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Serum amyloid A1: Structure, function and gene polymorphism

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Abstract

Inducible expression of serum amyloid A (SAA) is a hallmark of the acute-phase response, which is a conserved reaction of vertebrates to environmental challenges such as tissue injury, infection and surgery. Human SAA1 is encoded by one of the four SAA genes and is the best-characterized SAA protein. Initially known as a major precursor of amyloid A (AA), SAA1 has been found to play an important role in lipid metabolism and contributes to bacterial clearance, the regulation of inflammation and tumor pathogenesis. *SAA1* has five polymorphic coding alleles (*SAA1.1* – *SAA1.5*) that encode distinct proteins with minor amino acid substitutions. Single nucleotide polymorphism (SNP) has been identified in both the coding and non-coding regions of human *SAA1*. Despite high levels of sequence homology among these variants, *SAA1* polymorphisms have been reported as risk factors of cardiovascular diseases and several types of cancer. A recently solved crystal structure of SAA1.1 reveals a hexameric bundle with each of the SAA1 subunits assuming a 4-helix structure stabilized by the C-terminal tail. Analysis of the native SAA1.1 structure has led to the identification of a competing site for high-density lipoprotein (HDL) and heparin, thus providing the structural basis for a role of heparin and heparan sulfate in the conversion of SAA1 to AA. In this brief review, we compares human SAA1 with other forms of human and mouse SAAs, and discuss how structural and genetic studies of SAA1 have advanced our understanding of the physiological functions of the SAA proteins.

1. Introduction

Serum amyloid A1 (SAA1) was originally identified as a serum component recognized by antisera against amyloid fibrils known as amyloid A (AA) (Levin et al., 1973; Husby and Natvig, 1974; Rosenthal and Franklin, 1975). It was subsequently found that SAA1 serves as a major precursor of AA (Rosenthal et al., 1976) and is an apolipoprotein of high-density lipoprotein (HDL) in the acute-phase serum (Benditt and Eriksen, 1977; Benditt et al.,

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Conflict of interest statement

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1982). SAA is the generic name of a family of proteins with 103–104 amino acids that share high levels of sequence homology but are encoded by different genes (Figures 1, 2). In humans, there are 4 SAA genes (*SAA1, SAA2, SAA3* and *SAA4*) mapped in a 150-kb region of chromosome 11p15.1 (Betts et al., 1991; Sellar et al., 1994). Of these genes, *SAA1* and *SAA2* code for acute-phase SAA proteins that are highly inducible during the acute-phase response (Gabay and Kushner, 1999; Uhlar and Whitehead, 1999). *SAA4* encodes a constitutively expressed SAA protein, and *SAA3* is a pseudogene (Kluve-Beckerman et al., 1991). Mice also have 4 SAA genes with one of them (*Saa4*) coding for a constitutively expressed SAA. As will be discussed below, mouse SAA proteins are highly homologous to their human counterparts and are excellent models for functional studies. A major difference between the mouse and human SAA genes is that mouse *Saa3* encodes a functional product expressed mainly in extrahepatic tissues such as adipocytes and macrophages (Meek and Benditt, 1986; Benditt and Meek, 1989). Among all known SAA proteins, the human SAA1 is best characterized for its physiological functions and gene polymorphisms. This review summarizes progress made in our understanding of the physiological functions of human SAA1, its gene polymorphism, as well as recent findings in its structure. The review also discusses technical limitations such as the widespread use of a recombinant human SAA1 hybrid protein for *in vitro* studies, and new tools including genetically altered mice that became available only recently. Where applicable, other forms of SAA such as the mouse Saa3 are included for comparison. For comprehensive reviews on acute-phase SAAs, the interested reader is referred to a wealth of literature on the subject (Uhlar and Whitehead, 1999; Yamada, 1999; O'Brien and Chait, 2006; Malle et al., 2009; King et al., 2011; Eklund et al., 2012; Kisilevsky and Manley, 2012; Ye and Sun, 2015).

2. The human *SAA1* gene product and its structure

SAA1 encodes a pre-protein of 122 amino acids, including an 18-amino acid signal peptide (Uhlar and Whitehead, 1999). Cleavage of the signal peptide results in a mature SAA1 protein of 104 amino acids (Figure 1) that is secreted by hepatocytes and released to blood circulation. As a result of the massive production of SAA1 and SAA2 during the acute-phase response, the plasma level of these SAA proteins rises by up to 1,000-fold (Gabay and Kushner, 1999). The acute-phase SAA in plasma is tightly bound with high-density lipoprotein (HDL). There has been an ongoing effort to understand how inducible SAA expression and HDL remodeling are functionally associated with the protection against environmental insults (Getz and Reardon, 2008), which is thought to be a primary function of the acute-phase response (Gabay and Kushner, 1999).

The structure of SAA1 was not available until recently (Lu et al., 2014). In the absence of detailed structural information, computer modeling was used to simulate SAA1 structure based on a protein with sequence homology (Stevens, 2004). Combined with data from functional studies, the N-terminal region of SAA1 is predicted to harbor an HDL binding site, whereas the C-terminal segment constrains the overall SAA1 structure such that its removal facilitates aggregation of the cleavage product AA, forming highly ordered β-sheets as in amyloid fibrils. Structural studies of SAA1 were also carried out using β-sheet binding fluorescent dyes such as thioflavin T, revealing structural differences between SAA1.1 and SAA1.3 (Takase et al., 2014). Using deep UV resonance Raman, far UV-circular dichroism,

atomic force microscopy and fibrillation cross-seeding, a study has identified differences between SAA1.1 and SAA2.2 in their fibrillation kinetics, fibril morphology and quaternary structure (Srinivasan et al., 2013). Recently, the crystal structure of SAA1.1 was solved at a 2.2-Å resolution (Lu et al., 2014). In its native state, SAA1.1 exists as a hexamer of identical subunits showing a four-helix bundle structure. The previously predicted functional regions of human SAA1 partially overlap with these α-helices (Figure 1). Surprisingly, there is a lack of the previously predicted β-strands in this structure. An analysis of the SAA1.1 structure revealed that the C-terminal tail stabilizes the helix bundle structure by providing multiple contact sites. The apex of the cone-shaped SAA1.1 helix bundle serves as the site for HDL binding, which is inhibited by heparin (Lu et al., 2014). These findings provide structural insights into a mechanism by which heparin and heparan sulfate facilitate AA formation, which requires the dissociation of SAA1.1 from HDL.

The crystal structure of SAA1.1 was compared with that of the mouse Saa3, another SAA protein with its structure determined recently (Derebe et al., 2014). Both structures have four α-helices, forming a cone-shaped bundle. The α1 helix in the mouse Saa3 is longer than the one in human SAA1. In mouse SAA3, these subunits oligomerize to form a hollow, largely non-polar interior that serves as a binding pocket for retinol (Derebe et al., 2014). Unlike the hexameric bundle structure formed by the human SAA1, mouse Saa3 forms a tetrameric bundle structure. Differences in the primary sequence of the N-terminal regions in these SAA proteins may account for the discrepancy in their oligomeric states. The human SAA1.1, which has a tendency to form pathogenic amyloid fibrils, contains a more hydrophobic N-terminal region than the mouse Saa3, which is nonamyloidogenic. Therefore, fibrillogenecity and amyloidogenecity can be explained at the structural level as more structural information becomes available.

