Chapter

Serum Amyloid A and Immunomodulation

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Abstract

Serum amyloid A1 (SAA1), a major isoform of acute-phase SAA, is a well-known precursor of amyloid A (AA) that contributes to secondary amyloidosis with its tissue deposition. Acute-phase SAA is also a biomarker of inflammation. Recent studies have focused on the roles for acute-phase SAA in the regulation of immunity and inflammation. *In vitro* characterization of recombinant human SAA identified its chemotactic and cytokine-like properties, whereas the use of SAA isoform-specific transgenic and knockout mice has led to the discovery of new functions of SAA proteins in host defense and tissue homeostasis. Characterization of SAA-derived peptides has shown that fragments of SAA, generated through proteolysis, are bioactive and may contribute to a growing list of functions related to inflammation. This chapter summarizes recent progress in the studies of acute-phase SAA and its fragments in inflammation and immunomodulation.

Keywords: SAA, inflammation, immunity

1. Introduction

Serum amyloid A (SAA) was identified in early studies as the precursor of amyloid A (AA), the tissue deposit of which causes secondary amyloidosis [1–4]. SAA was also found as one of the major acute-phase proteins that are produced in large quantities by hepatocytes and released to blood circulation in response to trauma, infection, late-stage malignancy and severe stress [5, 6]. Extending from these early findings, increased levels of SAA were found both in plasma and in injured and inflammatory tissues. A large body of literature reports SAA as a biomarker in a variety of diseases ranging from acute inflammation, chronic inflammation, type-2 diabetes, malignancy and postsurgical complications [7–9]. However, the biological functions of SAA remained largely unknown for many years [10] despite efforts in it biochemical characterization, gene cloning of its isoforms, studies of the interactions between SAA and high-density lipoprotein (HDL), and delineation of its regulatory activities in inflammation and immunity. The widespread use of recombinant human SAA proteins has accelerated the characterization of the biological functions of SAA *in vitro*, but at the same time produced data that are not fully compatible with those obtained from *in vivo* studies. In the past decade, mice with genetically altered genes were prepared and their use in a number of diseases models has begun to delineate the pathophysiological functions of SAA *in vivo*. This chapter provides an overview of the studies of SAA that have been published and summarizes recent findings of the immunomodulatory functions of different SAA

proteins. For other functions of SAA, the interested reader is referred to several excellent reviews that have been published recently [9, 11–15].

2. SAA and its role in amyloidosis

SAA is the general name of a family of proteins with high sequence homology but encoded by distinct genes [16]. Both humans and mice have 4 SAA genes, but in human the *SAA3* is a pseudogene that does not express [17]. SAA4 is constitutively expressed in both humans and mice. In contrast, the expression of SAA1, SAA2 and in mice, SAA3, is highly inducible [18]. These SAA proteins are therefore termed acute-phase SAAs based on their induced expression during the acute-phase response [18, 19]. The human SAA genes are located on chromosome 11 while the mouse SAA genes are found in a cluster on chromosome 7 [20, 21].

At the primary sequence level, the human and mouse SAA proteins share high sequence homology (**Figure 1**), suggesting that these proteins may have similar functions although their modes of expression vary. Of note, although mouse SAA3 has an expression profile different from that of SAA1 and SAA2, its sequence is as homologous to human SAA1 as mouse SAA1 and SAA2 (**Figure 1**). The sequence homology suggests that the functions of SAA3, expressed upon induction by inflammatory cues in various mouse tissues, may be similar to those of human SAA1 and SAA2.

Human SAA1 has been widely studied for its functions. SAA1 was first identified as a serum component recognized by antibodies raised against the amyloid fibril protein known as amyloid A (AA). In one of the studies, antisera were prepared against the major nonimmunoglobulin component of secondary amyloidosis. The antisera were able to detect a serum component that was present at much higher levels in more than half of the pathological samples collected from

