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](image-url)

*(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.)*
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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 immunoprecipitated 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 tested 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<sup>high</sup>CD11b<sup>high</sup>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-1Rrn, 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].
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<sup>−/−</sup> and Ager<sup>−/−</sup> 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 scavenger-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-B1 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. Immuno-modulatory 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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References

[1] Levin M, Pras M, Franklin EC. Immunologic studies of the major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. The Journal of Experimental Medicine. 1973;138(2):373-380

[2] Husby G, Natvig JB. A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. The Journal of Clinical Investigation. 1974;53(4):1054-1061

[3] Rosenthal CJ, Franklin EC. Variation with age and disease of an amyloid A protein-related serum component. The Journal of Clinical Investigation. 1975;55(4):746-753

[4] Rosenthal CJ, Franklin EC, Frangione B, Greenspan J. Isolation and partial characterization of SAA-an amyloid-related protein from human serum. Journal of Immunology. 1976;116(5):1415-1418

[5] Kushner I. The phenomenon of the acute phase response. Annals of the New York Academy of Sciences. 1982;389:39-48

[6] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. The New England Journal of Medicine. 1999;340(6):448-454

[7] Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. Current Opinion in Hematology. 2000;7(1):64-69

[8] Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. Journal of the American Society of Nephrology. 2005;16(Suppl 1):S78-S82

[9] Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: An acute-phase protein involved in tumour pathogenesis. Cellular and Molecular Life Sciences. 2009;66(1):9-26

[10] Kisilevsky R. Serum amyloid A (SAA), a protein without a function: Some suggestions with reference to cholesterol metabolism. Medical Hypotheses. 1991;35(4):337-341

[11] Getz GS, Reardon CA. SAA, HDL biogenesis, and inflammation. Journal of Lipid Research. 2008;49(2):269-270

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

[13] De Buck M, Gouwy M, Wang JM, Van Snick J, Proost P, Struyf S, et al. The cytokine-serum amyloid A-chemokine network. Cytokine & Growth Factor Reviews. 2016;30:55-69. DOI: 10.1016/j.cytogfr.2015.12.010

[14] Sun L, Ye RD. Serum amyloid A1: Structure, function and gene polymorphism. Gene. 2016;583(1):48-57. DOI: 10.1016/j.gene.2016.02.044

[15] Sack GH Jr. Serum amyloid A—A review. Molecular Medicine. 2018;24(1):46. DOI: 10.1186/s10020-018-0047-0

[16] Uhlar CM, Burgess CJ, Sharp PM, Whitehead AS. Evolution of the serum amyloid A (SAA) protein superfamily. Genomics. 1994;19(2):228-235

[17] Kluve-Beckerman B, Drumm ML, Benson MD. Nonexpression of the human serum amyloid A three (SAA3) gene. DNA and Cell Biology. 1991;10(9):651-661
[18] Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. European Journal of Biochemistry. 1999;265(2):501-523

[19] Kushner I, Rzewnicki D. Acute phase response. In: Gallin JI, Snyderman R, editors. Inflammation: Basic Principles and Clinical Correlates. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999. pp. 317-329

[20] Butler A, Whitehead AS. Mapping of the mouse serum amyloid A gene cluster by long-range polymerase chain reaction. Immunogenetics. 1996;44(6):468-474

[21] Mori M, Tian G, Ishikawa A, Higuchi K. Diversity and complexity of the mouse Saa1 and Saa2 genes. Experimental Animals. 2014;63(1):99-106

[22] Anders RF, Natvig JB, Michaelsen TE, Husby G. Isolation and characterization of amyloid-related serum protein SAA as a low molecular weight protein. Scandinavian Journal of Immunology. 1975;4(4):397-401

[23] Pinney JH, Hawkins PN. Amyloidosis. Annals of Clinical Biochemistry. 2012;49(Pt 3):229-241. DOI: 10.1258/acb.2011.011225

[24] Westermark GT, Fändrich M, Westermark M. AA amyloidosis: Pathogenesis and targeted therapy. Annual Review of Pathology: Mechanisms of Disease. 2015;10(1):321-344. DOI: 10.1146/annurev-pathol-020712-163913