3. Inducible expression of human *SAA1*

Human *SAA1* is located in the short arm on chromosome 11 and contains 4 exons with the coding sequences residing in exon 2, exon 3 and part of exon 4, respectively (Figure 2A), based on two of the published sequences (NM_199161, NM_000331). Exon 1 and part of exon 4 contains non-coding sequences including the *SAA1* promoter. The third published gene sequence (NM_001178006) suggests the presence of 5 exons, with non-coding sequence in two exons upstream of the first coding exon (Figure 2).

Inducible expression is characteristic of all acute-phase SAAs including SAA1 and SAA2. In addition, Saa3 in mice is also an inducible SAA. The resulting increase in SAA production in various tissues has been a well-established clinical biomarker for inflammatory disorders. It is widely believed that proinflammatory cytokines such as IL-1β, IL-6 and TNF-α, as well as glucocorticoids, play important roles in hepatic expression of SAA1 and SAA2 during the acute-phase response (Uhlar and Whitehead, 1999). This notion is further supported by the observation that genetic deletion of the IL-1β gene led to an impaired acute-phase inflammatory response in mice (Zheng et al., 1995). Likewise, deletion of the IL-6 gene in mice resulted in compromised acute-phase response to tissue injury (Kopf et al., 1994). Along with the reduced acute-phase response, hepatic production of SAA decreases significantly. Transcriptional regulation is primarily responsible for the

marked induction of SAA in the acute-phase response (Uhlar and Whitehead, 1999). A variety of transcription factors including NF-κB, C/EBP, YY1, AP-2, SAF, Sp1, and STAT3, are involved in the induced expression of the acute-phase SAA (Betts et al., 1993; Uhlar and Whitehead, 1999). Hepatocyte-specific mutation of both NF-κB and STAT3, but not either one alone, abrogated the acute-phase response to tissue injury and bacterial infection in mice, leading to markedly reduced expression of hepatic SAA1 and its concentration in plasma (Quinton et al., 2014).

4. Major functions of human SAA1

Our understanding of the functions of SAA1 lags far behind our appreciation of its structure and induced expression. The presence of multiple isoforms and single nucleotide polymorphisms (SNPs) further complicates functional studies of SAA1. As a result of these limitations, information presented in this section only serves as a starting point for more detailed investigation of the functions of SAA in physiological and pathological conditions.

4.1. Amyloidogenesis

Type AA amyloidosis, also known as inflammatory amyloidosis, is a complication of chronic inflammatory conditions and is characterized by the deposit of insoluble amyloid fibrils in the affected organs and tissues. The amyloid protein amyloid A (AA) is mainly a degradation product of the acute-phase SAA1 (Tape et al., 1988; Liepnieks et al., 1995). In this regard, amyloidogenesis is not exactly a function of SAA1 *per se* but the consequence of over-production and aberrant processing of SAA1. In a commonly identified form, AA consists of the N-terminal 76 amino acids of human SAA1 and SAA2, with SAA1 being the predominant precursor (Prelli et al., 1987; Liepnieks et al., 1995). Fibrillar AA derives mostly from circulating SAA1, which dissociates from HDL before its conversion to amyloid fibrils. This process occurs through an interaction with heparan sulfate, a glycosaminoglycan component of the extracellular matrix. A recent study of the SAA1 structure has identified an overlapping binding site for HDL and heparin, thus explaining the effect of heparan sulfate in the dissociation of SAA1 from HDL before its conversion to amyloid fibrils (Lu et al., 2014). As will be discussed below, polymorphic variants of *SAA1* have different propensity for conformational transformation to amyloid fibrils.

4.2. HDL remodeling and lipid metabolism

During the acute-phase response, marked elevation in plasma SAA protein level causes HDL remodeling, with the newly synthesized SAA1 and SAA2 displacing apoA-1 and becoming an apolipoprotein of HDL (Benditt and Eriksen, 1977; Coetzee et al., 1986). However, SAA cannot replace apoA-1 for its role in HDL particle formation (Webb et al., 1997). HDL remodeling during the acute-phase response also requires plasma components such as the group IIA secretory phospholipase A2 and cholesteryl ester transfer protein (Jahangiri et al., 2009). It is well recognized that SAA plays an important role in lipid metabolism, but how SAA impacts lipid metabolism remains incompletely understood. Reverse cholesterol transport is a process by which cholesterol in non-hepatic tissues is transported back to the liver, via plasma components such as HDL and the ATP-binding cassette (ABC) transporters such as ABCA1 and ABCG1. In this process, apoA-1 acts as an acceptor for cholesterol.

Several studies have shown that SAA in lipid-free form can also act as a lipid acceptor for ABCA1 (Stonik et al., 2004; Abe-Dohmae et al., 2006). The effect of SAA on cholesterol removal, however, requires more than SAA. In human apoA-1 transgenic mice, adenovirus-mediated over-expression of human SAA1 alone does not significantly alter the distribution of cholesterol among lipoproteins (Hosoai et al., 1999). Using mice lacking both *Saa1* and *Saa2* (the *Saa1/2* double-knockout mice), a study found that the ABCG1-mediated transport of cellular cholesterol to HDL was not significantly affected compared to wild type controls, suggesting that these major forms of mouse SAA proteins are not absolutely required (de Beer et al., 2011). Another study using these double-knockout mice resulted in the conclusion that the impact of SAA on HDL levels and apoA-1 clearance may be very limited (de Beer et al., 2010). An *in vivo* study using the macrophage-to-feces reverse cholesterol transport assay has shown that the LPS-induced impairment of reverse cholesterol transport does not involve SAA (de Beer et al., 2013). These findings from the use of genetically altered mice indicate that the *in vivo* functions of SAA in lipid metabolism are more complicated than previously thought based on mostly *in vitro* studies.

It was reported that SAA binding to HDL increases its affinity for macrophage, while the binding affinity for hepatocytes decreases (Kisilevsky and Subrahmanyan, 1992). This change is believed to favor the removal of cholesterol from sites of inflammation (Kisilevsky, 1991). The scavenger receptor SR-BI was identified as one of the SAA receptors (Baranova et al., 2005; Cai et al., 2005). SAA inhibits HDL binding to SR-BI; however, the efflux of free cholesterol is enhanced in a SR-BI-dependent manner (van der Westhuyzen et al., 2005). Efficient efflux of cholesterol requires both lipid-free SAA and the expression of ABCA1, indicating that the lipidation status of SAA is a determinant for cholesterol efflux through SR-BI (Marsche et al., 2007). It is believed that SR-BI-mediated re-uptake of cholesterol underlies the role SAA plays in cholesterol recycling during tissue repair after injury, when cholesterol is much needed (Kisilevsky and Manley, 2012).