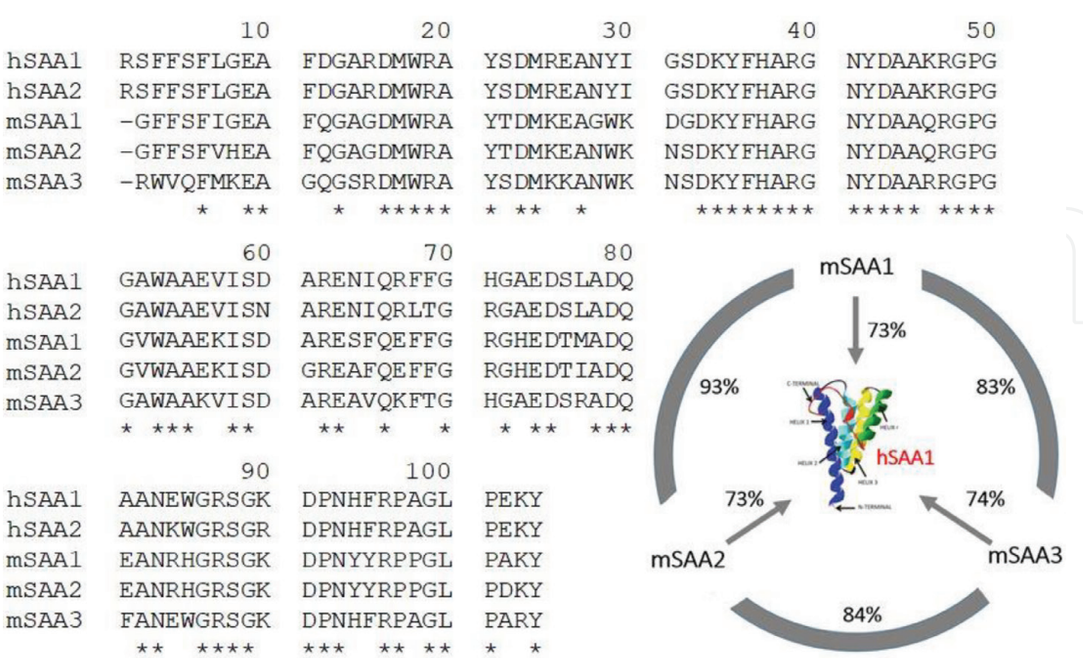


Figure 1.
Comparison of the amino acid sequence of human and mouse inducible SAA proteins. The amino acid sequences of mature SAA protein (without signal peptides) are shown, and identical amino acids are marked with asterisks (*). Inset shows the percent of sequence homology between the 3 inducible mouse SAA proteins and human SAA1.

patients compared to only 7% of normal controls [1]. Husby and Natvig found that the serum component detected by the antisera against AA was larger and its circulation level was increased with age and during pregnancy [2]. The protein immuoprecipitated by the antisera was of low molecular weight with similar but not identical amino acid composition of the AA fibrils [22]. It was thought that AA could be a subunit of the SAA protein [22], which was identified as a cleavage product of SAA.

Amyloidosis develops when insoluble amyloid fibrils accumulate in the extracellular space of the tissues and organs in the body. Patients with chronic inflammatory diseases may develop AA amyloidosis, also termed secondary amyloidosis [23, 24]. SAA as an amyloid protein has the propensity of fibril formation. However, how SAA forms fibril is not fully understood. A number of observations suggest that SAA produced in inflammatory tissues is endocytosed into macrophages [25], where the acidic environment of lysosome promotes fibril formation [26]. The small amount of fibril formed is then exocytosed to the cell surface, prompting a nucleation-dependent incorporation of additional SAA into fibrils [27]. More recent studies have shown that SAA forms stable oligomers at pH of 3.5–4.5, that are resistant to proteolysis and undergo α-helix to β-sheet conversion. The SAA accumulated in lysosomes eventually escape from the cells [28]. Based on these studies, AA fibril formation is a biphasic process [27, 29] that involves an intracellular phase and an extracellular phase. Proteolysis is involved probably in both phases [27, 30]. In the second phase, additional SAA proteins may be recruited with nucleation of AA fibrils, and cleavage of SAA may be a post-fibrillogenic event [31].

Recent delineation of the crystal structure of human SAA1 provides a structural basis for AA amyloidosis [32]. Despite high levels of sequence homology, different SAA isoforms have different propensity in forming AA fibrils. Human SAA1.1 has a high tendency of amyloidogenicity, whereas SAA2.2 found in the CE/J mice did not form amyloid fibrils [33, 34] despite sequence homology as high as 94% with SAA1.1. It was found that the structural determinants for amyloidogenicity reside in the first 10–15 residues of mature SAA protein [35]. In a more recent study, SAA2.2 was found to form small fibrils within a few hours, in contrast to the long lag time of SAA1.1 that was characteristically oligomer-rich [36]. These fibrils exhibited different morphology and the fibrils of SAA1.1 were found to be pathogenic. The results of this study suggest that fibrillation kinetics and prefibrillar oligomers of different SAA isoforms may determine their pathogenicity even though they all possess intrinsic amyloidogenicity.

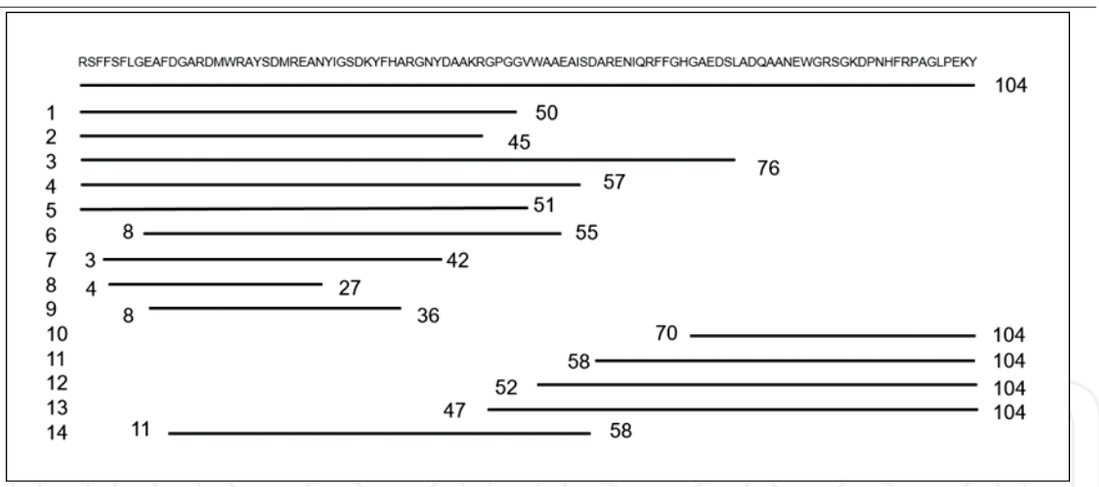
3. Production and characterization of SAA fragments