[25] Chan SL, Chronopoulos S, Murray J, Laird DW, AliKhan Z. Selective localization of murine ApoSAA(1)/SAA(2) in endosomes-lysosomes in activated macrophages and their degradation products. Amyloid-International Journal of Experimental and Clinical Investigation. 1997;4(1):40-48. DOI: 10.3109/13506129708995267

[26] Kluve-Beckerman B, Manaloor J, Liepnieks JJ. Binding, trafficking and accumulation of serum amyloid A in peritoneal macrophages. Scandinavian Journal of Immunology. 2001;53(4):393-400

[27] Magy N, Benson MD, Liepnieks JJ, Kluve-Beckerman B. Cellular events associated with the initial phase of AA amyloidogenesis: Insights from a human monocyte model. Amyloid. 2007;14(1):51-63. DOI: 10.1080/13506120601116575

[28] Jayaraman S, Gantz DL, Haupt C, Gursky O. Serum amyloid A forms stable oligomers that disrupt vesicles at lysosomal pH and contribute to the pathogenesis of reactive amyloidosis. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(32):E6507-E6515. DOI: 10.1073/pnas.1707120114

[29] Sipe JD, McAdam KP, Uchino F. Biochemical evidence for the biphasic development of experimental amyloidosis. Laboratory Investigation. 1978;38(1):110-114

[30] Phipps-Yonas H, Pinard G, Ali-Khan Z. Humoral proinflammatory cytokine and SAA generation profiles and spatio-temporal relationship between SAA and lysosomal cathepsin B and D in murine splenic monocytoid cells during AA amyloidosis. Scandinavian Journal of Immunology. 2004;59(2):168-176

[31] Kisilevsky R, Narindrasorasesak S, Tape C, Tan R, Boudreau L. During Aa-Amyloidogenesis is proteolytic attack on serum amyloid-a a pre-fibrillogenic or post-fibrillogenic event. Amyloid-International Journal of Experimental and Clinical Investigation. 1994;1(3):174-183. DOI: 10.3109/13506129409148449
[32] Lu J, Yu Y, Zhu I, Cheng Y, Sun PD. Structural mechanism of serum amyloid A-mediated inflammatory amyloidosis. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(14):5189-5194

[33] de Beer MC, de Beer FC, McCubbin WD, Kay CM, Kindy MS. Structural prerequisites for serum amyloid A fibril formation. The Journal of Biological Chemistry. 1993;268(27):20606-20612

[34] Sipe JD, Carreras I, Gonnerman WA, Cathcart ES, Debeer MC, Debeer FC. Characterization of the inbred Ce/J mouse strain as amyloid resistant. American Journal of Pathology. 1993;143(5):1480-1485

[35] Westermark GT, Engstrom U, Westermark P. The N-terminal segment of protein AA determines its fibrillogenic property. Biochemical and Biophysical Research Communications. 1992;182(1):27-33

[36] Srinivasan S, Patke S, Wang Y, Ye Z, Litt J, Srivastava SK, et al. Pathogenic serum amyloid A 1.1 shows a long oligomer-rich fibrillation lag phase contrary to the highly amyloidogenic non-pathogenic SAA2.2. The Journal of Biological Chemistry. 2013;288(4):2744-2755. DOI: 10.1074/jbc.M112.394155

[37] Westermark GT, Westermark P, Sletten K. Amyloid fibril protein AA. Characterization of uncommon subspecies from a patient with rheumatoid arthritis. Laboratory Investigation. 1987;57(1):57-64

[38] Levin M, Franklin EC, Frangiore B, Pras M. The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils. The Journal of Clinical Investigation. 1972;51(10):2773-2776. DOI: 10.1172/JCI107098

[39] Keyszer G, Lambiri I, Keysser M, Keysser C, Nagel R, Burmester GR, et al. Matrix metalloproteinases, but not cathepsins B, H, and L or their inhibitors in peripheral blood of patients with rheumatoid arthritis are potentially useful markers of disease activity. Zeitschrift für Rheumatologie. 1998;57(6):392-398