4.3. Tumor pathogenesis

Elevated plasma level of SAA, consisting of SAA1 and SAA2, has been a clinical biomarker for inflammatory diseases (Malle and De Beer, 1996). Accumulating evidence indicates that increased expression of SAA may be associated with tumor pathogenesis as well (Cho et al., 2004; Malle et al., 2009; Liu, 2012). SAA1 and SAA4 are highly expressed in uterine cervical carcinomas and therefore can serve as a biomarker for this type of cancer (Ren et al., 2014). In non-small cell lung cancer patients undergoing EGF receptor tyrosine-kinase inhibitor therapy, the increase in SAA1 expression level in plasma serves as an indicator of poor prognosis (Milan et al., 2012). Likewise, a correlation between SAA1 expression and poor clinical outcome has been established in patients with conventional renal cell carcinoma (Paret et al., 2010). Because of the close link between chronic inflammation and malignant transformation, SAA may affect tumorigenesis and tumor metastasis. A recent study has shown a close link between certain SAA1 allelic variants and the nasopharyngeal carcinoma, with some of these variants having anti-angiogenic and tumor-suppressive activities (Lung et al., 2014). Induced expression of matrix metalloproteases (Lee et al., 2005) and induction of angiogenesis (Mullan et al., 2006) may contribute to tumor metastasis and growth. A member of the SAA family, Saa3 has been shown to facilitate

tumor cell metastasis to lung tissue through a SAA3-TLR4 dependent process that favors an inflammation-like state termed pre-metastasis phase (Hiratsuka et al., 2008). A more recent study identified a link between the S100 family protein S100A4, Saa1 and Saa3, and tumor metastasis. In this study, it was found that S100A4 could stimulate the expression of mouse Saa1 and Saa3, which in turn stimulated the expression of RANTES, G-CSF, MMP2, S100A8 and S100A9, thereby promoting tumor metastasis (Hansen et al., 2015).

4.4. Antibacterial functions

SAA1 has been found to bind to the outer membrane of Gram-negative bacteria including *Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Vibrio cholerae*, and *Pseudomonas aeruginosa* (Hari-Dass et al., 2005). The outer membrane protein A (OmpA) is shown to be responsible for the binding of SAA1 that acts as an opsonin for increased neutrophil uptake of the invading bacteria (Shah et al., 2006). In addition, peripheral blood monocyte-derived macrophages respond to the SAA-opsonized bacteria with enhanced production of TNF-α and IL-10. These findings suggest a potentially important role for SAA1 in host defense against invading Gram-negative bacteria.

A recent study identified SAA1 as a retinol binding protein with a K_d of 259 nM (Derebe et al., 2014). Retinol plays an important role in host defense. Hence this finding suggests that SAA may limit bacterial burden, particularly in the intestine. The study reports that SAA recovered from serum following bacterial infection is associated with retinol, and mice lacking both *Saa1* and *Saa2* have higher bacterial burdens in the spleen and liver following an acute bacterial infection (Derebe et al., 2014). Together, these new findings suggest that SAA1 plays a role in combating bacterial infection.

In addition to systemic SAA1, epithelial cell-derived SAA1 has bactericidal activities as over-expression of Saa1 and Saa2 in cultured epithelial cell lines reduced co-cultured *E. coli* (Eckhardt et al., 2010). In mice, all 3 inducible SAA isoforms are found in intestinal epithelium, which has close contact with bacteria in the gut. The finding that SAA1 has antibacterial property leads to the prediction that the acute-phase protein may protect host in tissues and organs that are exposed to the outside environment.

4.5. Regulation of inflammation and immunity

A number of studies have been conducted using a chimeric SAA1/SAA2 protein, which is a commercially available recombinant human SAA (rhSAA from PeproTech) containing amino acid substitutions at positions 61 and 72 of the mature protein with a Met added to the N-terminus. These studies identified chemotactic activity of the rhSAA for phagocytes (Badolato et al., 1994) as well as its ability to induce proinflammatory cytokines *ex vivo* (Patel et al., 1998; Furlaneto and Campa, 2000; He et al., 2003; Cai et al., 2007; Sun et al., 2015). These activities are mediated by several receptors for rhSAA including FPR2 (FPR2/ALX, formerly FPRL1) (Su et al., 1999; Ye et al., 2009), TLR2 (Cheng et al., 2008) and TLR4 (Sandri et al., 2008). Because of the presence of multiple receptors for rhSAA, its cytokine-inducing profile is different from that of LPS and of the TLR2 ligands (He et al., 2006; Yan et al., 2014). rhSAA has been shown to extend the lifespan of neutrophils by delaying their constitutive apoptosis (El Kebir et al., 2007). In addition, rhSAA stimulates

the expression of G-CSF, resulting in neutrophil expansion (He et al., 2009). These findings suggest that rhSAA has cytokine-like activities and may play a role in the regulation of inflammation and immunity.

The physiological relevance of the above findings was questioned when a recombinant human SAA1 (rhSAA1), provided by the same vendor without the added N-terminal Met and the two amino acid substitutions, was found to have much lower activity in cytokine induction experiment (Christenson et al., 2013). A similar finding was made in another study (van den Brand et al., 2013). Together, these results suggest the possibility that the chimeric rhSAA may have acquired additional cytokine-inducing activity because of the altered amino acid sequence. An independent study has led to the conclusion that purified SAA does not induce cytokine production in physiological conditions (Kim et al., 2013). There are, however, published data showing that rhSAA1 (from PeproTech) stimulates MMP9 production in the HD2 and HA344 renal carcinoma cell lines (Paret et al., 2010). Recombinant human SAA1 prepared in different laboratories are able to induce the transcripts of proinflammatory cytokines including IL-8, MCP-1 and TNF-α (Leow et al., 2013) as well as the anti-inflammatory cytokine IL-10 (Chen et al., 2014). Of note, SAA1.1 prepared with the same protocol was as potent as SAA2.2 in the induction of chemotaxis and calcium mobilization, while both SAA variants were able to stimulate TLR2-dependent NF-κB activation (Chen et al., 2014). It was also reported that the THP-1-derived macrophages responded to rhSAA1 with the upregulation of 55 genes, many of which being proinflammatory cytokines induced by the chimeric rhSAA (Leow et al., 2012). A recent study has shown that rhSAA and rhSAA1 are both capable of promoting macrophage switch to the M2 phenotype, characterized by the induced expression of the M2 cytokines (Sun et al., 2015). This latter study suggests that SAA1 may be a homeostatic regulator of inflammation. Further supporting a role for SAA1 in cytokine induction, a recent report has shown that transgenic expression of human SAA1 in mouse liver led to induced expression of the chemokines MCP-1, MIP-1α, MIP-1β, IP-10 and eotaxin, suggesting that SAA1 may be functional *in vivo* for cytokine induction (Ji et al., 2015). As will be discussed below in Section 6, more recent studies identified Saa1 and Saa2 as local mediators for a Th17 response to segmented filamentous bacteria (Atarashi et al., 2015; Sano et al., 2015). These new findings suggest an active role for SAA1 in the regulation of inflammation and immunity in specific organs and tissues. Studies using better controlled experimental conditions will be required for further characterization of rhSAA and rhSAA1 for their inflammation-modulatory functions.