In AA amyloidosis, the insoluble AA amyloid protein is derived from the proteolytic cleavage of SAA, generating an N-terminal fragment of SAA. In some cases, this AA amyloid protein lacks amino acids at both N- and C-terminus compared to the full-length SAA. One reported study found that the AA fibril protein purified from rheumatoid arthritis patients with secondary amyloidosis contained 2 fragments with residues 1–50 and 1–45 [37]. However, a SAA fragment with residues 1–76 (or 2–76) was most commonly found in amyloid fibrils, such as those from the livers and spleens of patients with familial Mediterranean fever (FMF), tuberculosis, Hodgkin's diseases and bronchiectasis [24, 38].

In patients with rheumatoid arthritis, higher serum levels of metalloproteinases (MMPs)-1, -2, -3 and -9 were detected compared to healthy controls [39, 40], and

the production of these enzymes could be stimulated by SAA [41, 42]. Besides, these MMPs were shown to cleave SAA and AA amyloid protein *in vitro* to produce various sizes of SAA fragments (see **Table 1**). In addition to generating the AA fragments commonly identified in secondary amyloidosis, MMP-1, -2 and -3 cleaved SAA into fragments with residues 1–57, 1–51 and 8–55, respectively [43]. The spanning region (residues 51–57) contains sites that may be cleaved by all three MMPs. In addition, MMP-2 and MMP-3 can also cleave at other residues including residues 7–8 (MMP-2 and -3), 16–17 (MMP-3) and 23–24 (MMP-3). In other species studied, MMP-1 and -3 are able to cleave rabbit SAA3 at residues 50–57, showing conservation between the rabbit SAA3 and human SAA1 [44]. Therefore it was suggested that these MMPs might contribute to the pathogenesis of AA amyloidosis by generating SAA fragments.

In addition to their roles in AA amyloidosis, SAA-derived fragments may have other biological functions. A recent study demonstrated that MMP-9 could rapidly cleave human SAA1 within 30 minutes *in vitro* to produce COOH-terminal fragments, SAA1 (58–104), SAA1 (52–104) and SAA1 (57–104) [46]. These fragments account for 50, 30 and 20% of the total cleaved fragments by MMP-9, respectively. The synthetic peptides of these fragments failed to induce CXCL8 production in human monocytes and diploid fibroblasts, as well as neutrophil chemotaxis; however they potentiated CXCL8-induced neutrophil chemotaxis in a dose-dependent manner via FPR2 [46]. The authors of this report suggested that intact SAA first initiates the inflammatory response and induces the release of MMP-9, which cleaves SAA and modulates the response of SAA by potentiating activities of selected chemokines to prolong the inflammation process. In addition to MMPs,



Numbers	Sources of SAA fragments	References
1–2	An AA amyloidosis patient with rheumatoid arthritis	[37]
3	Patients with FMF, tuberculosis, Hodgkin's disease and bronchiectasis	[38]
4–6	Degradation products of human SAA with MMP1, MMP2, MMP3	[43]
7–10	Recombinant SAA cleaved with cathepsin B and cathepsin L	[45]
11–12	MMP-9 cleaved recombinant SAA1	[46]
13	Chemically synthesized fragment based on bovine serum SAA1 fragment	[47]
14	Recombinant protein based on human SAA1 sequence	[48]

The table lists known SAA fragments and synthetic peptides that have been identified. References are provided on the column to the right.

Table 1.
Generation of SAA fragments.

cathepsins, endosomal and lysosomal proteases, were also shown to cleave SAA and might also be involved in AA amyloidosis. Cathepsin B was shown to cleave SAA at residues 76–77 to produce the most common form of AA found in amyloidosis [49]. Another study also reported that both cathepsin B and L completely cleaved SAA, and cathepsin B could produce 9 AA amyloid-like proteins; however, cathepsin L produced no fragments resembling AA amyloid proteins by cleaving within the N-terminus [45]. All amyloid-like SAA fragments described to date have either an intact N-terminus or one that only lacks 1–2 amino acids. Elastase and cathepsin D that cleave SAA further along the N-terminus can prevent the formation of AA amyloid protein [35, 49, 50].