[40] Keyszer G, Lambiri I, Nagel R, Keysser C, Keysser M, Gronmica-Ihle E, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. The Journal of Rheumatology. 1999;26(2):251-258

[41] Migita K, Kawabe Y, Tominaga M, Origuchi T, Aoyagi T, Eguchi K. Serum amyloid A protein induces production of matrix metalloproteinases by human synovial fibroblasts. Laboratory Investigation. 1998;78(5):535-539

[42] Mullan RH, Bresnihan B, Golden-Mason L, Markham T, O’Hara R, Fitz Gerald O, et al. Acute-phase serum amyloid A stimulation of angiogenesis, leukocyte recruitment, and matrix degradation in rheumatoid arthritis through an NF-kappa B-dependent signal transduction pathway. Arthritis and Rheumatism. 2006;54(1):105-114

[43] Stix B, Kahne T, Sletten K, Raynes J, Roessner A, Rocken C. Proteolysis of AA amyloid fibril proteins by matrix metalloproteinases-1, -2, and -3. The American Journal of Pathology. 2001;159(2):561-570. DOI: 10.1016/S0002-9440(10)61727-0

[44] Mitchell TI, Jeffrey JJ, Palmiter RD, Brinckerhoff CE. The acute phase reactant serum amyloid A (SAA3) is a novel substrate for degradation by the metalloproteinases collagenase and
stromelysin. Biochimica et Biophysica Acta. 1993;1156(3):245-254

[45] Röcken C, Menard R, Bühling F, Vöckler S, Raynes J, Stix B, et al. Proteolysis of serum amyloid A and AA amyloid proteins by cysteine proteases: Cathepsin B generates AA amyloid proteins and cathepsin L may prevent their formation. Annals of the Rheumatic Diseases. 2005;64(6):808

[46] Gouwy M, De Buck M, Abouelasrar Salama S, Vandooren J, Knoops S, Pörtner N, et al. Matrix metalloproteinase-9-generated COOH-, but not NH(2)-terminal fragments of serum amyloid A1 retain potentiating activity in neutrophil migration to CXCL8, with loss of direct chemotactic and cytokine-inducing capacity. Frontiers in Immunology. 2018;9:1081. DOI: 10.3389/fimmu.2018.01081

[47] De Buck M, Gouwy M, Berghmans N, Opdenakker G, Proost P, Struyf S, et al. COOH-terminal SAA1 peptides fail to induce chemokines but synergize with CXCL8 and CCL3 to recruit leukocytes via FPR2. Blood. 2018;131(4):439-449. DOI: 10.1182/blood-2017-06-788554

[48] Zhou H, Chen M, Zhang G, Ye RD. Suppression of lipopolysaccharide-induced inflammatory response by fragments from serum amyloid A. Journal of Immunology. 2017;199(3):1105-1112. DOI: 10.4049/jimmunol.1700470

[49] Yamada T, Liepnieks JJ, Kluve-Beckerman B, Benson MD. Cathepsin B generates the most common form of amyloid A (76 residues) as a degradation product from serum amyloid A. Scandinavian Journal of Immunology. 1995;41(1):94-97

[50] Yamada T, Kluve-Beckerman B, Liepnieks JJ, Benson MD. In vitro degradation of serum amyloid A by cathepsin D and other acid proteases: Possible protection against amyloid fibril formation. Scandinavian Journal of Immunology. 1995;41(6):570-574

[51] Yavin EJ, Preciado-Patt L, Rosen O, Yaron M, Suessmuth RD, Levartowsky D, et al. Serum amyloid A-derived peptides, present in human rheumatic synovial fluids, induce the secretion of interferon-gamma by human CD(4) (+) T-lymphocytes. FEBS Letters. 2000;472(2-3):259-262

[52] de Jesus Rodriguez B, Chevaleyre C, Henry G, Molle D, Virlogeux-Payant I, Berri M, et al. Identification in milk of a serum amyloid A peptide chemoattractant for B lymphoblasts. BMC Immunology. 2009;10:4. DOI: 10.1186/1471-2172-10-4