5. Human *SAA1* polymorphism and disease disposition

SAA1 has 5 polymorphic coding alleles, *SAA1.1, SAA1.2, SAA1.3, SAA1.4* and *SAA1.5* (Sipe, 1999). The gene products of these coding alleles vary by only a few amino acids at positions 52, 57, 60 and 72 of the mature SAA1 protein (highlighted in red in Figure 1B). Despite these minor differences, the allelic variants of SAA1 have shown differences in various *in vitro* assays as well as linkage to several diseases.

5.1. Amyloidogenesis

Studies have shown that homozygosity at the *SAA1.1* allele is a strong predictor for type AA amyloidosis. In one study, SAA1.1 was found to be more susceptible than SAA1.5 to MMP-1 degradation (van der Hilst et al., 2008). The difference lies in sequence variation at position 57, where an Ala in SAA1.1 becomes a Val in SAA1.5 (Figure 1B). This finding may partially explain the clinical observation that individuals with the *SAA1.1/1.1* genotype have higher risk for type AA amyloidosis than individuals with the *SAA1.5/1.5* or *SAA1.1/1.5* genotype (Booth et al., 1998). In addition, *SAA1.3* homozygosity has been shown as a univariate predictor of survival as well as a risk factor for AA amyloidosis, based on a study with an enrollment of 182 Japanese RA patients (Nakamura et al., 2006).

5.2. Tumor suppression

A recent study links the high frequency of the *SAA1.5/1.5* genotype in nasopharyngeal carcinoma patients with abrogated anti-angiogenic activity of the SAA1.5 protein (Lung et al., 2014). The study shows that SAA1.1 and SAA1.3 have higher affinity for the integrin αvβ3 than SAA1.5. Because αvβ3 integrin is required for angiogenesis (Brooks et al., 1994), these two SAA1 variants display strong anti-angiogenic effect by inhibition of stress fiber assembly and focal adhesion of vascular endothelial cells. Restoration of SAA1.1 expression in the deficient nasopharyngeal carcinoma cell lines corrects the absence of the tumor suppressive effect (Lung et al., 2014), supporting the conclusion that genetic variation in *SAA1* has a functional impact on the susceptibility to nasopharyngeal carcinomas.

5.3. Differential preference for SAA receptors

At least 6 functional receptors for SAA, including the receptor for advanced glycation end products (RAGE), the formyl peptide receptor 2 (FPR2), the scavenger receptor class B type I (SR-BI), the Toll-like receptors 2 (TLR2) and 4 (TLR4) and the purinoceptor P2X7 receptor, have been identified to date (recently reviewed in (Malle et al., 2009; Sun et al., 2015)). These receptors mediate various functions of SAA1 and possibly other forms of SAA proteins. For example, facilitation of cholesterol efflux by SAA1 is mediated through the scavenger receptor SR-BI (Cai et al., 2005; van der Westhuyzen et al., 2005), whereas the two TLRs are thought to be responsible for the cytokine-inducing activities of SAA (Cheng et al., 2008; Sandri et al., 2008). The effects of amino acid substitutions in the SAA1 variants on receptor interaction have been evaluated in transfected cell lines and primary mouse macrophages lacking functional TLR4 (Chen et al., 2014). Of the 3 SAA1 variants compared, SAA1.1 displayed higher efficacy than SAA1.3 and SAA1.5 in mobilizing intracellular Ca^{2+}, a function mediated by the G protein-coupled chemoattractant receptor FPR2 (Su et al., 1999; Ye et al., 2009). In comparison, SAA1.3 showed strong capability in stimulating the phosphorylation of ERK and p38 MAPK. SAA1.5 is most effective in the induction of IL-10 expression, a function mediated primarily though SAA binding to TLR2 (Cheng et al., 2008; Chen et al., 2014). These findings provide the structural basis for the interaction of SAA1 variants with their receptors as well as an explanation for the different effects produced by these SAA1 variants.

5.4. Other SAA1 nucleotide polymorphisms

Single-nucleotide polymorphisms (SNPs) have been identified in coding and non-coding regions of the human SAA1 gene (Figure 2B). The presence of these SNPs of *SAA1* has been associated with disposition to a variety of diseases. A C/T switch at −13 of *SAA1.3* (−13C/T) is associated with increased transcriptional activity of this gene, correlating with susceptibility to type-AA amyloidosis in a Japanese rheumatoid arthritis population (Moriguchi et al., 2005). Individuals carrying this SNP also have an increased susceptibility to familial Mediterranean fever in the Japanese population (Migita et al., 2013). Studies of the SNP rs12218 has been conducted in Chinese Han subjects, and it was found that individuals with the CC genotype of this SNP have lower HDL-C level (Xie et al., 2010) as well as higher risk for peripheral arterial disease, with a decreased ankle-to-brachial index (Xie et al., 2011). The CC genotype of rs12218 was more frequent among patients suffering from coronary artery disease (Xie et al., 2015). This genotype was also more frequent in patients suffering from cerebral infarction (Zhang et al., 2013a). In comparison, the TT genotype of rs12218 is associated with an increased serum uric acid level (Xie et al., 2012) and with susceptibility to osteoporosis (Feng et al., 2013). There is an association of the SNP rs4638289 with increased risk for carotid artery intima-media thickness in obese individuals (Carty et al., 2009). In obese children, the frequency of this SNP and SNP rs12218 is increased (Zhang et al., 2013b). The mechanisms by which these SNPs affect the functions of SAA1 remain poorly understood. SNP rs4638289 has been reported to increase the expression of SAA1 (Zhang et al., 2013b). In comparison to these SNPs, non-synonymous SNPs such as rs79681911 (c.269G>A) lead to missense or nonsense mutations. In this case, rs79681911 causes an amino acid substitution (Gly72 to Asp, as seen in SAA1.2 at this position). The resulting SAA1 protein displayed reduced capability in the induction of IL-8, MCP-1 and TNFα in THP-1 cells (Leow et al., 2013).

6. SAA in other species

6.1. Comparison of human, mouse and pig SAAs

Genes coding for SAA or SAA-like proteins have been identifies in a variety of vertebrates ranging from zebrafish to humans. Of particular interest are the SAA proteins in mice, which have been widely used in basic research laboratories; and pigs, which have seen rapid increase in their use as models of human diseases. Like the human SAA genes, the mouse and pig SAA genes have the same genomic organization with 4 exons and 3 introns. In mice, the major acute-phase SAA proteins are encoded by *Saa1.1* and *Saa2.1*, located on chromosome 7 (Lowell et al., 1986). Mouse Saa1.1 as a mature protein has 103 amino acids and shares 76% sequence identity with the human counterpart (Figure 3). Mice predominantly expressing Saa1.1 and 2.1 are susceptible to AA amyloidosis, whereas mice expressing Saa2.2 (the CE/J mice) are resistant to AA amyloidosis (Mori et al., 2014), mainly due to its proline-rich cluster of 13 amino acids in the C-terminus (Patke et al., 2012). The two mouse Saa proteins share many properties with human acute-phase SAA (see comparison of human SAA1.1 and the mouse counterpart in Figure 3), and are therefore used often in *in vivo* studies. Mice with deletion of both *Saa1.1* and *Saa2.1* have been generated (de Beer, 2010) and used in several recent studies with exciting new findings.

