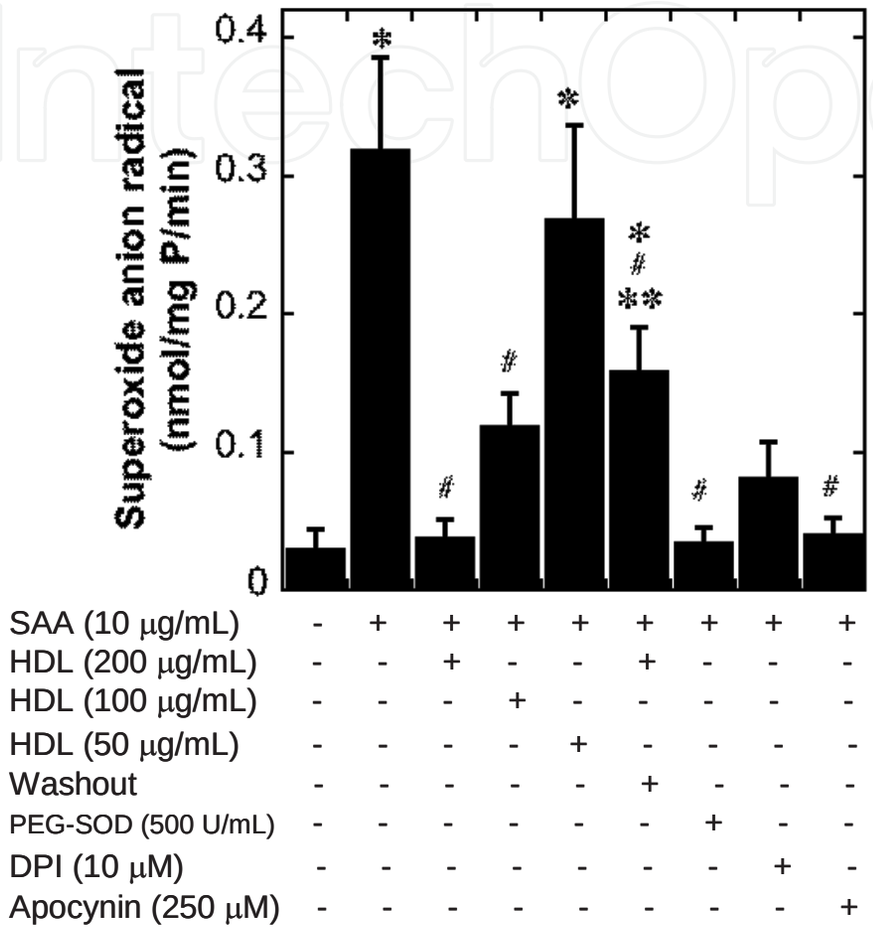

Figure 3. Increased $O_2^{\bullet-}$ production in SAA-stimulated HCAEC. HCAEC (1–2 ×10^6 cells) were treated with 2 μM acetylated ferric cytochrome *c*, SAA (10 μg/mL) was added, and PEG-SOD-inhibitable ferric cytochrome *c* reduction was monitored at 550 nm. Other HCAEC were incubated with 200, 100, or 50 μg HDL/mL for 30 min, then HDL was left in the well or was thoroughly washed out before SAA addition. Other cells were pre-incubated with 10 μM DPI or 250 μM apocynin before SAA stimulation. Data represent means ± SD; n=4 experiments. * Different to unstimulated cells; P<0.05. # Different to HCAEC treated with SAA; P < 0.05. ** Different to the corresponding cells with HDL present; P<0.05. Data represent n=3 experiments. Figure derived from Ref [44] with permission from the Publisher.

Uncontrolled ROS production coupled with impaired antioxidant enzyme activity such as SOD, catalase and glutathione peroxidase (GPx) [57], may also contribute to SAA-mediated EC activation. Further studies implicate P38, JNK/Erk and angiotension II pathways in the deterioration of endothelial function [58, 59]. Overall, the underlying mechanisms implicated in SAA-mediated endothelial dysfunction are multifactorial and include the damage to the NO/eNOS system [45]; enhanced $O_2^{\bullet-}$ production in response to ACh [44], increases in Arg-1 expression [44], and the deficiency of antioxidant systems [53, 57, 60]. Regulation of argi-

nase-1/2 is linked to the up-stream expression of TNF that is induced by SAA and itself promotes vascular dysfunction by decreasesing the pool of substrate available to eNOS [61].

4. SAA and atherosclerosis

Atherosclerosis may be considered as a chronic inflammatory disease [38]. Circulating SAA levels increase in subjects with CAD and changes with the disease severity [4, 62-65]. Levels of SAA also increase in conditions subject to increased cardiovascular risk, such as obesity [62], diabetes [66, 67], rheumatoid arthritis (RA) [68, 69] and angiographically demonstrable CAD [65]. Although this accumulated supporting evidence is mainly observational, the correlative data have provided the basis of a link between SAA and chronic inflammatory processes associated with atherogenesis.

In the artery wall, various inflammatory cell types are recruited and this may be attributed by SAA's chemo-attractant activity [16]. The stimulation of vascular EC to promote the production of TF and TNF-α, combined with the SAA-induced accumulation of adherent monocytes / macrophages, particularly within lymphocyte-rich areas of vascular plaque, may trigger a focal TF response in addition to SAA action on circulating monocytes, thereby contributing to the highly pro-thrombotic properties of the lipid-rich core within atherosclerotic lesions. Subsequent expression of matrix degrading enzymes will result in plaque instability [21]. Potentially, reoccurring acute inflammation will give rise to cyclical increases in circulating SAA that may incite monocyte adhesion and chemotaxis to the artery wall leading to altered barrier function (*i.e.*, endothelial dysfunction) and an increase in lipid content in the sub-endothelial space [12]. At this point, SAA associated HDL may impact on lipid metabolism and possibly reverse cholesterol transportation in the developing lesion through an intensified affinity to macrophages within the atheroma [70]. The retention of SAA-containing HDL in the arterial wall may be promoted due to SAA's ability to strongly bind to vascular proteoglycans [71, 72]. Increased resident time for SAA in the vascular wall may conceivably stimulate the formation of macrophage foam cells implicating SAA in different stages of atherogenesis [38].

Therefore, in addition to accumulating pro-atherogeneic LDL [73] in the arterial wall, the presence of both SAA-associated HDL and oxidized HDL in close proximity to macrophage scavenger receptors may act in concert to potentiate atherogenesis [73-76]. In support of this idea levels of SAA, but not cholesterol, predict lesion area in cholesterol-fed rabbits [77], suggesting a critical role for SAA in the early stages of lesion development. It is also found that SAA deposition in the vessel wall is present at all stages of atherosclerosis [12, 16]. These chronically elevated SAA concentrations in the arterial wall are commonly associated with the pathogenesis of secondary amyloidosis [78] where SAA retains its ability to induce cytokine and chemokine production, matrix-degrading enzymes, such as collagenases and MMP, and interfere with platelet function [78]. Not surprisingly, SAA is co-located with apolipoprotein A-I (apoA-I) in the vascular wall of patients with peripheral atherosclerosis, particularly in the arterial intima [79], an observation confirmed by presence of co-localization of SAA with both

apoA-I and proteoglycans in atherosclerotic lesions [80]. Interestingly, SAA is also present within different compartments of HCAEC such as cytoskeletal filaments including microtubules, inside the nucleus and within nanotubules [43]. The expression of SAA in these compartments may favor the progression of atherosclerosis in the vascular wall.