[53] Cheng N, Liang Y, Du X, Ye RD. Serum amyloid A promotes LPS clearance and suppresses LPS-induced inflammation and tissue injury. EMBO Reports. 2018;19(10). pii:e45517. DOI: 10.15252/embr.201745517

[54] Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, Bausserman LL, et al. Serum amyloid A is a chemoattractant: Induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. The Journal of Experimental Medicine. 1994;180(1):203-209

[55] Xu L, Badolato R, Murphy WJ, Longo DL, Anver M, Hale S, et al. A novel biologic function of serum amyloid A. Induction of T lymphocyte migration and adhesion. Journal of Immunology. 1995;155(3):1184-1190

[56] Derebe MG, Zlatkov CM, Gattu S, Ruhn KA, Vaishnava S, Diehl GE, et al. Serum amyloid A is a retinol binding protein that transports retinol during bacterial infection. eLife. 2014;3:e03206
Serum Amyloid A and Immunomodulation
DOI: http://dx.doi.org/10.5772/intechopen.81617

[57] Clore GM, Gronenborn AM. Three-dimensional structures of alpha and beta chemokines. The FASEB Journal. 1995;9(1):57-62

[58] Patel H, Fellowes R, Coade S, Woo P. Human serum amyloid A has cytokine-like properties. Scandinavian Journal of Immunology. 1998;48(4):410-418

[59] Furlaneto CJ, Campa A. A novel function of serum amyloid A: A potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. Biochemical and Biophysical Research Communications. 2000;268(2):405-408

[60] He R, Sang H, Ye RD. Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. Blood. 2003;101(4):1572-1581

[61] Sodin-Semrl S, Spagnolo A, Mikus R, Barbaro B, Varga J, Fiore S. Opposing regulation of interleukin-8 and NF-kappaB responses by lipoxin A4 and serum amyloid A via the common lipoxin A receptor. International Journal of Immunopharmacology. 2004;17(2):145-156

[62] Bozinovski S, Uddin M, Vlahos R, Thompson M, McQualter JL, Merritt AS, et al. Serum amyloid A opposes lipoxin A(4) to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(3):935-940. DOI: 10.1073/pnas.1109382109

[63] Cai H, Song C, Endoh I, Goyette J, Jessup W, Freedman SB, et al. Serum amyloid A induces monocyte tissue factor. Journal of Immunology. 2007;178(3):1852-1860

[64] He RL, Zhou J, Hanson CZ, Chen J, Cheng N, Ye RD. Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. Blood. 2009;113(2):429-437. DOI: 10.1182/blood-2008-03-139923

[65] He R, Shepard LW, Chen J, Pan ZK, Ye RD. Serum amyloid A is an endogenous ligand that differentially induces IL-12 and IL-23. Journal of Immunology. 2006;177(6):4072-4079

[66] Sun L, Zhu Z, Cheng N, Ye RD. Serum amyloid A induces interleukin-33 expression through an IRF7-dependent pathway. European Journal of Immunology. 2014;44(7):2153-2164

[67] Sun L, Zhou H, Zhu Z, Yan Q, Wang L, Liang Q, et al. Ex vivo and in vitro effect of serum amyloid A in the induction of macrophage m2 markers and efferocytosis of apoptotic neutrophils. Journal of Immunology. 2015;194(10):4891-4900. DOI: 10.4049/jimmunol.1402164

[68] Yan Q, Sun L, Zhu Z, Wang L, Li S, Ye RD. Jmjd3-mediated epigenetic regulation of inflammatory cytokine gene expression in serum amyloid A-stimulated macrophages. Cellular Signalling. 2014;26(9):1783-1791

[69] Anthony D, McQualter JL, Bishara M, Lim EX, Yatmaz S, Seow HJ, et al. SAA drives proinflammatory heterotypic macrophage differentiation in the lung via CSF-1R-dependent signaling. The FASEB Journal. 2014;28(9):3867-3877. DOI: 10.1096/fj.14-250332