Unlike the human *SAA3* which is a pseudogene (*hSAA3P*), the mouse *Saa3* encodes a functional gene product expressed mainly in extrahepatic tissues and cells, including adipocytes and macrophages (Meek and Benditt, 1986; Benditt and Meek, 1989). The expression of *Saa3* is highly inducible in inflammatory tissues and in obese mice (Chiba et al., 2009). It is interesting to note that SAA3 transcripts are detected in many species based on a GenBank search, with the few exceptions including humans (Kluve-Beckerman et al., 1991). Research on mouse Saa3, therefore, may help to understand the potential functions of tissue-derived SAA proteins in physiological and pathological conditions. The mouse Saa3 has several distinct properties. It has an alkaline isoelectric point (10.03), as opposed to the much lower isoelectric points of the mouse Saa1 (6.07) and human SAA1 (5.89). Although the mouse Saa3 is abundant in inflammatory tissues, it is not amyloidogenic and does not contribute to the rise of plasma SAA level during the acute-phase response (Chiba et al., 2009). In 3T3-L1 adipocytes, Saa3 expression is induced with hypoxia (de Oliveira et al., 2013). In immune cells, Saa3 binds MD-2 for activation of p38 MAPK and NF-κB through TLR4 (Deguchi et al., 2013). Saa3 serves as an endogenous ligand for TLR4 that facilitates lung tumor metastasis (Hiratsuka et al., 2008). It also functions as a paracrine factor for bone homeostasis (Thaler et al., 2015). A recent study implies that Saa3 signaling through TLR2 contributes to an enhanced suppressive capacity of myeloid-derived suppressor cells, thus exacerbating tumor growth (Lee et al., 2014).

Several porcine SAA genes have been identified to date, but the function of the gene products remains elusive. The major SAA protein in pig differs from SAA1 in other mammals, but with some characteristics of SAA3 (Soler et al., 2011; Soler et al., 2013). These include higher sequence homology to SAA3 of other species rather than SAA1 (Figure 3), a highly alkaline isoelectric point, and absence from the HDL-rich serum fraction (Soler et al., 2013). Porcine SAAs differ from their human and mouse counterparts with an insertion of 8 amino acids in the predicted 4^{th} helix structure (Figure 3). Based on these properties, it is unlikely that the major porcine SAA participates in HDL metabolism. Taken together, studies of SAAs from other species may shed light on the mechanisms by which these evolutionarily conserved proteins function in vertebrate, thus helping to understand the roles human SAAs play systemically and in specific tissues.

6.2. Genetically altered mouse models for SAA research

Several approaches have been taken to create mouse models for SAA research. In early studies, adenovirus-mediated systemic expression of mouse and human SAA1 was used for evaluation of the *in vivo* properties of acute-phase SAA in mice (Webb et al., 1997; Kindy et al., 1998; Hosoai et al., 1999). These studies took advantage of the available apoA-1 deficient mice, the ApoE deficient mice, and the CE/J strain. By expressing human or mouse SAAs of interest, these studies have led to the conclusions that SAA cannot replace apoA-1 in HDL particle formation (Webb et al., 1997), that the increases in plasma SAA level alone is insufficient to alter HDL cholesterol or apoA-1 levels (Kindy et al., 2000), and that SAA may alter vascular proteoglycans through stimulation of TGF-β (Wilson et al., 2008).

Transgenic mice expressing human SAA1 in adipocytes have been prepared (Olsson et al., 2011). Using these mice, the involvement of SAA1 in the development of insulin resistance

or obesity-related inflammation was examined. However, there was no evidence that adipose tissue-derived hSAA1 could influence the development of insulin resistance and obesity-related inflammation (Ahlin et al., 2013). When the mice expressing transgenic human SAA1 was bred with ApoE-deficient mice and the atherosclerotic lesion areas were compared with that of ApoE-deficient mice, no significant difference was detected (Ahlin et al., 2014). These findings suggest that the contribution of adipose tissue-derived SAA1 to atherosclerotic lesion and insulin resistance in obese mice may be limited. Whether the same can apply to humans remains unclear at this time, and other mouse models such as the LDLR-deficient mice should be compared. A doxycycline-inducible Saa1.1 transgene has been expressed in mice for the study of the relationship between inflammation and AA amyloidosis (Simons et al., 2013). The use of this transgenic line has allowed separation of amyloid A deposit from inflammation. In a more recent study, transgenic mice expressing human SAA1 was created to study the effect of SAA1 over-production in the liver (Ji et al., 2015). This work led to the finding that the transgenic SAA1 aggravates T cell-mediated hepatitis in part through TLR2-dependent induction of chemokines such as MCP-1, MIP-1α, MIP-1β, and IP-10.

Mice lacking both *Saa1.1* and *Saa2.1* have been generated (de Beer et al., 2010). These mice were used to study the role for SAA in lipid metabolism. Deletion of these two major acute-phase SAA genes apparently did not alter plasma apoA-1 level and its clearance. The expected size increase of the acute-phase HDL in wild type mice was also seen in the *Saa1/2* double-knockout mice. In another study, it was observed that the acute-phase HDL isolated from the *Saa1/2* double-knockout mice was more effective in promoting ABCG1 efflux (de Beer et al., 2011). These findings challenge the previously accepted role for acute-phase SAA in HDL remodeling and cholesterol efflux. However, caution must be taken in the interpretation of these results due to the differences between human and mouse SAA proteins and the environment in which these SAA proteins function.

There have been close associations between increased plasma SAA levels and many cardiovascular diseases (Filep and El Kebir, 2008). Atherosclerosis is one of these diseases linked to the acute-phase SAA (Dong et al., 2011; King et al., 2011; Thompson et al., 2015). It is of interest to note that deletion of *Saa1.1* and *Saa2.1* does not affect atherosclerotic lipid deposition in ApoE-deficient mice (De Beer et al., 2014). The *Saa1/2* double-knockout mice, however, are protected against experimental abdominal aortic aneurysm, accompanied with reduced matrix metalloproteinase-2 (MMP-2) activity (Webb et al., 2015). Induction of MMPs by rhSAA is well documented and is a part of the cytokine-like activities of the chimeric rhSAA (Migita et al., 1998; O'Hara et al., 2004; Lee et al., 2005; Mullan et al., 2006). The finding from the *Saa1/2* knocout study confirms the presence of this biological activity of SAA *in vivo*.

The availability of the *Saa1/2* knockout mice has spurred research in other areas where the acute-phase SAA is suspected to play a role. In an experimental colitis model, the *Saa1/2* knockout mice were found to be more susceptible to dextran sodium sulfate-induced weight loss, colon shortening and hematocrit decrease (Eckhardt et al., 2010). This finding led the authors to speculate that the expression of Saa1 and Saa2 in intestinal epithelial cells might protect intestinal epithelium against bacterial invasion. This notion has been further

supported by two recent studies that identified the Saa1 and Saa2 expressed in gut epithelium to be mediators of a local Th17 response (Atarashi et al., 2015; Sano et al., 2015).