5. Over-expression of SAA in apolipoportein E deficient mice

The apolipoprotein E-deficient (apoE$^{-/-}$) mouse is widely employed as an animal model of atherosclerosis because of its propensity to develop atherosclerotic lesions [81-83]. A growing body of research supports the idea that SAA can initiate endothelial dysfunction. For example, construction of SAA lentivirus in apoE$^{-/-}$ mice stimulates pro-atherogenic changes in the vessel wall [13]. Furthermore, elevated plasma levels of SAA are detected in an apoE$^{-/-}$ model of obesity that exhibits accelerated atherosclerosis [84].

Interestingly, high levels of SAA are found to be associated with both HDL and LDL in mice fed with a high-fat diet. The later is primarily localized with apoB-containing lipoproteins and biglycan in the vascular wall [84]. Similarly, the use of viral vectors to increase SAA levels in apoE$^{-/-}$ mice result in substantially enhanced plasma levels of IL-6 and TNF-α and increased macrophage infiltration into the sub-endothelial space of early developing vascular lesions [13]. Over-expression of SAA also causes marked increase in the expression of MCP-1 and VCAM-1 in HAEC, thus providing direct evidence that chronic elevation of SAA in the vasculature enhances the progression of atherosclerosis in apoE$^{-/-}$ mice [13]. However, in contrast with this idea, extravascular inflammatory stimuli (*i.e.*, croton oil-induced skin inflammation, *aspergillus fumigatus* antigen-induced allergic lung disease and *A.fumigatus* antigen-induced peritonitis), which also stimulates an increase in circulating SAA levels and has no effect on the progression of atherosclerosis in the same mouse model [85]: the gender of mice employed in these two studies differed and this may be important to the study outcome [86]. Interestingly, the latter model likely elicits multiple inflammatory and antioxidant pathways independent of SAA and this may explain at least in part the differences in lesion size reported.

6. Regulation of endothelial function by HDL bound SAA

One of the principal roles of SAA is its association with HDL and the subsequent modulation of the metabolic properties of HDL. In general, SAA is an apolipoprotein largely associated with HDL_3 (density 1.125–1.21 g/mL) in plasma where it can displace apoA-1 if in sufficiently high concentration in the circulating blood [87-89]: apoA-1 is the major protein responsible for the bioactivities associated with anti-atherogenic HDL [90]. Displacement of apoA-I by SAA results in substantial altered metabolic properties of its main physiological carrier. These changes in the apolipoprotein moieties may transform an originally anti-atherogenic into a pro-atherogenic lipoprotein particle [88], although this is yet to be corroborated by other

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independent researchers and further studies are warranted to establish this hypothesis. Nevertheless, factors that affect remodeling of HDL are complex due to putative roles of both apoA-1 and SAA.

A recent HDL proteome study confirmed that protein compositions of HDL from acute coronary syndrome (ACS) patients are shifted to a pro-inflammatory profile that co-incidentally show increased circulating SAA [91]. Similarly, studies involving end-stage renal disease (ESRD) patients, indicate that SAA enriched HDL has reduced anti-inflammatory capacity compared to normal HDL [92, 93]. Such SAA-enriched HDL exerts lower anti-inflammatory properties partly due to enhanced binding capacity of SAA-containing HDL to macrophages [94] and proteoglycans [71] relative to native HDL. Furthermore, SAA impedes HDL's hepatocytic affinity and occurs concomitantly with a decrease in apoA-1 content in SAA - containing HDL [94, 95]. The decrease in HDL apoA-1 may be related to the prevention of apoA-I lipidation caused by SAA-elicited inflammation, resulting in an overall decrease in nascent HDL formation [96-99].

However not all studies support a role for SAA in altering HDL function. Increasing the expression of SAA1 or SAA4 (28 -72 mg/dL) in transgenic mice do not significantly alter apoA-I or HDL cholesterol or affect lipoprotein profiles compared with the wild-type [6]. In other studies, adenoviral vector mediated over expression of SAA in ApoAI$^{-/-}$ mice is unable to substitute for apoA-I in HDL particle formation [100]. Interestingly, in SAA deficient mice (dual SAA-1/2 gene deletion), increased size of HDL is found in relation to surface phospholipids, not proteins. Total HDL levels and apoA-I clearance are resistant to change during inflammation [89, 101].

The proportion of SAA incorporating into HDL as an apolipoprotein may impact on the function of this lipoprotein. A recent perspective review on SAA by Kisilevsky *et al* [99] has detailed estimates of molar ratios between HDL and SAA in different clinical settings (Table 1). In the setting of developing CAD, ~10% of circulating HDL contains SAA, whereas in an acute inflammatory response every HDL particle contains at lease one SAA. By contrast in a normal physiological setting few HDL particles contain SAA [99]. It is estimated that when SAA constitutes 10-20% or more of total HDL protein, HDL binding capacity to PBMC and EC is increased relative to native HDL [95].

Furthermore, 8-10% incorporation of SAA in total HDL protein causes slight increases in the release of pro-inflammatory cytokines from adipocytes [102]. Outcomes from cell culture studies suggest that reduction of cholesterol efflux by SAA bound HDL is not pronounced unless SAA constitutes more than 50% of the total HDL protein [17]. A similar study confirms that impaired ABCG1-dependent efflux by HDL is independent of SAA during inflammation, although the amount of SAA contained in HDL was not determined in this study [103]. Conflicting with this data others have suggested that SAA does play a role in cholesterol metabolism during acute inflammation [104], although again the relative SAA-to-HDL ratios are not available. It is feasible that SAA can alter HDL function beyond specifically influencing apoA-I concentration, for example by impacting HDL-scavenger receptor VI interactions and scavenger receptor class B member 1 (SR-B1) [71, 105] and / or acting to facilitate the binding

Experimental model	Amount of SAA in HDL	Comments	Ref
Human adipocyte	8-10% SAA in total protein	Slightly induce pro-inflammatory adipose secretion	[102]
ESRD patients	Enriched with SAA, amount not known, apoA-I not detectable.	Reduced anti-inflammatory capacity with reduced MCP-1 inhibition	[92, 93]
C57BL/6 mice	Co-expression of SAA and endothelial lipase	Reduced levels of HDL cholesterol and apo A-I; impeded ABCA1-mediated lipidation of apoA-I	[98]
ACS and CAD subjects	Increased SAA in HDL, amount not known.	Pro-inflammtory profile of HDL in patients; ABCA-1, ABCG-1 and SR-B1 mediated cholesterol efflux are changed	[91]
$SAA^{-/-}$ mice	Amount not available	No impact on HDL cholesterol and apoA-I level	
Human endotoxemia	Unchanged HDL proteome; higher expression of SAA in low HDL-c subjects	Low HDL-c levels are more responsive to inflammatory stimuli compared to high HDL-c	[106]
Mouse HDL	3 apoA-I and 3-5 SAA molecules per HDL particle	Increased binding of HDL to vascular proteoglycans	[71]
U937, THP-1, PBMC, EA.hy. 926 / HuH-7 cells	10-20% SAA in HDL	Increased HDL binding capacity to PBMC / EC	[95]
$SAA^{-/-}$ mice	no SAA level available in HDL	No impact on HDL cholesterol and apoA-I level	[101]
Transgenic mice from C57BL/6	Levels of SAA in HDL not available	No alteration to HDL cholesterol and apoA-I level	[6]
Human THP-1 cells	>50% SAA constituted in HDL	Reduced cellular cholesterol efflux	[17]
$ApoAI^{-/-}$ mice	Overexpression of SAA, only 4% is associated with HDL	Not able to replace apoA-I	[100]

Table 1. Levels of SAA associated in HDL and the influence on HDL activity

of HDL to vascular proteoglycans [71]. Also, SR-B1 mediating is co-expressed with SAA in EC in RA synovial membrane [105] suggesting multiple factors participate in SAA-mediated changes to HDL function.