[70] Niemi K, Teirila L, Lappalainen J, Rajamaki K, Baumann MH, Oorni K, et al. Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a cathepsin B-sensitive pathway. Journal of Immunology. 2011;186(11):6119-6128
[71] Ather JL, Ckless K, Martin R, Foley KL, Suratt BT, Boyson JE, et al. Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. Journal of Immunology. 2011;187(1):64-73. DOI: 10.4049/jimmunol.1100500

[72] Migeotte I, Communi D, Parmentier M. Formyl peptide receptors: A promiscuous subfamily of G protein-coupled receptors controlling immune responses. Cytokine & Growth Factor Reviews. 2006;17(6):501-519

[73] Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, et al. International union of basic and clinical pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. Pharmacological Reviews. 2009;61(2):119-161. DOI: 10.1124/pr.109.001578

[74] Su SB, Gong W, Gao JL, Shen W, Murphy PM, Oppenheim JJ, et al. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. The Journal of Experimental Medicine. 1999;189(2):395-402

[75] Ye RD, Cavanagh SL, Quehenberger O, Prossnitz ER, Cochrane CG. Isolation of a cDNA that encodes a novel granulocyte N-formyl peptide receptor. Biochemical and Biophysical Research Communications. 1992;184(2):582-589

[76] Murphy PM, Ozcelik T, Kenney RT, Tiffany HL, Mcdermott D, Francke U. A structural homolog of the N-formyl peptide receptor-characterization and chromosome mapping of a peptide chemotactant receptor family. Journal of Biological Chemistry. 1992;267(11):7637-7643

[77] Lu B, Gerard NP, Eddy RL, Shows TB, Gerard C. Mapping of genes for the human-C5a receptor (C5ar), human Fmlp receptor (Fpr), and 2 Fmlp receptor homolog orphan receptors (Fprh1, Fprh2) to Chromosome-19. Genomics. 1992;13(2):437-440

[78] Bjorkman L, Karlsson J, Karlsson A, Rabiet MJ, Boulay F, Fu H, et al. 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. DOI: 10.1189/jlb.0607-408

[79] Chen M, Zhou H, Cheng N, Qian F, Ye RD. Serum amyloid A1 isoforms display different efficacy at Toll-like receptor 2 and formyl peptide receptor 2. Immunobiology. 2014;219(12):916-923

[80] Lee HY, Kim MK, Park KS, Bae YH, Yun J, Park JI, et al. Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochemical and Biophysical Research Communications. 2005;330(3):989-998

[81] Lee HY, Kim SD, Baek SH, Choi JH, Bae YS. Role of formyl peptide receptor 2 on the serum amyloid A-induced macrophage foam cell formation. Biochemical and Biophysical Research Communications. 2013;433(2):255-259

[82] Lee HY, Kim SD, Shim JW, Lee SY, Lee H, Cho KH, et al. Serum amyloid A induces CCL2 production via formyl peptide receptor-like-1-mediated signaling in human monocytes. Journal of Immunology. 2008;181(6):4332-4339

[83] Lee MS, Yoo SA, Cho CS, Suh PG, Kim WU, Ryu SH. Serum amyloid A binding to formyl peptide receptor-like 1 induces synovial hyperplasia and angiogenesis. Journal of Immunology. 2006;177(8):5585-5594

[84] O’Hara R, Murphy EP, Whitehead AS, Fitz Gerald O, Bresnihan B. Local
expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. Arthritis and Rheumatism. 2004;50(6):1788-1799

[85] Ren SW, Qi X, Jia CK, Wang YQ. Serum amyloid A and pairing formyl peptide receptor 2 are expressed in corneas and involved in inflammation-mediated neovascularization. International Journal of Ophthalmology. 2014;7(2):187-193

[86] Cheng N, He R, Tian J, Ye PP, Ye RD. Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. Journal of Immunology. 2008;181(1):22-26

[87] Sandri S, Rodriguez D, Gomes E, Monteiro HP, Russo M, Campa A. Is serum amyloid A an endogenous TLR4 agonist? Journal of Leukocyte Biology. 2008;83:1174-1180