In addition to mice lacking *Saa1.1* and *Saa2.1*, mice deficient in *Saa3* has been generated. When fed with high fat and high sucrose diet, the commonly seen weight gain and adipose tissue inflammation were improved. Female $Saa3^{-/-}$ mice also saw improved plasma cholesterol, triglycerides and lipoproteins profiles than the wild type controls (den Hartigh et al., 2014). Interestingly, hepatic production of Saa1 and Saa2 was reduced in $Saa3^{-/-}$ mice. These findings provide *in vivo* evidence for a regulatory function of Saa3, which is expressed in extrahepatic tissues but may regulate hepatic acute-phase SAA secretion.

7. Conclusion

This review briefly summarizes studies conducted on SAA1, a major acute-phase SAA with substantial gene polymorphism. These studies have shown pleiotropic functions of SAA1 ranging from HDL remodeling and lipid metabolism to regulation of inflammation and host defense. Despite extensive research in the past 40 years, there are still unanswered questions concerning the true physiological functions of SAA1. These questions include whether the physical state of SAA1 being studied (*e.g.*, HDL-bound *vs.* lipid-poor) is actually relevant to the physiological and pathological conditions *in vivo*, and whether the source material used (*e.g.*, recombinant human SAA as a chimera of SAA1 and SAA2) may bias result interpretation. These concerns have been raised in some of the cited papers and discussed extensively in two recent reviews on SAA (Kisilevsky and Manley, 2012; Ye and Sun, 2015). It is with great hope that recent advancements in SAA1 research have provided the much needed tools for eventual resolution of the disputes. The crystal structure of SAA1.1 has shown for the first time the α-helix bundle of this acute-phase protein, and provides insights into the mechanism by which heparin facilitates the conversion of SAA1 to AA (Lu et al., 2014). Genetically altered mice carrying the SAA1 transgenes (Olsson et al., 2011; Ji et al., 2015) or deletion of the *Saa1* and *Saa2* loci (de Beer et al., 2010) have allowed examination of the SAA protein of interest for its functions in whole animals. The use of these new approaches is expected to greatly facilitate research on SAA1 and other SAA proteins.

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Abbreviations

AA	amyloid A
HDL	high-density lipoprotein

OMA	outer membrane protein A
PBMC	peripheral blood mononuclear cells
rhSAA	recombinant human serum amyloid A
SAA	serum amyloid A
MAPK	mitogen-activated protein kinases
SNP	single-nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1

References

Abe-Dohmae S, Kato KH, Kumon Y, Hu W, Ishigami H, Iwamoto N, Okazaki M, Wu CA, Tsujita M, Ueda K, Yokoyama S. Serum amyloid A generates high density lipoprotein with cellular lipid in an ABCA1- or ABCA7-dependent manner. J Lipid Res. 2006; 47:1542–50. [PubMed: 16607034]

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:e72204. [PubMed: 23967285]

Ahlin S, Olsson M, Wilhelmson AS, Skalen K, Boren J, Carlsson LM, Svensson PA, Sjoholm K. Adipose tissue-derived human serum amyloid a does not affect atherosclerotic lesion area in hSAA1+/−/ApoE−/− mice. PLoS One. 2014; 9:e95468. [PubMed: 24751653]

Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, Suda W, Imaoka A, Setoyama H, Nagamori T, Ishikawa E, Shima T, Hara T, Kado S, Jinnohara T, Ohno H, Kondo T, Toyooka K, Watanabe E, Yokoyama S, Tokoro S, Mori H, Noguchi Y, Morita H, Ivanov, Sugiyama T, Nunez G, Camp JG, Hattori M, Umesaki Y, Honda K. Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. Cell. 2015; 163:367–80. [PubMed: 26411289]

Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, Bausserman LL, Kelvin DJ, Oppenheim JJ. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. J Exp Med. 1994; 180:203–209. [PubMed: 7516407]

Baranova IN, Vishnyakova TG, Bocharov AV, Kurlander R, Chen Z, Kimelman ML, Remaley AT, Csako G, Thomas F, Eggerman TL, Patterson AP. 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. J Biol Chem. 2005; 280:8031–40. [PubMed: 15576377]

Benditt EP, Eriksen N. Amyloid protein SAA is associated with high density lipoprotein from human serum. Proc Natl Acad Sci U S A. 1977; 74:4025–8. [PubMed: 198813]

Benditt EP, Hoffman JS, Eriksen N, Parmelee DC, Walsh KA. SAA, an apoprotein of HDL: its structure and function. Ann N Y Acad Sci. 1982; 389:183–9. [PubMed: 7046575]

Benditt EP, Meek RL. Expression of the third member of the serum amyloid A gene family in mouse adipocytes. J Exp Med. 1989; 169:1841–6. [PubMed: 2715765]

Betts JC, Cheshire JK, Akira S, Kishimoto T, Woo P. The role of NF-kappa B and NF-IL6 transactivating factors in the synergistic activation of human serum amyloid A gene expression by interleukin-1 and interleukin-6. J Biol Chem. 1993; 268:25624–31. [PubMed: 8244997]

Betts JC, Edbrooke MR, Thakker RV, Woo P. The human acute-phase serum amyloid A gene family: structure, evolution and expression in hepatoma cells. Scand J Immunol. 1991; 34:471–82. [PubMed: 1656519]

Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB. SAA1 alleles as risk factors in reactive systemic AA amyloidosis. Amyloid. 1998; 5:262–5. [PubMed: 10036584]

Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science. 1994; 264:569–71. [PubMed: 7512751]

Cai H, Song C, Endoh I, Goyette J, Jessup W, Freedman SB, McNeil HP, Geczy CL. Serum amyloid A induces monocyte tissue factor. J Immunol. 2007; 178:1852–60. [PubMed: 17237436]

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. J Biol Chem. 2005; 280:2954–61. [PubMed: 15561721]

Carty CL, Heagerty P, Heckbert SR, Enquobahrie DA, Jarvik GP, Davis S, Tracy RP, Reiner AP. Association of genetic variation in serum amyloid-A with cardiovascular disease and interactions with IL6, IL1RN, IL1beta and TNF genes in the Cardiovascular Health Study. J Atheroscler Thromb. 2009; 16:419–30. [PubMed: 19729864]

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; 219:916–23. [PubMed: 25154907]

Cheng N, He R, Tian J, Ye PP, Ye RD. Cutting Edge: TLR2 Is a Functional Receptor for Acute-Phase Serum Amyloid A. J Immunol. 2008; 181:22–6. [PubMed: 18566366]

Chiba T, Han CY, Vaisar T, Shimokado K, Kargi A, Chen MH, Wang S, McDonald TO, O'Brien KD, Heinecke JW, Chait A. Serum amyloid A3 does not contribute to circulating SAA levels. J Lipid Res. 2009; 50:1353–62. [PubMed: 19286646]

Cho WC, Yip TT, Yip C, Yip V, Thulasiraman V, Ngan RK, Yip TT, Lau WH, Au JS, Law SC, Cheng WW, Ma VW, Lim CK. Identification of serum amyloid a protein as a potentially useful biomarker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. Clin Cancer Res. 2004; 10:43–52. [PubMed: 14734450]

Christenson K, Bjorkman L, Ahlin S, Olsson M, Sjoholm K, Karlsson A, Bylund J. Endogenous Acute Phase Serum Amyloid A Lacks Pro-Inflammatory Activity, Contrasting the Two Recombinant Variants That Activate Human Neutrophils through Different Receptors. Front Immunol. 2013; 4:92. [PubMed: 23626589]