Independent of the conflicting data on the potential for SAA to impact HDL activity, the relative ratio of SAA to HDL consistently impacts on HDL activity. For example, SAA-induced TNF-α and IL-1β release in THP-1 cells are dose dependently inhibited by the addition of HDL, suggesting that HDL protects against the effects of SAA during SAA transport in the blood-stream [107]. That is, HDL retains its ability to protect HCAEC from SAA stimulation when cells are pre-treated with HDL before addition of pathological SAA [44]. Similarly, endothe-lium-dependent relaxation was partially restored by pretreatment of aorta with PEG-SOD compared to control (Fig. 4A). Whereas pretreatment of aorta with 100, 200, and 400 (but not 50) μg HDL/mL before stimulation with 10 μg SAA/mL protected from EC dysfunction either partially (~50%) or completely (effective HDL doses *vs* SAA alone; $P < 0.05$, Fig. 4B). Further-more, reduced NO production in HCAEC stimulated SAA then ACh is restored by pre-incubation of HCAEC with 200 μg HDL/mL before SAA stimulation (see Fig. 2). Conversely, lower HDL-to-SAA ratios are less able to inhibit SAA activity on EC and therefore, increase the likelihood of endothelial dysfunction. The data underscore HDL's protective roles in

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regulating SAA-mediated damage to EC particularly when high levels of HDL are present relative to SAA.

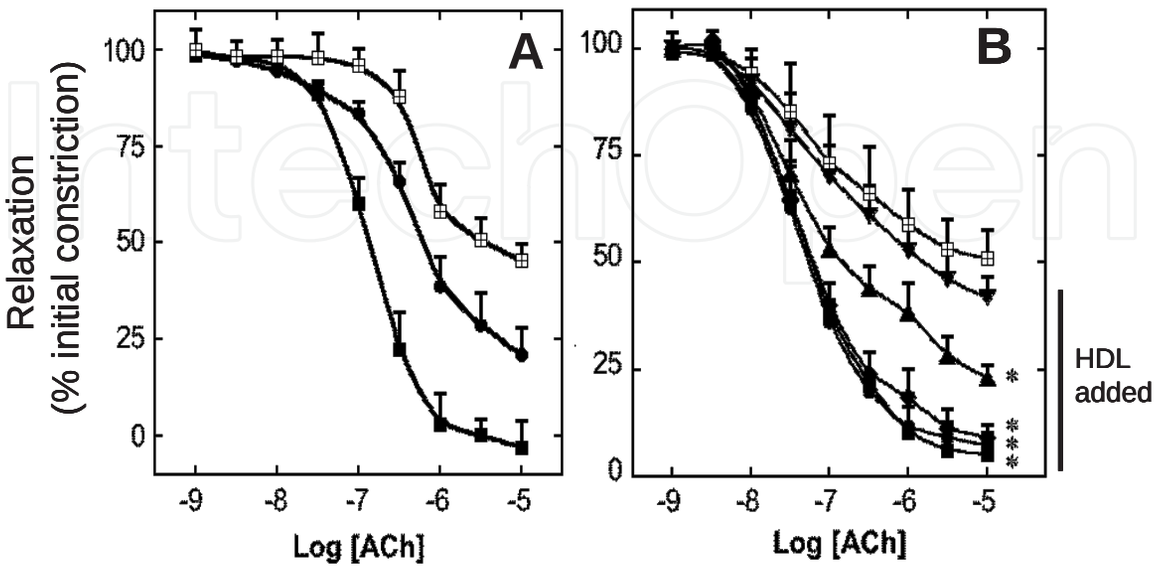

Figure 4. Added PEG-SOD or HDL reverses SAA-provoked vascular dysfunction. Aortic rings were pre-incubated with (A) PEG-SOD (500 U/mL, filled circle) or (B) HDL at 400 (circles), 200 (diamonds), 100 (triangles), or 50 µg/mL (inverted triangles), or vehicle (control). Next, ring segments were treated with vehicle (filled square) or 10 µg SAA/mL (hatched squares) and incubated at 37 °C. After 4 h, rings were treated with phenylephrine and then ACh. Data represent means ± SD, n=5. * P<0.05, different to vessels treated with 10 µg SAA/mL in the absence of HDL.

7. Other actions of SAA on the vascular endothelium

Angiogenesis is defined as the formation of new capillaries from existing vessels, whereas vasculogenesis is a process that involves neo-capillary formation and involves endothelial precursor cells such as angioblast [108]. In addition to activating the vascular endothelium toward pro-inflammatory and pro-thrombotic states, SAA also promotes EC migration and proliferation and this has been linked to its chemokine-like properties that regulate cellular migration and stimulate cell proliferation [69]. The ability of SAA mediating pro-inflammatory response is also to promote neo-capillary formation through a process termed inflammatory angiogenesis [109].

The proliferation of EC is a pathological hallmark of RA where significantly elevated levels of serum SAA and CRP are also a characteristic feature of this pathology [110]. Mullan and co-workers have demonstrated that SAA (i) enhances levels of ICAM-1 and VCAM-1 in RA fibroblast-like synoviocytes (FLS) and human microvascular endothelial cells (HMVECs) and (ii) SAA significantly induces EC tube formation and HMVEC migration emphasising SAA's role in angiogenesis [111]. Presently it is understood that SAA binding to the formyl peptide receptor-like 1 (FPR-1) stimulates this mode of EC activation [112]. Interestingly, activation of FPR-1 by synthetic agonists readily induces macrophage TNF-α production [113] with a

parallel increase in ROS production [48]. The latter increases Nfκβ activation [114], which itself enhances TNF-α production [115, 116] and the downstream production of vascular EC growth factor (VEGF) [117]. Furthermore, VEGF signaling activates the expression of EC-derived MMPs that is essential for initiation of EC sprouting [118]. At this point, Notch signaling, an evolutionary conserved protein pathway directing cell-fate determination [119], acts downstream of VEGF signaling to regulate EC morphogenesis via induction and activation of specific MMPs demonstrating that Notch mediates VEGF-induced MMP expression [120]. Notch is also reported to promote extracellular matrix components, such as type I collagen; at the same setting, Notch also induces differentiation of resting fibroblasts into myofibroblasts [121]. In fact, due to high expression Notch 1,2 and 3 in RA patients [122], as well we its particular regulation on cell proliferation and differentiation, Notch is being considered as the direction of a new therapeutic target for RA [123].

Another important mediator: serum amyloid A activating factor-1 (SAF-1), is identified as a critical regulator of a variety of cellular genes including MMP-1 and FLS, and acting as VEGF promoter [124]. Recent studies on experimentally induced arthritis in a SAF-1 transgenic mouse showed a phenotype with markedly higher levels of angiogenesis, synovial inflammation and inflammatory cell infiltration all mediated by induction of VEGF by SAF-1 [125]. SAA-stimulation of ROS production in the endothelium also represents a feasible mechanism that leads to the production of pro-angiogenic factors. Alternately, blockade of SAA binding or direct inhibition to cell surface receptors that binding VEGF may lead to some benefit in this inflammatory condition.

In addition to SAA's participation in angiogenesis during RA, SAA may also be related to the pathogenesis of cancer. Indeed, high levels of SAA in serum concentrations have been associated with gastric [126], lung [127], renal [128] colorectal [129], breast [130], prostatatic [131] and pancreatic cancers [132], where cancer cells themselves have been implicated in localized SAA production. Irrespective of the source of SAA, within the tumor microenvironment, SAA is enriched together with tumor promoting-cytokines produced by activated innate immune cells. Therefore, cancers are likely to stimulate angiogenesis through multiple mechanisms and this should be taken into account in the development of therapeutic drugs that target the inhibition of angiogenesis as a means to limit cancer growth.