[88] Hansen MT, Forst B, Cremers N, Quagliata L, Ambartsumian N, Grum-Schwensen B, et al. Oncogene. 2015;34(4):424-435. DOI: 10.1038/onc.2013.568

[89] Ji YR, Kim HJ, Bae KB, Lee S, Kim MO, Ryoo ZY. Hepatic serum amyloid A1 aggravates T cell-mediated hepatitis by inducing chemokines via Toll-like receptor 2 in mice. The Journal of Biological Chemistry. 2015;290(20):12804-12811. DOI: 10.1074/jbc.M114.635763

[90] Lee JM, Kim EK, Seo H, Jeon I, Chae MJ, Park YJ, et al. Serum amyloid A3 exacerabtes cancer by enhancing the suppressive capacity of myeloid-derived suppressor cells via TLR2-dependent STAT3 activation. European Journal of Immunology. 2014;44(6):1672-1684

[91] O’Reilly S, Cant R, Ciechomska M, Finnigan J, Oakley F, Hambleton S, et al. Serum amyloid A induces interleukin-6 in dermal fibroblasts via Toll-like receptor 2, interleukin-1 receptor-associated kinase 4 and nuclear factor-kappaB. Immunology. 2014;143(3):331-340. DOI: 10.1111/imm.12260

[92] Zhang G, Liu J, Wu L, Fan Y, Sun L, Qian F, et al. Elevated expression of serum amyloid A3 protects colon epithelium against acute injury through TLR2-dependent induction of neutrophil IL-22 expression in a mouse model of colitis. Frontiers in Immunology. 2018;9(1503):1-11. DOI: 10.3389/fimmu.2018.01503

[93] Yan SD, Zhu H, Zhu A, Golabek A, Du H, Roher A, et al. Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis. Nature Medicine. 2000;6(6):643-651

[94] Okamoto H, Katagiri Y, Kiire A, Momohara S, Kamatani N. Serum amyloid A activates nuclear factor-kappaB in rheumatoid synovial fibroblasts through binding to receptor of advanced glycation end-products. The Journal of Rheumatology. 2008;35(5):752-756

[95] Belmokhtar K, Robert T, Ortillon J, Braconnier A, Vuiblet V, Boulagnon-Rombi C, et al. Signaling of serum amyloid A through receptor for advanced glycation end products as a possible mechanism for uremia-related atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016;36(5):800-809. DOI: 10.1161/ATVBAHA.115.306349

[96] van der Westhuyzen DR, Cai L, de Beer MC, de Beer FC. Serum amyloid A promotes cholesterol efflux mediated by scavenger receptor B-I. The Journal of Biological Chemistry. 2005;280(43):35890-35895

[97] Cai L, de Beer MC, de Beer FC, van der Westhuyzen DR. Serum amyloid
A is a ligand for scavenger receptor class B type I and inhibits high density lipoprotein binding and selective lipid uptake. The Journal of Biological Chemistry. 2005;280(4):2954-2961

[98] Baranova IN, Vishnyakova TG, Bocharov AV, Kurlander R, Chen Z, Kimelman ML, et al. Serum amyloid A binding to CLA-1 (CD36 and LIMPII analogous-1) mediates serum amyloid A protein-induced activation of ERK1/2 and p38 mitogen-activated protein kinases. The Journal of Biological Chemistry. 2005;280(9):8031-8040

[99] Baranova IN, Souza ACP, Bocharov AV, Vishnyakova TG, Hu X, Vaisman BL, et al. Human SR-BII mediates SAA uptake and contributes to SAA pro-inflammatory signaling in vitro and in vivo. PLoS One. 2017;12(4):e0175824. DOI: 10.1371/journal.pone.0175824

[100] Christenson K, Bjorkman L, Tangemo C, Bylund J. Serum amyloid A inhibits apoptosis of human neutrophils via a P2X7-sensitive pathway independent of formyl peptide receptor-like 1. Journal of Leukocyte Biology. 2008;83(1):139-148