Accumulating evidence suggests that some of the observed biological functions of SAA, other than those related to amyloidosis, may be attributed to SAA-derived fragments rather than the intact protein. In some of these studies, synthetic peptides based on SAA protein sequence were prepared to verify or identify the potential functions. SAA-derived peptides with IFNγ-inducing capability were found in human rheumatic synovial fluid [51]. An SAA2-derived peptide with chemotactic activity for B lymphocytes was found in cow milk [52]. In a recent study, a fragment of SAA1 (46–112) was found in bovine serum and is equivalent to human SAA1 (47–104). The synthetic peptides of this fragment failed to directly induce chemotaxis and chemokine production (CXCL8 and CCL3) in human neutrophils and monocytes, but it synergized with CXCL8 or CCL3 to induce chemotaxis via FPR2 [47]. Studies were also conducted to examine potential functions of SAA and its peptides in LPS-induced inflammatory response. SAA-derived fragments lacking both N- and C-terminal residues were expressed as recombinant proteins and texted for their activities *in vitro*. Fragments such as one with amino acids 11–58 of human SAA1 exhibited minimal proinflammatory activity but enhanced ability to induce IL-10 expression and to counteract LPS-induced inflammation and lung injury [48]. In a recent study, a peptide consisting amino acids 32–47 of human SAA1 was found to disrupt the binding of SAA1 to LPS, suggesting the involvement of this region of SAA1 in LPS binding [53].

4. The cytokine-like activities of recombinant SAA

Recombinant SAA was used in an early study that identified the SAA protein as a chemoattractant for phagocytes [54]. Xu et al. reported that SAA also induced the migration and adhesion of lymphocytes [55]. These studies were among the first to identify leukocyte-activating activities of the recombinant SAA protein. SAA differs from chemokines as it lacks the characteristic cysteine residues that form disulfide bonds for structural stabilization. It was not until 2014 when the crystal structures of two SAA proteins were solved [32, 56]. The 4-helix bundle structure of the SAA monomers and the propensity of forming multimers [32, 56] are strikingly different from the known structural properties of chemokines [57].

Studies conducted by Patel et al. [58] and Fulaneto et al. [59] revealed cytokine-like activities of SAA for its induction of IL-1β, TNFα, IL-1RA and IL-8. Of note, the study conducted by Patel and coworkers used both the recombinant human SAA (rhSAA) and purified SAA-HDL complex, although they found that the cytokine-inducing activity of the SAA-HDL complex was much lower than that of rhSAA. These studies were followed by reports that SAA in neutrophils could induce IL-8 expression through one of the chemoattractant receptors [60] that also mediates anti-inflammatory activities when stimulated by the eicosanoid lipoxin A4 [61, 62]. In addition to proinflammatory cytokines, rhSAA was found to stimulate monocyte expression of tissue factor [63]. Injection of rhSAA to mice increased G-CSF

production and neutrophil expansion [64]. SAA also induced the expression of immunomodulatory cytokines including selective induction of IL-23 over IL-12 [65] and the induction of IL-33 expression [66]. The transcription factors NF-κB, IRF4 and IRF7 have been implicated in SAA-induced gene expression [66, 67]. In addition, SAA appears to be involved in epigenetic regulation of gene expression [68].

One of the cellular targets of SAA is macrophages, a major source of cytokines and most if not all SAA receptors. Macrophages may be differentiated into M1 or M2 phenotypes. Studies have shown that SAA may influence macrophage differentiation. Anthony et al. examined the effects of SAA *in vitro*, using human blood monocytes from chronic obstructive pulmonary disease patients and healthy controls, and *in vivo* using a mouse model with airway SAA challenge [69]. Their work showed that SAA-rendered human monocytes secrete IL-6 and IL-1β concurrently with the M2 markers CD163 and IL-10. Moreover, these cells responded to subsequent LPS stimulation with markedly higher levels of IL-6 and IL-1β. In the mouse model, SAA induced a $CD11c^{high}$ $CD11b^{high}$ macrophage population in a CSF-1R signaling-dependent manner, with concurrent inhibition of neutrophilic inflammation. Sun et al. investigated the potential effect of SAA on macrophage plasticity, and found that SAA treatment led to increased expression of macrophage M2 markers including IL-10, Ym1, Fizz-1, MRC1, IL-1Rn, and CCL17 [67]. Moreover, SAA enhanced efferocytosis of mouse macrophages. Silencing IRF4 by small interfering RNA abrogated the SAA-induced expression of M2 markers, suggesting a potential role for SAA to alter macrophage phenotype and modulate macrophage functions.

SAA has been identified as an endogenous activator of the NLRP3 inflammasome, which is critical to the process of pro-IL-1β. Niemi et al. reported that SAA provided a signal for pro-IL-1β expression and for inflammasome activation [70]. At least 3 SAA receptors, including TLR2, TLR4 and the ATP receptor P2X7, were involved. Interestingly, inflammasome activation was dependent on the activity of cathepsin B, the expression of which was induced by SAA. Therefore, SAA-induced secretion of cathepsin B could facilitate extracellular processing of SAA and development of AA amyloidosis. Ather et al. showed SAA3 expression in the lungs of mice exposed to mixed Th2/Th17-polarizing allergic sensitization regimens [71]. SAA instillation into the lungs elicited pulmonary neutrophilic inflammation and activation of the NLRP3 inflammasome, thereby promoting IL-1β secretion by dendritic cells and macrophages. SAA administered into the lungs also served as an adjuvant that sensitized mice to inhaled OVA, promoting IL-17 production from restimulated splenocytes and leukocyte influx. Collectively, these findings illustrate a stimulatory function of SAA in the induced expression of IL-1β.