8. Future perspectives

Overall, SAA is now increasingly seen as an independent pathogenic risk factor that plays a role in EC activation, and ultimately the development of vascular complications associated with atherosclerosis. Through a concerted relationship with HDL, SAA's pro-atherogenic action on the vascular endothelium may be regulated and this has potential implications for the management of CVD patients that typically show a high SAA/HDL ratio. In terms of clinical impact it is of importance to fully understand the relatioship between native HDL, SAA associated HDL and free SAA and their impact on atherogenesis. Thus, SAA may not be simply a biomarker of inflammatory status, but be actively involved in pro-atherogenic activation of the vascular endothelium.

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Abbreviations

ABCG1, ATP-binding cassette sub-family G member 1; ACh, Acetylcholine; ACS, Acute coronary syndrome; apoA-I, Apolipoprotein A-I; apoE$^{-/-}$, Apolipoprotein E-deficient; cGMP, Cyclic guanosine monophosphate; CRP, C-reactive protein; CAD, Coronary artery disease; CVD, Cardiovascular disease; DPI, Diphenyliodonium; EC, Endothelial cells; eNOS, Endothelial nitric oxide synthase; ERK1/2, Extracellular-signal-regulated kinases; ESRD, End-stage renal disease; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HCAEC, Human coronary artery endothelial cells; HDL, High-density lipoproteins; ICAM-1, Intracellular adhesion molecule-1; IL, Interleukins; JNK, c-Jun N-terminal kinases; LDL, Low-density lipoproteins; LPS, Lipolysaccaride; MAPK, Mitogen-activated protein kinase; MCP-1, Monocyte chemotactic protein 1; MIP-1α, Macrophage inflammatory protein 1 α; MMP, Matrix metalloproteases; NADPH, Nicotinamide adenine dinucleotide phosphate; NFκB, Nuclear factor kappa beta; NO, Nitric oxide; $O_2^{\bullet-}$, Superoxide radical anion; ODQ, 1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one; PBMC, Peripheral blood mononuclear cells; PEG-SOD, Polyethylene glycol–superoxide dismutase; RA, Rheumatoid arthritis; ROS, Reactive oxygen species; SAA, Serum amyloid A; SNP, s-nitrosopenicillamine; SOD, Superoxide dismutase; SR-B1, Scavenger receptor class B member 1; TF, Tissue factor; TNF, Tumor necrosis factor-α; THP-1, Human acute monocytic leukemia cell line; VCAM-1, Vascular cell adhesion molecule-1; VEGF, Vascular endothelial cell growth factor.

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References

[1] Steel, D. M, Sellar, G. C, Uhlar, C. M, Simon, S, Debeer, F. C, & Whitehead, A. S. A Constitutively Expressed Serum Amyloid A Protein Gene (SAA4) Is Closely Linked to, and Shares Structural Similarities with, an Acute-Phase Serum Amyloid A Protein Gene (SAA2). Genomics (1993). , 16(2), 447-454.

[2] Meek, R. L, Eriksen, N, & Benditt, E. P. Serum amyloid A in the mouse. Sites of uptake and mRNA expression. Am J Pathol (1989). , 135(2), 411-9.

[3] Jensen, L. E, & Whitehead, A. S. Regulation of serum amyloid A protein expression during the acute-phase response. Biochem J (1998). Pt 3) 489-503.

[4] Hua, S, Song, C, Geczy, C. L, Freedman, S. B, & Witting, P. K. A role for acute-phase serum amyloid A and high-density lipoprotein in oxidative stress, endothelial dysfunction and atherosclerosis. Redox Rep (2009). , 14(5), 187-96.

[5] Kumon, Y, Hosokawa, T, Suehiro, T, Ikeda, Y, Sipe, J. D, & Hashimoto, K. Acute-phase, but not constitutive serum amyloid A (SAA) is chemotactic for cultured human aortic smooth muscle cells. Amyloid (2002). , 9(4), 237-41.

[6] Kindy, M. S, De Beer, M. C, Yu, J, & De Beer, F. C. Expression of Mouse Acute-Phase (SAA1.1) and Constitutive (SAA4) Serum Amyloid A Isotypes : Influence on Lipoprotein Profiles. Arteriosclerosis, Thrombosis, and Vascular Biology (2000). , 20(6), 1543-1550.

[7] Larson, M. A, Wei, S. H, Weber, A, Weber, A. T, & Mcdonald, T. L. Induction of human mammary-associated serum amyloid A3 expression by prolactin or lipopolysaccharide. Biochemical and Biophysical Research Communications (2003). , 301(4), 1030-1037.

[8] Fasshauer, M, Klein, J, Kralisch, S, Klier, M, Lossner, U, Bluher, M, & Paschke, R. Serum amyloid A3 expression is stimulated by dexamethasone and interleukin-6 in 3T3-L1 adipocytes. Journal of Endocrinology (2004). , 183(3), 561-567.

[9] Gabay, C, & Kushner, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. New England Journal of Medicine (1999). , 340(6), 448-454.

[10] Uhlar, C. M, & Whitehead, A. S. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem (1999). , 265(2), 501-23.

[11] Lachmann, H. J, Goodman, H. J. B, Gilbertson, J. A, Gallimore, J. R, Sabin, C. A, Gillmore, J. D, & Hawkins, P. N. Natural History and Outcome in Systemic AA Amyloidosis. New England Journal of Medicine (2007). , 356(23), 2361-2371.

[12] King, V. L, Thompson, J, & Tannock, L. R. Serum amyloid A in atherosclerosis. Current Opinion in Lipidology (2011). MOL.0b013e3283488c39., 22(4), 302-307.

[13] Dong, Z, Wu, T, Qin, W, An, C, Wang, Z, Zhang, M, Zhang, Y, Zhang, C, & An, F. Serum amyloid a directly accelerates the progression of atherosclerosis in apolipoprotein e-deficient mice. Mol Med (2011).

[14] Malle, E, Sodin-semrl, S, & Kovacevic, A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. Cell Mol Life Sci (2009). , 66(1), 9-26.

[15] Yamada, T, Kakihara, T, Kamishima, T, Fukuda, T, & Kawai, T. Both acute phase and constitutive serum amyloid A are present in atherosclerotic lesions. Pathology International (1996). , 46(10), 797-800.

[16] Chait, A, Han, C. Y, Oram, J. F, & Heinecke, J. W. Thematic review series: The Immune System and Atherogenesis. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? Journal of Lipid Research (2005). , 46(3), 389-403.

[17] Banka, C. L, Yuan, T, De Beer, M. C, Kindy, M, Curtiss, L. K, & De Beer, F. C. Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux. J Lipid Res (1995). , 36(5), 1058-65.

[18] Filep, J. G, & El Kebir, D. Serum amyloid A as a marker and mediator of acute coronary syndromes. Future Cardiology (2008). , 4(5), 495-504.

[19] Cai, H, Song, C, Endoh, I, Goyette, J, Jessup, W, Freedman, S. B, Mcneil, H. P, & Geczy, C. L. Serum amyloid A induces monocyte tissue factor. J Immunol (2007). , 178(3), 1852-60.

[20] Song, C, Hsu, K, Yamen, E, Yan, W, Fock, J, Witting, P. K, Geczy, C. L, & Freedman, S. B. Serum amyloid A induction of cytokines in monocytes/macrophages and lymphocytes. Atherosclerosis (2009). , 207(2), 374-383.

[21] Song, C, Shen, Y, Yamen, E, Hsu, K, Yan, W, Witting, P. K, Geczy, C. L, & Freedman, S. B. Serum amyloid A may potentiate prothrombotic and proinflammatory events in acute coronary syndromes. Atherosclerosis (2009). , 202(2), 596-604.

[22] Furlaneto, C. J, & Campa, A. A Novel Function of Serum Amyloid A: A Potent Stimulus for the Release of Tumor Necrosis Factor-α, Interleukin-1β, and Interleukin-8 by Human Blood Neutrophil. Biochemical and Biophysical Research Communications (2000). , 268(2), 405-408.