[101] Shridas P, De Beer MC, Webb NR. High-density lipoprotein inhibits serum amyloid A-mediated reactive oxygen species generation and NLRP3 inflammasome activation. The Journal of Biological Chemistry. 2018;293(34):13257-13269. DOI: 10.1074/jbc.RA118.002428

[102] Bjorkman L, Raynes JG, Shah C, Karlsson A, Dahlgren C, Bylund J. The proinflammatory activity of recombinant serum amyloid A is not shared by the endogenous protein in the circulation. Arthritis and Rheumatism. 2010;62(6):1660-1665

[103] Kim MH, de Beer MC, Wroblewski JM, Webb NR, de Beer FC. SAA does not induce cytokine production in physiological conditions. Cytokine. 2013;61(2):506-512

[104] Christenson K, Bjorkman L, Ahlin S, Olsson M, Sjoholm K, Karlsson A, et al. Endogenous acute phase serum amyloid A lacks pro-inflammatory activity, contrasting the two recombinant variants that activate human neutrophils through different receptors. Frontiers in Immunology. 2013;4:92

[105] Burgess EJ, Hoyt LR, Randall MJ, Mank MM, Bivona JJ 3rd, Eisenhauer PL, et al. Bacterial lipoproteins constitute the TLR2-stimulating activity of serum amyloid A. Journal of Immunology. 2018. DOI: 10.4049/jimmunol.1800503. [Epub ahead of print]

[106] Webb NR, de Beer MC, van der Westhuizen DR, Kindy MS, Banka CL, Tsukamoto K, et al. Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice. Journal of Lipid Research. 1997;38(8):1583-1590

[107] Kindy MS, King AR, Yu J, Gerardot C, Whitley J, de Beer FC. Adenoviral expression of murine serum amyloid A proteins to study amyloid fibrillogenesis. The Biochemical Journal. 1998;332(Pt 3):721-728

[108] Thompson JC, Wilson PG, Shridas P, Ji A, de Beer M, de Beer FC, et al. Serum amyloid A3 is pro-atherogenic. Atherosclerosis. 2018;268:32-35. DOI: 10.1016/j.atherosclerosis.2017.11.011

[109] Eckhardt ER, Witta J, Zhong J, Arsenescu R, Arsenescu V, Wang Y, et al. Intestinal epithelial serum amyloid A modulates bacterial growth in vitro and pro-inflammatory responses in mouse experimental colitis. BMC Gastroenterology. 2010;10:133. DOI: 10.1186/1471-230X-10-133

[110] Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK,
et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. The Journal of Clinical Investigation. 2008;118(2):534-544. DOI: 10.1172/JCI33194

[111] Sano T, Huang W, Hall JA, Yang Y, Chen A, Gavzy SJ, et al. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. Cell. 2015;163(2):381-393. DOI: 10.1016/j.cell.2015.08.061

[112] Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. Cell. 2015;163(2):367-380. DOI: 10.1016/j.cell.2015.08.058

[113] De Santo C, Arscott R, Booth S, Karydis I, Jones M, Asher R, et al. Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. Nature Immunology. 2010;11(11):1039-1046. DOI: 10.1038/ni.1942

[114] Ahlin S, Olsson M, Olsson B, Svensson PA, Sjoholm K. No evidence for a role of adipose tissue-derived serum amyloid A in the development of insulin resistance or obesity-related inflammation in hSAA1(+/-) transgenic mice. PLoS One. 2013;8(8):e72204

[115] De Beer MC, Wroblewski JM, Noffsinger VP, Rateri DL, Howatt DA, Balakrishnan A, et al. Deficiency of endogenous acute phase serum amyloid A does not affect atherosclerotic lesions in apolipoprotein E-deficient mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014;34(2):255-261. DOI: 10.1161/ATVBAHA.113.302247

[116] Liu J, Wang D, Li SQ, Yu Y, Ye RD. Suppression of LPS-induced tau hyperphosphorylation by serum amyloid A. Journal of Neuroinflammation. 2016;13:28. DOI: 10.1186/s12974-016-0493-y

[117] Shah C, Hari-Dass R, Raynes JG. Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. Blood. 2006;108(5):1751-1757