Coetzee GA, Strachan AF, van der Westhuyzen DR, Hoppe HC, Jeenah MS, de Beer FC. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. J Biol Chem. 1986; 261:9644–51. [PubMed: 3525531]

de Beer MC, Ji A, Jahangiri A, Vaughan AM, de Beer FC, van der Westhuyzen DR, Webb NR. ATP binding cassette G1-dependent cholesterol efflux during inflammation. J Lipid Res. 2011; 52:345–53. [PubMed: 21138980]

de Beer MC, Webb NR, Wroblewski JM, Noffsinger VP, Rateri DL, Ji A, van der Westhuyzen DR, de Beer FC. Impact of serum amyloid A on high density lipoprotein composition and levels. J Lipid Res. 2010; 51:3117–25. [PubMed: 20667817]

de Beer MC, Wroblewski JM, Noffsinger VP, Ji A, Meyer JM, van der Westhuyzen DR, de Beer FC, Webb NR. The Impairment of Macrophage-to-Feces Reverse Cholesterol Transport during Inflammation Does Not Depend on Serum Amyloid A. J Lipids. 2013; 2013:283486. [PubMed: 23431457]

De Beer MC, Wroblewski JM, Noffsinger VP, Rateri DL, Howatt DA, Balakrishnan A, Ji A, Shridas P, Thompson JC, van der Westhuyzen DR, Tannock LR, Daugherty A, Webb NR, De Beer FC. Deficiency of endogenous acute phase serum amyloid A does not affect atherosclerotic lesions in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2014; 34:255–61. [PubMed: 24265416]

de Oliveira EM, Sandri S, Knebel FH, Contesini CG, Campa A, Filippin-Monteiro FB. Hypoxia increases serum amyloid A3 (SAA3) in differentiated 3T3-L1 adipocytes. Inflammation. 2013; 36:1107–10. [PubMed: 23605472]

Deguchi A, Tomita T, Omori T, Komatsu A, Ohto U, Takahashi S, Tanimura N, Akashi-Takamura S, Miyake K, Maru Y. Serum amyloid A3 binds MD-2 to activate p38 and NF-kappaB pathways in a MyD88-dependent manner. J Immunol. 2013; 191:1856–64. [PubMed: 23858030]

den Hartigh LJ, Wang S, Goodspeed L, Ding Y, Averill M, Subramanian S, Wietecha T, O'Brien KD, Chait A. Deletion of serum amyloid A3 improves high fat high sucrose diet-induced adipose tissue inflammation and hyperlipidemia in female mice. PLoS One. 2014; 9:e108564. [PubMed: 25251243]

Derebe MG, Zlatkov CM, Gattu S, Ruhn KA, Vaishnava S, Diehl GE, MacMillan JB, Williams NS, Hooper LV. Serum amyloid A is a retinol binding protein that transports retinol during bacterial infection. Elife. 2014; 3:e03206. [PubMed: 25073702]

Dong Z, Wu T, Qin W, An C, Wang Z, Zhang M, Zhang Y, Zhang C, An F. Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Mol Med. 2011; 17:1357–64. [PubMed: 21953420]

Eckhardt ER, Witta J, Zhong J, Arsenescu R, Arsenescu V, Wang Y, Ghoshal S, de Beer MC, de Beer FC, de Villiers WJ. Intestinal epithelial serum amyloid A modulates bacterial growth in vitro and pro-inflammatory responses in mouse experimental colitis. BMC Gastroenterol. 2010; 10:133. [PubMed: 21067563]

Eklund KK, Niemi K, Kovanen PT. Immune functions of serum amyloid A. Crit Rev Immunol. 2012; 32:335–48. [PubMed: 23237509]

El Kebir D, Jozsef L, Khreiss T, Pan W, Petasis NA, Serhan CN, Filep JG. Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. J Immunol. 2007; 179:616–22. [PubMed: 17579083]

Feng ZP, Li XY, Jiang R, Deng HC, Yang M, Zhou Q, Que WJ, Du J. Associations of SAA1 gene polymorphism with lipid lelvels and osteoporosis in Chinese women. Lipids Health Dis. 2013; 12:39. [PubMed: 23522429]

Filep JG, El Kebir D. Serum amyloid A as a marker and mediator of acute coronary syndromes. Future Cardiol. 2008; 4:495–504. [PubMed: 19804343]

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. Biochem Biophys Res Commun. 2000; 268:405–8. [PubMed: 10679217]

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999; 340:448–454. [PubMed: 9971870]

Getz GS, Reardon CA. SAA, HDL biogenesis, and inflammation. J Lipid Res. 2008; 49:269–70. [PubMed: 18199807]

Hansen MT, Forst B, Cremers N, Quagliata L, Ambartsumian N, Grum-Schwensen B, Klingelhofer J, Abdul-Al A, Herrmann P, Osterland M, Stein U, Nielsen GH, Scherer PE, Lukanidin E, Sleeman JP, Grigorian M. A link between inflammation and metastasis: serum amyloid A1 and A3 induce metastasis, and are targets of metastasis-inducing S100A4. Oncogene. 2015; 34:424–35. [PubMed: 24469032]

Hari-Dass R, Shah C, Meyer DJ, Raynes JG. Serum amyloid A protein binds to outer membrane protein A of gram-negative bacteria. J Biol Chem. 2005; 280:18562–7. [PubMed: 15705572]

He R, Sang H, Ye RD. Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. Blood. 2003; 101:1572–81. [PubMed: 12393391]

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. J Immunol. 2006; 177:4072–9. [PubMed: 16951371]

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; 113:429–37. [PubMed: 18952897]

Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, Shibuya M, Akira S, Aburatani H, Maru Y. The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. Nat Cell Biol. 2008; 10:1349–55. [PubMed: 18820689]

Hosoai H, Webb NR, Glick JM, Tietge UJ, Purdom MS, de Beer FC, Rader DJ. Expression of serum amyloid A protein in the absence of the acute phase response does not reduce HDL cholesterol or apoA-I levels in human apoA-I transgenic mice. J Lipid Res. 1999; 40:648–53. [PubMed: 10191288]

Husby G, Natvig JB. A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. J Clin Invest. 1974; 53:1054–61. [PubMed: 4205522]

Jahangiri A, de Beer MC, Noffsinger V, Tannock LR, Ramaiah C, Webb NR, van der Westhuyzen DR, de Beer FC. HDL remodeling during the acute phase response. Arterioscler Thromb Vasc Biol. 2009; 29:261–7. [PubMed: 19008529]

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. J Biol Chem. 2015; 290:12804–11. [PubMed: 25847238]

Kim MH, de Beer MC, Wroblewski JM, Webb NR, de Beer FC. SAA does not induce cytokine production in physiological conditions. Cytokine. 2013; 61:506–12. [PubMed: 23165195]

Kindy MS, de Beer MC, Yu J, de Beer FC. Expression of mouse acute-phase (SAA1.1) and constitutive (SAA4) serum amyloid A isotypes: influence on lipoprotein profiles. Arterioscler Thromb Vasc Biol. 2000; 20:1543–50. [PubMed: 10845870]

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. Biochem J. 1998; 332(Pt 3):721–8. [PubMed: 9620875]

King VL, Thompson J, Tannock LR. Serum amyloid A in atherosclerosis. Curr Opin Lipidol. 2011; 22:302–7. [PubMed: 21734573]

Kisilevsky R. Serum amyloid A (SAA), a protein without a function: some suggestions with reference to cholesterol metabolism. Med Hypotheses. 1991; 35:337–41. [PubMed: 1943886]

Kisilevsky R, Manley PN. Acute-phase serum amyloid A: perspectives on its physiological and pathological roles. Amyloid. 2012; 19:5–14.