[23] Cassatella, M. A. The production of cytokines by polymorphonuclear neutrophils. Immunology Today (1995). , 16(1), 21-26.

[24] Furie, B, & Furie, B. C. Mechanisms of Thrombus Formation. New England Journal of Medicine (2008). , 359(9), 938-949.

[25] Zhao, Y, Zhou, S, & Heng, C. K. Impact of Serum Amyloid A on Tissue Factor and Tissue Factor Pathway Inhibitor Expression and Activity in Endothelial Cells. Arteriosclerosis, Thrombosis, and Vascular Biology (2007). , 27(7), 1645-1650.

[26] Jijon, H. B, Madsen, K. L, Walker, J. W, Allard, B, & Jobin, C. Serum amyloid A activates NF-κB and proinflammatory gene expression in human and murine intestinal epithelial cells. European Journal of Immunology (2005). , 35(3), 718-726.

[27] Baranova, I. N, Bocharov, A. V, Vishnyakova, T. G, Kurlander, R, Chen, Z, Fu, D, Arias, I. M, Csako, G, Patterson, A. P, Eggerman, T. L, & Is, C. D. a Novel Serum Amyloid A (SAA) Receptor Mediating SAA Binding and SAA-induced Signaling in Human and Rodent Cells. Journal of Biological Chemistry (2010). , 285(11), 8492-8506.

[28] Utoguchi, N, Ikeda, K, Saeki, K, Oka, N, Mizuguchi, H, Kubo, K, Nakagawa, S, & Mayumi, T. Ascorbic acid stimulates barrier function of cultured endothelial cell monolayer. J Cell Physiol (1995). , 163(2), 393-9.

[29] Napoli, C, & Ignarro, L. J. Nitric Oxide and Atherosclerosis. Nitric Oxide (2001). , 5(2), 88-97.

[30] Phinikaridou, A, Andia, M. E, Protti, A, Indermuehle, A, Shah, A, Smith, A, Warley, A, & Botnar, R. M. Noninvasive MRI Evaluation of Endothelial Permeability in Murine Atherosclerosis Using an Albumin-Binding Contrast Agent. Circulation (2012).

[31] Gonzalez, M. A, & Selwyn, A. P. Endothelial function, inflammation, and prognosis in cardiovascular disease. The American Journal of Medicine (2003). Supplement 1): 99-106.

[32] Murdaca, G, Colombo, B. M, Cagnati, P, Gulli, R, Spanò, F, & Puppo, F. Endothelial dysfunction in rheumatic autoimmune diseases. Atherosclerosis (2012).

[33] Ludmer, P. L, Selwyn, A. P, Shook, T. L, Wayne, R. R, Mudge, G. H, Alexander, R. W, & Ganz, P. Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. New England Journal of Medicine (1986). , 315(17), 1046-1051.

[34] Thomas, S. R, Witting, P. K, & Drummond, G. R. Redox Control of Endothelial Function and Dysfunction: Molecular Mechanisms and Therapeutic Opportunities Antioxidants & Redox Signaling (2008). , 10(10), 1713-1765.

[35] Bredt, D. S. Endogenous nitric oxide synthesis: Biological functions and pathophysiology. Free Radical Research (1999). , 31(6), 577-596.

[36] Ignarro, L. J, Cirino, G, Casini, A, & Napoli, C. Nitric Oxide as a Signaling Molecule in the Vascular System: An Overview. Journal of Cardiovascular Pharmacology (1999). , 34(6), 879-886.

[37] Anderson, T. J, Gerhard, M. D, Meredith, I. T, Charbonneau, F, Delagrange, D, Creager, M. A, Selwyn, A. P, & Ganz, P. Systemic nature of endothelial dysfunction in atherosclerosis. The American journal of cardiology (1995). Supplement 1): 71B-74B.

[38] Stocker, R, & Keaney, J. F. Role of Oxidative Modifications in Atherosclerosis. Physiological Reviews (2004). , 84(4), 1381-1478.

[39] Maron, B. A, Zhang, Y, White, Y, Chan, K, Handy, S. Y, Mahoney, D. E, Loscalzo, C. E, & Leopold, J. J.A. Aldosterone Inactivates the Endothelin-B Receptor via a Cysteinyl Thiol Redox Switch to Decrease Pulmonary Endothelial Nitric Oxide Levels and Modulate Pulmonary Arterial Hypertension. Circulation (2012).

[40] Van Bussel, B. C. T, Soedamah-muthu, S. S, Henry, R. M. A, Schalkwijk, C. G, Ferreira, I, Chaturvedi, N, Toeller, M, Fuller, J. H, & Stehouwer, C. D. A. Unhealthy dietary patterns associated with inflammation and endothelial dysfunction in type 1 diabetes: The EURODIAB study. Nutrition, Metabolism and Cardiovascular Diseases (2012).

[41] Olsson, N, Siegbahn, A, & Nilsson, G. Serum Amyloid A Induces Chemotaxis of Human Mast Cells by Activating a Pertussis Toxin-Sensitive Signal Transduction Pathway. Biochemical and Biophysical Research Communications (1999). , 254(1), 143-146.

[42] Badolato, R, Johnston, J. A, Wang, J. M, Mcvicar, D, Xu, L. L, Oppenheim, J. J, & Kelvin, D. J. Serum amyloid A induces calcium mobilization and chemotaxis of human monocytes by activating a pertussis toxin-sensitive signaling pathway. The Journal of Immunology (1995). , 155(8), 4004-10.

[43] Lakota, K, Resnik, N, Mrak-poljsak, K, Sodin-semrl, S, & Veranic, P. Colocalization of serum amyloid a with microtubules in human coronary artery endothelial cells. J Biomed Biotechnol (2011).

[44] Witting, P. K, Song, C, Hsu, K, Hua, S, Parry, S. N, Aran, R, Geczy, C, & Freedman, S. B. The acute-phase protein serum amyloid A induces endothelial dysfunction that is inhibited by high-density lipoprotein. Free Radic Biol Med (2011). , 51(7), 1390-8.

[45] Wang, X, Chai, H, Wang, Z, Lin, P. H, Yao, Q, & Chen, C. Serum amyloid A induces endothelial dysfunction in porcine coronary arteries and human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol (2008). H, 2399-408.

[46] Zhao, Y, & Brandish, P. E. DiValentin, M., Schelvis, J.P.M., Babcock, G.T., and Marletta, M.A. Inhibition of Soluble Guanylate Cyclase by ODQ†. Biochemistry (2000). , 39(35), 10848-10854.

[47] Witting, P. K, Rayner, B. S, Wu, B. J, Ellis, N. A, & Stocker, R. Hydrogen Peroxide Promotes Endothelial Dysfunction by Stimulating Multiple Sources of Superoxide Anion Radical Production and Decreasing Nitric Oxide Bioavailability. Cellular Physiology and Biochemistry (2007). , 20(5), 255-268.

[48] Björkman, L, Karlsson, J, Karlsson, A, Rabiet, M, Boulay, J, Fu, F, Bylund, H, & Dahlgren, J. C. Serum amyloid A mediates human neutrophil production of reactive oxygen species through a receptor independent of formyl peptide receptor like-1. Journal of Leukocyte Biology (2008). , 83(2), 245-253.

[49] Cathcart, M. K. Regulation of Superoxide Anion Production by NADPH Oxidase in Monocytes/Macrophages. Arteriosclerosis, Thrombosis, and Vascular Biology (2004). , 24(1), 23-28.

[50] Thomas, S. R, Witting, P. K, & Drummond, G. R. Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. Antioxid Redox Signal (2008). , 10(10), 1713-65.