5. SAA receptors

It has long been suspected that the diverse functions of SAA are mediated by cell surface receptors. Studies conducted in the past 20 years have led to the identification of several cell surface receptors for SAA in addition to a number of binding proteins (**Figure 2**). In 1999, Su et al. reported the involvement of formyl peptide receptor 2 (FPR2, also termed FPRL1 [72, 73]), in the chemotactic activity of SAA [74]. FPR2 is a G protein-coupled chemoattractant receptor initially identified as a homolog of human FPR1 with low-affinity binding of formylated peptide [75–77]. The identification of FPR2 as a receptor for SAA is consistent with reports that SAA induces migration of phagocytes and to a lesser extent, lymphocytes [54, 55]. Subsequent studies have shown that a number of biological functions of SAA, ranging from chemotaxis and superoxide generation to induced expression of proinflammatory cytokines and matrix metalloproteases, are mediated through FPR2 [47, 60, 78–85].

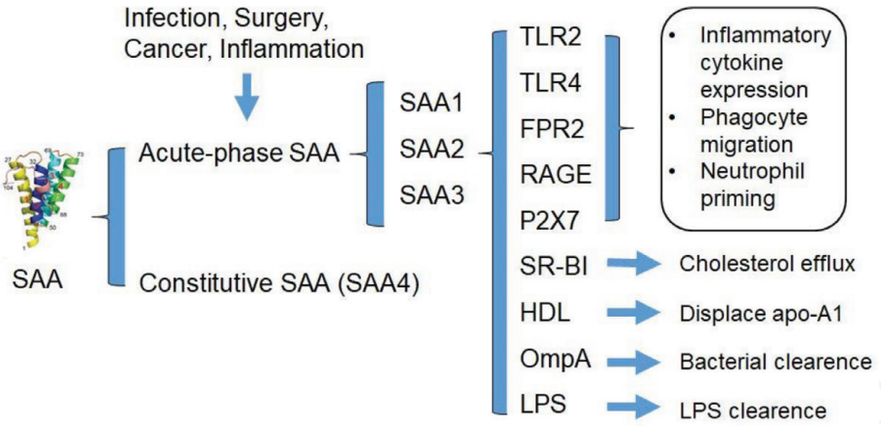


Figure 2.
SAA receptors. The major receptors of human and mouse SAA proteins and their projected functions are listed. Also shown in the figure are selected binding partners of SAA. OmpA, bacterial outer membrane protein a; HDL, high-density lipoprotein. Permission from the publisher was obtained for the use of the crystal structure of SAA1 [32].

The identification of cytokine-like activities of recombinant SAA protein suggests the involvement of receptors that typically mediate phagocyte cytokine production. The finding that SAA selectively induces IL-23 but not IL-12 expression suggests a pattern similar to that of Toll-like receptor (TLR)-mediated cytokine induction [65]. In 2008, two of the TLRs were identified as SAA receptors. TLR2, and more specifically the TLR2-TLR1 heterodimer, was found to mediate SAA-induced NF-κB activation leading to the expression of several proinflammatory cytokines and chemokines [86]. TLR2 is also responsible for SAA-induced neutrophil expansion through upregulation of G-CSF [64]. TLR4 was found to mediate SAA-induced expression of iNOS and activation of the related signaling pathways [87]. Despite differences in primary and high-level structures between SAA and the microbial ligands for these receptors, the two TLRs mediate SAA functions both in transfected cells expressing the receptors and *in vivo* [48, 71, 79, 88–92]. The identification of the two TLRs as SAA receptors illustrates the possible roles for TLRs in detecting host-derived molecules as a mechanism for alerting immune cells upon exposure to environmental stress.

RAGE (receptor for advanced glycation end product) is a multiligand immunoglobulin superfamily cell surface molecule. In a study of AA amyloidosis, RAGE was identified as a receptor of SAA [93]. The expression of RAGE and its interaction with SAA coincide with cell stress, and RAGE has been shown to mediate the NF-κB activating effect of SAA [93, 94]. SAA also binds to soluble RAGE [63]. NF-κB activation induced by SAA interaction with RAGE apparently contributed to the expression of tissue factor in monocytes through MAP kinase activation. Inhibition of RAGE by a RAGE competitor, by soluble RAGE, and by anti-RAGE IgG reduced the SAA-stimulated tissue factor expression [63]. RAGE is also reported to mediate the proinflammatory activity of SAA in uremia-related atherosclerosis, based on a study using the *Apoe*$^{-/-}$ and *Ager*$^{-/-}$ mice [95]. These studies identify RAGE as an endothelial and monocyte-expressed molecule that mediates selected activities of SAA.