[51] Brennan, L. A, Steinhorn, R. H, Wedgwood, S, Mata-greenwood, E, Roark, E. A, Russell, J. A, & Black, S. M. Increased Superoxide Generation Is Associated With Pulmonary Hypertension in Fetal Lambs. Circulation Research (2003). , 92(6), 683-691.

[52] Jin, N, & Rhoades, R. A. Activation of tyrosine kinases in H2Oinduced contraction in pulmonary artery. Am J Physiol (1997). Pt 2): H2686-92., 2.

[53] Ülker, S, Mcmaster, D, Mckeown, P. P, & Bayraktutan, U. Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. Cardiovascular Research (2003). , 59(2), 488-500.

[54] Griendling, K. K, & Ushio-fukai, M. NADH/NADPH Oxidase and Vascular Function. Trends in Cardiovascular Medicine (1997). , 7(8), 301-307.

[55] Pieper, G. M, Langenstroer, P, & Siebeneich, W. Diabetic-induced endothelial dysfunction in rat aorta: role of hydroxyl radicals. Cardiovascular Research (1997). , 34(1), 145-156.

[56] Wassmann, S, Wassmann, K, & Nickenig, G. Modulation of Oxidant and Antioxidant Enzyme Expression and Function in Vascular Cells. Hypertension (2004). , 44(4), 381-386.

[57] Ohashi, M, Runge, M. S, Faraci, F. M, & Heistad, D. D. MnSOD Deficiency Increases Endothelial Dysfunction in ApoE-Deficient Mice. Arteriosclerosis, Thrombosis, and Vascular Biology (2006). , 26(10), 2331-2336.

[58] Huang, A, Yang, Y, Yan, M, Kaley, C, Hintze, G, & Sun, T. H. D. Altered MAPK Signaling in Progressive Deterioration of Endothelial Function in Diabetic Mice. Diabetes (2012). , 61(12), 3181-3188.

[59] Shatanawi, A, Romero, M. J, Iddings, J. A, Chandra, S, Umapathy, N. S, Verin, A. D, Caldwell, R. B, & Caldwell, R. W. Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/mitogen-activated protein kinase/arginase pathway. American Journal of Physiology- Cell Physiology (2011). C1181-C1192., 38.

[60] Schulz, E, Anter, E, & Keaney, J. F. Jr. Oxidative stress, antioxidants, and endothelial function. Curr Med Chem (2004). , 11(9), 1093-104.

[61] Zhang, C, Wu, J, Xu, X, Potter, B. J, & Gao, X. Direct relationship between levels of TNF-alpha expression and endothelial dysfunction in reperfusion injury. Basic Res Cardiol (2010). , 105(4), 453-64.

[62] Jousilahti, P, Salomaa, V, Rasi, V, Vahtera, E, & Palosuo, T. The association of c-reactive protein, serum amyloid a and fibrinogen with prevalent coronary heart disease-baseline findings of the PAIS project. Atherosclerosis (2001). , 156(2), 451-456.

[63] Ridker, P. M, Hennekens, C. H, & Buring, J. E. and Rifai, N. C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. New England Journal of Medicine (2000). , 342(12), 836-843.

[64] Erren, M, Reinecke, H, Junker, R, Fobker, M, Schulte, H, Schurek, J. O, Kropf, J, Kerber, S, Breithardt, G, Assmann, G, & Cullen, P. Systemic Inflammatory Parameters in Patients With Atherosclerosis of the Coronary and Peripheral Arteries. Arteriosclerosis, Thrombosis, and Vascular Biology (1999). , 19(10), 2355-2363.

[65] Johnson, B. D, Kip, K. E, Marroquin, O. C, Ridker, P. M, Kelsey, S. F, Shaw, L. J, Pepine, C. J, & Sharaf, B. Bairey Merz, C.N., Sopko, G., Olson, M.B., and Reis, S.E. Serum Amyloid A as a Predictor of Coronary Artery Disease and Cardiovascular Outcome in Women. Circulation (2004). , 109(6), 726-732.

[66] Leinonen, E, Hurt-camejo, E, Wiklund, O, Hultén, L. M, Hiukka, A, & Taskinen, M. R. Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. Atherosclerosis (2003). , 166(2), 387-394.

[67] Haffner, S. M. Agostino Jr, R.D., Saad, M.F., O'Leary, D.H., Savage, P.J., Rewers, M., Selby, J., Bergman, R.N., and Mykkänen, L. Carotid artery atherosclerosis in type-2 diabetic and nondiabetic subjects with and without symptomatic coronary artery disease (The Insulin Resistance Atherosclerosis Study). The American journal of cardiology (2000). , 85(12), 1395-1400.

[68] Connolly, M, Veale, D. J, & Fearon, U. Acute serum amyloid A regulates cytoskeletal rearrangement, cell matrix interactions and promotes cell migration in rheumatoid arthritis. Annals of the Rheumatic Diseases (2011). , 70(7), 1296-1303.

[69] Connolly, M, Marrelli, A, Blades, M, Mccormick, J, Maderna, P, Godson, C, & Mullan, R. FitzGerald, O., Bresnihan, B., Pitzalis, C., Veale, D.J., and Fearon, U. Acute serum amyloid A induces migration, angiogenesis, and inflammation in synovial cells in vitro and in a human rheumatoid arthritis/SCID mouse chimera model. J Immunol (2010). , 184(11), 6427-37.

[70] Lewis, G. F, & Rader, D. J. New Insights Into the Regulation of HDL Metabolism and Reverse Cholesterol Transport. Circulation Research (2005). , 96(12), 1221-1232.

[71] Chiba, T, Chang, M. Y, Wang, S, Wight, T. N, Mcmillen, T. S, Oram, J. F, Vaisar, T, Heinecke, J. W, De Beer, F. C, De Beer, M. C, & Chait, A. Serum Amyloid A Facilitates the Binding of High-Density Lipoprotein From Mice Injected With Lipopolysac-

charide to Vascular Proteoglycans. Arteriosclerosis, Thrombosis, and Vascular Biology (2011). , 31(6), 1326-1332.

[72] Ancsin, J. B, & Kisilevsky, R. The Heparin/Heparan Sulfate-binding Site on Apo-serum Amyloid A. Journal of Biological Chemistry (1999). , 274(11), 7172-7181.

[73] Steinberg, D, Parthasarathy, S, Carew, T. E, Khoo, J. C, & Witztum, J. L. Beyond Cholesterol. New England Journal of Medicine (1989). , 320(14), 915-924.

[74] Wang, X. S, Shao, B, Oda, M. N, Heinecke, J. W, Mahler, S, & Stocker, R. A sensitive and specific ELISA detects methionine sulfoxide-containing apolipoprotein A-I in HDL. Journal of Lipid Research (2009). , 50(3), 586-594.

[75] Coetzee, G. A, Strachan, A. F, Van Der Westhuyzen, D. R, Hoppe, H. C, Jeenah, M. S, & De Beer, F. C. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. Journal of Biological Chemistry (1986). , 261(21), 9644-9651.

[76] Whitehead, A. S, De Beer, M. C, Steel, D. M, Rits, M, Lelias, J. M, Lane, W. S, & De Beer, F. C. Identification of novel members of the serum amyloid A protein superfamily as constitutive apolipoproteins of high density lipoprotein. Journal of Biological Chemistry (1992). , 267(6), 3862-3867.

[77] Van Lenten, B. J, Wagner, A. C, Navab, M, Anantharamaiah, G. M, Hama, S, Reddy, S. T, & Fogelman, A. M. Lipoprotein inflammatory properties and serum amyloid A levels but not cholesterol levels predict lesion area in cholesterol-fed rabbits. Journal of Lipid Research (2007). , 48(11), 2344-2353.

[78] Yamamoto, K, Shiroo, M, & Migita, S. Diverse gene expression for isotypes of murine serum amyloid A protein during acute phase reaction. Science (1986). , 232(4747), 227-9.