Scavenger receptors on macrophages play important roles in the removal of debris during tissue injury and in macrophage transport of lipids. The scavenge-receptor SR-BI has been known for mediating cholesterol efflux, in which SAA plays a role [96]. Two independent studies published in the same year reported the identification of SR-BI as an SAA receptor [97, 98]. Direct binding assays using radiolabeled SAA found its interaction with SR-B1 in cells that express this receptor [97]. SR-BI and its human homolog CLA-I mediate SAA uptake and its downstream signaling, including the activation of ERK and p38 MAPK that leads to IL-8

expression [98]. A more recent study reported that SR-BII, a splice variant of SR-BI, also serves as a SAA receptor for uptake and proinflammatory signaling through MAP kinase signaling [99].

The human P2X7 purinergic receptor is an ionotropic receptor found at high expression levels in immune cells such as macrophages and microglia. Activation of P2X7 receptor by extracellular ATP opens a cation channel, allowing K^+ efflux that is associated with processing of pro-interleukin IL-1β and IL-18. Christenson et al. found that SAA, either recombinant or purified from the plasma of rheumatoid arthritis patients, could suppress apoptosis of human neutrophils, an effect abrogated by antagonizing the nucleotide receptor P2X7 [100]. Niemi et al. reported that the P2X7 receptor plays a role in SAA-mediated activation of NLRP3, thereby explaining the involvement of SAA in the processing of pro-IL-1β [70]. However, a recently published work indicates that in murine J774 and bone marrow-derived macrophages, SAA stimulates IL-1β secretion through a mechanism that depends on NLRP3 expression and caspase-1 activity but not the P2X7 receptor [101].

Collectively, published reports have identified several functional receptors that mediate SAA signaling. It is likely that these receptors and their downstream signaling pathways have substantial cross-talk that together contributes to the diverse immunomodulatory and homeostatic functions of SAA.

Recent studies have shown that recombinant human SAA, which has been widely used in in *vitro* studies throughout the last two decades, has properties that differ from those of native SAA purified from human samples [102–104]. The rhSAA differs from human SAA1 in two sites, with amino acid substitutions at positions 60 and 71 in addition to gaining a methionine at the N-terminus. Since the rhSAA is made by *Escherichia coli* expression, the bacterial contaminants in the preparation may contribute to the observed cytokine-like activity. This is especially a concern because the contaminating bacterial products can activate the two TLRs that are known as the SAA receptors. A careful analysis of published literature found evidence that both support the use of the two TLRs by SAA and detract from the claim. Many of the published studies have included controls for LPS contamination, showing that the SAA protein is necessary for the reported biological functions. A recent study has shown that the bacterial contaminants may not be LPS that acts through TLR4 but lipoproteins that activate TLR2 [105]. The study also showed that adding bacterial lipopeptides into mammalian cell-expressed SAA1 protein could restore the cytokine-like activity that otherwise was missing from the SAA1 protein [105]. It is however unclear how much lipoproteins are carried by the *E. coli*-derived recombinant SAA. The *E. coli* expression system has been widely used in the production of reagents including proinflammatory cytokines such as TNFα and IL-1β, and there were not previous concerns over bacterial product contamination with these cytokines. Whereas the authors attributed the previously reported NLRP3 inflammasome-activating property of SAA to bacterial lipoprotein contaminants in the *E. coli*-derived SAA [105], another recent study demonstrated that SAA purified from human samples was able to stimulate NLRP3 inflammasome activation [101]. Taken together, these findings raise the possibility that bacterial contaminants may modify the biological properties of human SAA1 for a potent cytokine-inducing effect. Exactly how much bacterial contaminant is associated with a recombinant human SAA1 is still unknown, but published studies have shown that *E. coli*-produced SAA proteins can be processed to sufficient purity so they can form crystals [32, 56]. Moreover, CHO cell-derived SAA in the form of secreted Fc fusion protein has been shown to bind to the ectodomain of TLR2 [86]. While the contaminating lipoproteins may contribute to the cytokine-inducing activity through TLR2, these contaminants have not been known to stimulate the G protein-coupled FPR2 that mediates some of the biological activities of SAA [47, 60, 74, 78–85]. Based on available data, it is postulated that some of the observed functions of rhSAA are

attributable to bacterial contaminants. *In vivo* studies conducted in various models of diseases are therefore important for confirming the biological functions of SAA under physiologically relevant conditions.

6. Immunomodulatory functions of SAA in disease models

Since most of the early studies were conducted using cell lines and isolated primary cells such as monocytes and neutrophils, these experimental findings are now examined in an *in vivo* setting. An early model created for the *in vivo* studies of SAA employed adenoviral expression of human SAA1, raising the circulatory levels of human SAA1 in the infected mice [106]. This approach was used in studies of the involvement of SAA1 in lipid metabolism [106] and fibril formation [107]. In a more recent study, the same group that created the adenoviral approach found a role for SAA3 in atherosclerosis [108].