[79] Mucchiano, G. I, Jonasson, L, Häggqvist, B, Einarsson, E, & Westermark, P. Apolipoprotein A-I-Derived Amyloid in Atherosclerosis. American Journal of Clinical Pathology (2001). , 115(2), 298-303.

[80] Lewis, K. E, Kirk, E. A, Mcdonald, T. O, Wang, S, Wight, T. N, Brien, O, & Chait, K. D. A. Increase in Serum Amyloid A Evoked by Dietary Cholesterol Is Associated With Increased Atherosclerosis in Mice. Circulation (2004). , 110(5), 540-545.

[81] Plump, A. S, Smith, J. D, Hayek, T, Aalto-setälä, K, Walsh, A, Verstuyft, J. G, Rubin, E. M, & Breslow, J. L. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell (1992). , 71(2), 343-353.

[82] Meir, K. S, & Leitersdorf, E. Atherosclerosis in the Apolipoprotein E-Deficient Mouse. Arteriosclerosis, Thrombosis, and Vascular Biology (2004). , 24(6), 1006-1014.

[83] Letters, J. M, Witting, P. K, Christison, J. K, Eriksson, A. W, Pettersson, K, & Stocker, R. Time-dependent changes to lipids and antioxidants in plasma and aortas of apolipoprotein E knockout mice. Journal of Lipid Research (1999). , 40(6), 1104-1112.

[84] King, V. L, Hatch, N. W, Chan, H, De Beer, W, De Beer, M. C, & Tannock, F. C. L.R. A Murine Model of Obesity With Accelerated Atherosclerosis. Obesity (2009). , 18(1), 35-41.

[85] Ko, K. W. S, Corry, D. B, Brayton, C. F, Paul, A, & Chan, L. Extravascular inflammation does not increase atherosclerosis in apoE-deficient mice. Biochemical and Biophysical Research Communications (2009). , 384(1), 93-99.

[86] Daugherty, A. Mouse Models of Atherosclerosis. The American Journal of the Medical Sciences (2002). , 323(1), 3-10.

[87] Khovidhunkit, W, Kim, M, Memon, S, Shigenaga, R. A, Moser, J. K, Feingold, A. H, & Grunfeld, K. R. C. Thematic review series: The Pathogenesis of Atherosclerosis. Effects of infection and inflammation on lipid and lipoprotein metabolism mechanisms and consequences to the host. Journal of Lipid Research (2004). , 45(7), 1169-1196.

[88] Artl, A, Marsche, G, Lestavel, S, Sattler, W, & Malle, E. Role of Serum Amyloid A During Metabolism of Acute-Phase HDL by Macrophages. Arteriosclerosis, Thrombosis, and Vascular Biology (2000). , 20(3), 763-772.

[89] Benditt, E. P, & Eriksen, N. Amyloid protein SAA is associated with high density lipoprotein from human serum. Proc Natl Acad Sci U S A (1977). , 74(9), 4025-8.

[90] Barter, P. J, Nicholls, S, Rye, K, Anantharamaiah, A, Navab, G. M, & Fogelman, M. A.M. Antiinflammatory Properties of HDL. Circulation Research (2004). , 95(8), 764-772.

[91] Alwaili, K, Bailey, D, Awan, Z, Bailey, S. D, Ruel, I, Hafiane, A, Krimbou, L, Laboissiere, S, & Genest, J. The HDL proteome in acute coronary syndromes shifts to an inflammatory profile. Biochimica et Biophysica Acta (BBA)- Molecular and Cell Biology of Lipids (2012). , 1821(3), 405-415.

[92] Tölle, M, Huang, T, Schuchardt, M, Jankowski, V, Prüfer, N, Jankowski, J, Tietge, U. J. F, Zidek, W, & Van Der Giet, M. High-density lipoprotein loses its anti-inflammatory capacity by accumulation of pro-inflammatory-serum amyloid A. Cardiovascular Research (2012). , 94(1), 154-162.

[93] Weichhart, T, Kopecky, C, Kubicek, M, Haidinger, M, Döller, D, Katholnig, K, Suarna, C, Eller, P, Tölle, M, Gerner, C, Zlabinger, G. J, Van Der Giet, M, Hörl, W. H, Stocker, R, & Säemann, M. D. Serum Amyloid A in Uremic HDL Promotes Inflammation. Journal of the American Society of Nephrology (2012). , 23(5), 934-947.

[94] Kisilevsky, R, & Subrahmanyan, L. Serum amyloid A changes high density lipoprotein's cellular affinity. A clue to serum amyloid A's principal function. Lab Invest (1992). , 66(6), 778-85.

[95] Hayat, S, & Raynes, J. G. Acute phase serum amyloid A protein increases high density lipoprotein binding to human peripheral blood mononuclear cells and an endothelial cell line. Scand J Immunol (2000). , 51(2), 141-6.

[96] Duffy, D, & Rader, D. J. Update on strategies to increase HDL quantity and function. Nat Rev Cardiol (2009). , 6(7), 455-463.

[97] Bailey, D, Jahagirdar, R, Gordon, A, Hafiane, A, Campbell, S, Chatur, S, Wagner, G. S, Hansen, H. C, Chiacchia, F. S, Johansson, J, Krimbou, L, Wong, N. C, & Genest, J. RVX-208: a small molecule that increases apolipoprotein A-I and high-density lipoprotein cholesterol in vitro and in vivo. J Am Coll Cardiol (2010). , 55(23), 2580-9.

[98] Wroblewski, J. M, Jahangiri, A, Ji, A, De Beer, F. C, Van Der Westhuyzen, D. R, & Webb, N. R. Nascent HDL formation by hepatocytes is reduced by the concerted action of serum amyloid A and endothelial lipase. Journal of Lipid Research (2011). , 52(12), 2255-2261.

[99] Kisilevsky, R, & Manley, P. N. Acute-phase serum amyloid A: Perspectives on its physiological and pathological roles. Amyloid (2012). , 19(1), 5-14.

[100] Webb, N. R, De Beer, M. C, Van Der Westhuyzen, D. R, Kindy, M. S, Banka, C. L, Tsukamoto, K, Rader, D. L, & De Beer, F. C. Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice. Journal of Lipid Research (1997). , 38(8), 1583-90.

[101] De Beer, M. C, Webb, N. R, Wroblewski, J. M, Noffsinger, V. P, Rateri, D. L, Ji, A, Van Der Westhuyzen, D. R, & De Beer, F. C. Impact of serum amyloid A on high density lipoprotein composition and levels. Journal of Lipid Research (2010). , 51(11), 3117-3125.

[102] Faty, A, Ferre, P, & Commans, S. The acute phase protein Serum Amyloid A induces lipolysis and inflammation in human adipocytes through distinct pathways. PLoS One (2012). e34031.

[103] De Beer, M. C, Ji, A, Jahangiri, A, Vaughan, A. M, De Beer, F. C, Van Der Westhuyzen, D. R, & Webb, N. R. ATP binding cassette G1-dependent cholesterol efflux during inflammation. Journal of Lipid Research (2011). , 52(2), 345-353.

[104] Lindhorst, E, Young, D, Bagshaw, W, Hyland, M, & Kisilevsky, R. Acute inflammation, acute phase serum amyloid A and cholesterol metabolism in the mouse. Biochimica et Biophysica Acta (BBA)- Protein Structure and Molecular Enzymology (1997). , 1339(1), 143-154.

[105] Mullan, R. H, Mccormick, J, Connolly, M, Bresnihan, B, Veale, D. J, & Fearon, U. A Role for the High-Density Lipoprotein Receptor SR-B1 in Synovial Inflammation via Serum Amyloid-A. The American Journal of Pathology (2010). , 176(4), 1999-2008.

[106] Levels, J. H, Geurts, P, Karlsson, H, Maree, R, Ljunggren, S, Fornander, L, Wehenkel, L, Lindahl, M, Stroes, E. S, Kuivenhoven, J. A, & Meijers, J. C. High-density lipoprotein proteome dynamics in human endotoxemia. Proteome Sci (2011).

[107] Franco, A. G, Sandri, S, & Campa, A. High-density lipoprotein prevents SAA-induced production of TNF-± in THP-1 monocytic cells and peripheral blood mononuclear cells. MemÃ³rias do Instituto Oswaldo Cruz (2011). , 106-986.

[108] Patan, S. Vasculogenesis and angiogenesis. Cancer Treat Res (2004). , 117-3.

[109] Szekanecz, Z, Besenyei, T, Szentpétery, Á, & Koch, A. E. Angiogenesis and vasculogenesis in rheumatoid arthritis. Current Opinion in Rheumatology (2010). BOR. 0b013e328337c95a., 22(3), 299-306.

[110] Tak, P. P, & Bresnihan, B. The pathogenesis and prevention of joint damage in rheumatoid arthritis: Advances from synovial biopsy and tissue analysis. Arthritis & Rheumatism (2000). , 43(12), 2619-2633.

[111] Mullan, R.H, Bresnihan, B, Golden-Mason, L, Markham, T, Hara, O, & Fitz, R. ., and Fearon, U. Acute-phase serum amyloid A stimulation of angiogenesis, leukocyte recruitment, and matrix degradation in rheumatoid arthritis through an NF-κB-dependent signal transduction pathway. Arthritis & Rheumatism 2006. 54 (1): 105-114.

[112] Lee, M, Yoo, S, Cho, S. -A, Suh, C. -S, Kim, P. -G, & Ryu, W. -U. S.H. Serum Amyloid A Binding to Formyl Peptide Receptor-Like 1 Induces Synovial Hyperplasia and Angiogenesis. The Journal of Immunology (2006). , 177(8), 5585-5594.

[113] Schepetkin, I. A, Kirpotina, L. N, Tian, J, Khlebnikov, A. I, Ye, R. D, & Quinn, M. T. Identification of Novel Formyl Peptide Receptor-Like 1 Agonists That Induce Macrophage Tumor Necrosis Factor α Production. Molecular Pharmacology (2008). , 74(2), 392-402.

[114] Neuzil, J, Witting, P. K, Kontush, A, & Headrick, J. P. Role of vitamin E in nuclear factor-kB and nitric oxide signalling. Encyclopedia of Vitamin E, ed. V. Preedy and R. Watson. London: CABI Publishing. (2006).

[115] Stone, K. P, Kastin, A. J, & Pan, W. NFκB is an Unexpected Major Mediator of Interleukin-15 Signaling in Cerebral Endothelia. Cellular Physiology and Biochemistry (2011). , 28(1), 115-124.

[116] Li, W, Li, H, Bocking, A. D, & Challis, J. R. G. Tumor Necrosis Factor Stimulates Matrix Metalloproteinase 9 Secretion from Cultured Human Chorionic Trophoblast Cells Through TNF Receptor 1 Signaling to IKBKB-NFKB and MAPK1/3 Pathway. Biology of Reproduction (2010). , 83(3), 481-487.

[117] González-pacheco, F. R, Deudero, J. J. P, Castellanos, M. C, Castilla, M. A, Álvarez-arroyo, M. V, Yagüe, S, & Caramelo, C. Mechanisms of endothelial response to oxidative aggression: protective role of autologous VEGF and induction of VEGFR2 by

H2O2. American Journal of Physiology- Heart and Circulatory Physiology (2006). HH1401., 1395.

[118] Hollborn, M, Stathopoulos, C, Steffen, A, Wiedemann, P, Kohen, L, & Bringmann, A. Positive Feedback Regulation between MMP-9 and VEGF in Human RPE Cells. Investigative Ophthalmology & Visual Science (2007). , 48(9), 4360-4367.

[119] Shawber, C. J, & Kitajewski, J. Notch function in the vasculature: insights from zebrafish, mouse and man. BioEssays (2004). , 26(3), 225-234.

[120] Funahashi, Y, Shawber, C, Sharma, A, Kanamaru, E, Choi, Y, & Kitajewski, J. Notch modulates VEGF action in endothelial cells by inducing Matrix Metalloprotease activity. Vascular Cell (2011).

[121] Warde, N. Connective tissue diseases: Notch signaling: an important player in SSc fibrosis. Nat Rev Rheumatol (2011). , 7(6), 312-312.

[122] Ishii, H, Nakazawa, M, Yoshino, S, Nakamura, I, Nishioka, H, & Nakajima, K. T. Expression of Notch homologues in the synovium of rheumatoid arthritis and osteoarthritis patients. Rheumatology International (2001). , 21(1), 10-14.

[123] Sassi, N, Laadhar, L, Driss, M, Kallel-sellami, M, Sellami, S, & Makni, S. The role of the Notch pathway in healthy and osteoarthritic articular cartilage: from experimental models to ex vivo studies. Arthritis Research & Therapy (2011).

[124] Ray, A, Kuroki, K, Cook, J. L, Bal, B. S, Kenter, K, Aust, G, & Ray, B. K. Induction of matrix metalloproteinase 1 gene expression is regulated by inflammation-responsive transcription factor SAF-1 in osteoarthritis. Arthritis & Rheumatism (2003). , 48(1), 134-145.

[125] Ray, A, Kumar, D, Shakya, A, Brown, C. R, Cook, J. L, & Ray, B. K. Serum amyloid A-activating factor-1 (SAF-1) transgenic mice are prone to develop a severe form of inflammation-induced arthritis. J Immunol (2004). , 173(7), 4684-91.

[126] Chan, D, Chen, C, Chu, C. -J, Chang, H. -C, Yu, W. -K, Chen, J. -C, Wen, Y. -J, Huang, L. -L, Ku, S. -C, & Liu, C. -H. Y.-C.e.a. Evaluation of Serum Amyloid A as a Biomarker for Gastric Cancer. Annals of Surgical Oncology (2007). , 14(1), 84-93.

[127] Benson, M, Eyanson, S, & Fineberg, N. Serum amyloid A in carcinoma of the lung. Cancer (1986). , 57(9), 1783-7.

[128] Kimura, M, Tomita, Y, Imai, T, Saito, T, Katagiri, A, Ohara-mikami, Y, Matsudo, T, & Takahashi, K. Significance of serum amyloid A on the prognosis in patients with renal cell carcinoma. Cancer. (2001). , 92(8), 2072-5.

[129] Glojnaric, I, Casl, M, Šimic, T, & Lukac, D. J. Serum Amyloid A Protein (SAA) in Colorectal Carcinoma. Clinical Chemistry and Laboratory Medicine. (2005). , 39(2), 129-133.

[130] Zhang, G, Sun, X, Lv, H, Yang, X, & Kang, X. Serum amyloid A: A new potential serum marker correlated with the stage of breast cancer. Oncol Lett. (2012). , 3(4), 940-944.

[131] Firpo, M, Gay, D, Granger, S, & Scaife, C. DiSario, J., Boucher, K., and Mulvihill, S. Improved diagnosis of pancreatic adenocarcinoma using haptoglobin and serum amyloid A in a panel screen. World J Surg. (2009). , 33(4), 716-722.

[132] Yokoi, K, Shih, L, Kobayashi, C. N, Koomen, R, Hawke, J, Li, D, Hamilton, D, Tanley, S, Abbruzzese, R, Coombes, J. L, & Fidler, K. R. I, s.J. Serum amyloid A as a tumor marker in sera of nude mice with orthotopic human pancreatic cancer and in plasma of patients with pancreatic cancer. Int J Oncol, (2005). , 27, 1361-1369.

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