Transgenic expression of human SAA1 in mice is another approach used in studies of the *in vivo* functions of SAA. Ji et al. reported transgenic expression of human SAA1 in mouse liver [89]. These mice exhibited more severe liver injury, increased hepatocyte apoptosis, and higher levels of hepatic enzymes than in their wildtype controls. After induction of hepatitis, liver infiltration of $CD4^+$ T cells and macrophages was also increased more in the transgenic mice than in wildtype mice, along with elevated expression of several chemokines. The aggravated liver injury, increased hepatocyte apoptosis and elevated levels of hepatic enzymes in the transgenic mice were eased with the use of a TLR2 antagonist, suggesting that TLR2 mediates the effects of the transgenic SAA1. In a more recent study, Cheng et al. placed the human SAA1 under an inducible promoter of SR-A receptor, generating transgenic mice with elevated local production of SAA1 upon inflammatory stimulation [53]. The transgenic SAA1 was most abundant in mouse lungs and protected mice against acute lung injury caused by LPS administration and cecal ligation and puncture (CLP). Transgenic expression of SAA1 did not protect mice against acute lung injury induced by intratracheal instillation of TNFα. Binding studies showed that human SAA1, purified from either *E. coli* or transfected HEK293 cells, bound to LPS and formed a complex that promoted LPS clearance by macrophages. As a result, serum endotoxin concentration was significantly reduced in the transgenic mice than in their wildtype controls that went through the CLP procedure. Of note, injection of a SAA1-derived peptide that disrupted LPS-SAA1 interaction diminished the endotoxin-lowering effect in the SAA1 transgenic mice and increased serum endotoxin level in wildtype mice after CLP [53]. These findings suggest a mechanism by which acute-phase SAA protects host against bacterial infection-induced injury.

SAA gene knockout mice were generate to examine the physiological functions of the individual SAA proteins. After observing SAA1 and SAA2 expression in intestinal epithelial cells and conforming their cell-protecting effect in epithelial cell line co-cultured with *E. coli*, Eckhardt et al. examined the effect of Saa1/2 double knockout (DKO) in dextran sodium sulfate (DSS) induced colitis model [109]. They found that that epithelial expression of SAA1 and SAA2 protected colonic epithelium against bacterial infection. A more recent study using Saa3 gene knockout mice found that SAA3 is the predominant isoform of inducible SAA proteins in colonic epithelium following chemical injury [92]. Compared to wildtype mice, $Saa3^{-/-}$ mice exposed to DSS showed more severe damage to the colonic epithelial structure, significantly reduced expression of the anti-microbial peptides Reg3β and Reg3γ, and reduced lifespan of afflicted mice if not treated. Administration of exogenous SAA3 protein or adoptive transfer of SAA3-treated neutrophils partially ameliorated symptoms of DSS-induced colitis in part due to SAA3-induced

neutrophil expression of IL-22, a cytokine with epithelia-protection function [110]. Together, these results suggest that epithelial expression of SAA1 and SAA2 in healthy mice may be important for homeostasis of gut functions including host defense, whereas inducible expression of SAA3 serves to combat acute injury to the colonic epithelium.

A role for SAA as a mediator of local immune response has been reported recently. In a study of segmented filamentous bacteria (SFB) for its involvement in mucosal defenses and autoimmune diseases through RORγ^{+} Th17 cells, Sano et al. found that direct contact of SFB with epithelium in the ileum could induce SAA1 and SAA2 expression and promote local IL-17A expression in RORγ$^{(+)}$ T cells. The mechanisms involved an IL-23R/IL-22 circuit and the participation of type 3 innate lymphoid cells (ILC3) that secretes IL-22 [111]. Likewise, Atarashi et al. investigated a group of intestinal microbes for their ability to induce Th17 response, and found that SFB could stimulate intestinal epithelial cells to generate SAA and ROS, creating an amplification loop for sustained production of SAA by both epithelial cells and myeloid cells that led to local Th17 response [112]. These findings provide direct evidence for the contribution of epithelial SAA to intestinal homeostasis in an environment where host interaction with gut microbiota influences the health states of individuals.

In addition to studies of the *in vivo* functions of SAA in innate immunity and inflammation, mice with genetically altered SAA genes were used in the investigation of these acute-phase proteins in animal models of atherosclerosis, osteoclast activation, adipogenesis, and neurodegenerative disorders such as Alzheimer's disease. Ahlin et al. generated transgenic mouse model expressing human SAA1 in the adipose tissue, and used the hSAA1$^{+/-}$ mice in studies of the effect of SAA1 on glucose metabolism and insulin resistance [114]. They found no evidence that adipose tissue-derived hSAA1 could influence the development of insulin resistance or obesity-related inflammation. The potential involvement of SAA in atherogenesis was investigated using the Saa1/2 DKO mice in the *Apoe*$^{-/-}$ background [115]. Surprisingly, the absence of *Saa1.1* and *Saa2.1* did not affect atherosclerotic lesion in the ApoE-deficient mice that were fed with Western diets. It was later reported that SAA3, instead of SAA1/2, is pro-atherogenic based on experiments using adeno-associated virus for overexpression of SAA3 and antisense oligonucleotide-mediated suppression of Saa3 expression [108]. Using SAA3 KO mice, Liu et al. reported elevated Tau phosphorylation (hyperphosphorylation) compared to wildtype mice upon systemic LPS administration. Overexpression of SAA by intracerebral injection attenuated tau hyperphosphorylation in the brain, suggesting that SAA3 may be neuroprotective in the mouse AD model [116].

Several studies of the *in vivo* functions of SAA were conducted in wildtype mice. De Santo et al. reported that systemic SAA1 plays a role in the regulation of neutrophil plasticity through induction of the anti-inflammatory IL-10 and promotion of the interaction of invariant natural killer T cells (iNKT cells) with neutrophils. As a result, SAA1 indirectly limits the suppressive activity by diminishing IL-10 production and enhancing IL-12 production [113].

Collectively, results from the studies of SAA proteins in mice identified important functions of SAA that were previous unknown from *in vitro* studies. There are other functions revealed from the *in vivo* studies using genetically altered mice that are consistent with the *in vitro* findings. For example, the ability of SAA to interact with Gram-negative bacterial wall components [117] is consistent with the *in vivo* findings that SAA1 protects mice against LPS- and CLP-induced acute lung injury [53]. The *in vivo* findings strongly suggest that acute-phase SAA protects host against environmental insults such as chemical-induced intestinal epithelial injury and bacterial infection. Four of the animal models used in studies of SAA are

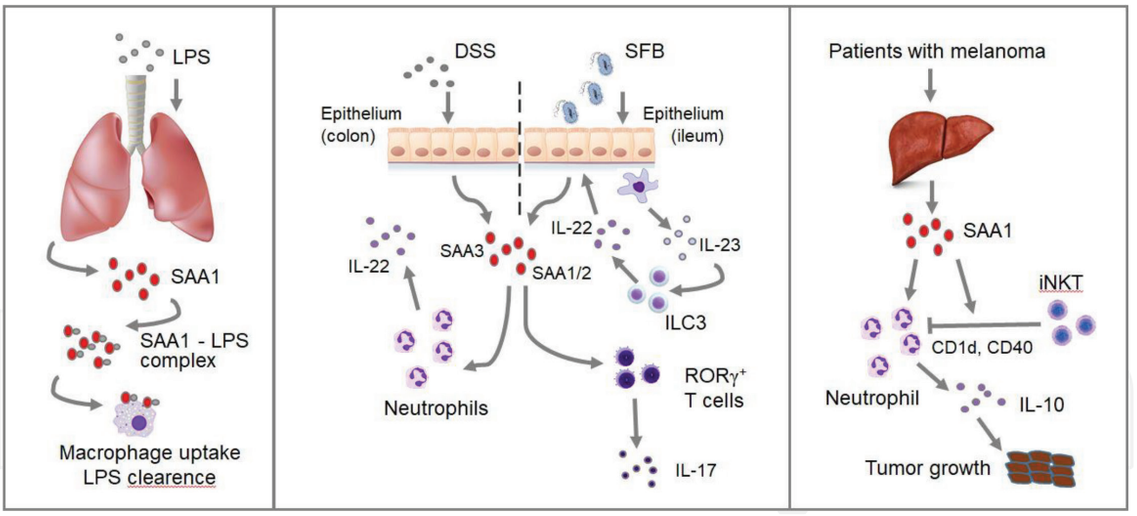


Figure 3.
Immunomodulatory functions of SAA in selected mouse models. Left: transgenic expression of human SAA1 in the lung tissue protects mice against LPS-induced acute lung injury [53]. The protection is conferred in part through SAA binding to LPS, forming a complex that promotes LPS clearance by macrophages. Middle: SAA1 and SAA2 expressed in epithelium of the ileum serves as a mediator of segmented filamentous bacteria-induced local Th17 response [111, 112], contributing to homeostasis of the microenvironment in the intestine [109]. In response to acute injury such as dextran sodium sulfate (DSS) treatment, SAA3 is induced in mouse colonic epithelium and serves as an inducer for neutrophil IL-22 expression [92]. Right: SAA1-producing melanomas induce neutrophil secretion of IL-10 for its suppressive effect. SAA1 also promotes neutrophil interaction with invariant natural killer T (iNKT) cells, thereby limiting IL-10 production but enhancing IL-12 production [113]. This mechanism may be explored to reduce the immunosuppressive neutrophils and restore tumor-specific immunity.

summarized in **Figure 3**. Due to page limitation, *in vivo* studies on SAA functions other than those related to immunomodulation are not discussed in this chapter.

7. Conclusion remarks

SAA has emerged from a precursor of AA to a modulator of immunity and inflammation. Several developments, including the ability to express recombinant SAA proteins, the generation of genetically altered mice expressing SAA transgenes or deletion of a specific SAA gene, and the availability of crystal structures of SAA proteins, have helped to advance our understanding of SAA for its functions in host defense, lipid metabolism, adipogenesis, and neuroprotection. In coming years, studies will likely focus on the comparison of SAA functions *in vitro* to those identified *in vivo,* and on the possible modifications and proteolytic processing of newly synthesized SAA in order to address several questions that remain unanswered today. A better understanding of SAA for its biological functions is expected to benefit human health through development of new diagnostic approaches and therapies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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