Kisilevsky R, Subrahmanyan L. Serum amyloid A changes high density lipoprotein's cellular affinity. A clue to serum amyloid A's principal function. Lab Invest. 1992; 66:778–85. [PubMed: 1602745]

Kluve-Beckerman B, Drumm ML, Benson MD. Nonexpression of the human serum amyloid A three (SAA3) gene. DNA Cell Biol. 1991; 10:651–61. [PubMed: 1755958]

Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Kohler G. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature. 1994; 368:339–42. [PubMed: 8127368]

Lee HY, Kim MK, Park KS, Bae YH, Yun J, Park JI, Kwak JY, Bae YS. Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochem Biophys Res Commun. 2005; 330:989–98. [PubMed: 15809093]

Lee JM, Kim EK, Seo H, Jeon I, Chae MJ, Park YJ, Song B, Kim YS, Kim YJ, Ko HJ, Kang CY. Serum amyloid A3 exacerbates cancer by enhancing the suppressive capacity of myeloid-derived suppressor cells via TLR2-dependent STAT3 activation. Eur J Immunol. 2014; 44:1672–84. [PubMed: 24659444]

Leow KY, Goh WW, Heng CK. Effect of serum amyloid A1 treatment on global gene expression in THP-1-derived macrophages. Inflamm Res. 2012; 61:391–8. [PubMed: 22228103]

Leow KY, Goh WW, Tan SZ, Lim J, Ng K, Oh VM, Low AF, Heng CK. Variant screening of the serum amyloid A1 gene and functional study of the p.Gly90Asp variant for its role in atherosclerosis. Atherosclerosis. 2013; 227:112–7. [PubMed: 23357645]

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. J Exp Med. 1973; 138:373–80. [PubMed: 4198200]

Liepnieks JJ, Kluve-Beckerman B, Benson MD. Characterization of amyloid A protein in human secondary amyloidosis: the predominant deposition of serum amyloid A1. Biochim Biophys Acta. 1995; 1270:81–6. [PubMed: 7827140]

Liu C. Serum amyloid a protein in clinical cancer diagnosis. Pathol Oncol Res. 2012; 18:117–21. [PubMed: 21901273]

Lowell CA, Potter DA, Stearman RS, Morrow JF. Structure of the murine serum amyloid A gene family. Gene conversion. J Biol Chem. 1986; 261:8442–52. [PubMed: 3013853]

Lu J, Yu Y, Zhu I, Cheng Y, Sun PD. Structural mechanism of serum amyloid A-mediated inflammatory amyloidosis. Proc Natl Acad Sci U S A. 2014; 111:5189–94. [PubMed: 24706838]

Lung HL, Man OY, Yeung MC, Ko JM, Cheung AK, Law EW, Yu Z, Shuen WH, Tung E, Chan SH, Bangarusamy DK, Cheng Y, Yang X, Kan R, Phoon Y, Chan KC, Chua D, Kwong DL, Lee AW, Ji MF, Lung ML. SAA1 polymorphisms are associated with variation in antiangiogenic and tumor-

suppressive activities in nasopharyngeal carcinoma. Oncogene. 2014; 34:878–89. [PubMed: 24608426]

Malle E, De Beer FC. Human serum amyloid A (SAA) protein: a prominent acute-phase reactant for clinical practice. Eur J Clin Invest. 1996; 26:427–435. [PubMed: 8817153]

Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. Cell Mol Life Sci. 2009; 66:9–26. [PubMed: 18726069]

Marsche G, Frank S, Raynes JG, Kozarsky KF, Sattler W, Malle E. The lipidation status of acute-phase protein serum amyloid A determines cholesterol mobilization via scavenger receptor class B, type I. Biochem J. 2007; 402:117–24. [PubMed: 17034364]

Meek RL, Benditt EP. Amyloid A gene family expression in different mouse tissues. J Exp Med. 1986; 164:2006–17. [PubMed: 3783088]

Migita K, Agematsu K, Masumoto J, Ida H, Honda S, Jiuchi Y, Izumi Y, Maeda Y, Uehara R, Nakamura Y, Koga T, Kawakami A, Nakashima M, Fujieda Y, Nonaka F, Eguchi K, Furukawa H, Nakamura T, Nakamura M, Yasunami M. The contribution of SAA1 polymorphisms to Familial Mediterranean fever susceptibility in the Japanese population. PLoS One. 2013; 8:e55227. [PubMed: 23437051]

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. Lab Invest. 1998; 78:535–9. [PubMed: 9605178]

Milan E, Lazzari C, Anand S, Floriani I, Torri V, Sorlini C, Gregorc V, Bachi A. SAA1 is over-expressed in plasma of non small cell lung cancer patients with poor outcome after treatment with epidermal growth factor receptor tyrosine-kinase inhibitors. J Proteomics. 2012; 76(Spec No):91–101. [PubMed: 22771314]

Mori M, Tiang G, Higuchi K. AA amyloidosis-resistant CE/J mice have Saa1 and Saa2 genes that encode an identical SAA isoform. Amyloid. 2014; 21:1–8. [PubMed: 24228751]

Moriguchi M, Kaneko H, Terai C, Koseki Y, Kajiyama H, Inada S, Kitamura Y, Kamatani N. Relative transcriptional activities of SAA1 promoters polymorphic at position -13(T/C): potential association between increased transcription and amyloidosis. Amyloid. 2005; 12:26–32. [PubMed: 16076608]

Mullan RH, Bresnihan B, Golden-Mason L, Markham T, O'Hara R, FitzGerald O, Veale DJ, Fearon U. Acute-phase serum amyloid A stimulation of angiogenesis, leukocyte recruitment, and matrix degradation in rheumatoid arthritis through an NF-kappaB-dependent signal transduction pathway. Arthritis Rheum. 2006; 54:105–14. [PubMed: 16385502]

Nakamura T, Higashi S, Tomoda K, Tsukano M, Baba S, Shono M. Significance of SAA1.3 allele genotype in Japanese patients with amyloidosis secondary to rheumatoid arthritis. Rheumatology (Oxford). 2006; 45:43–9. [PubMed: 16219644]

O'Brien KD, Chait A. Serum amyloid A: the "other" inflammatory protein. Curr Atheroscler Rep. 2006; 8:62–8. [PubMed: 16455016]

O'Hara R, Murphy EP, Whitehead AS, FitzGerald 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 Rheum. 2004; 50:1788–99. [PubMed: 15188355]

Olsson M, Ahlin S, Olsson B, Svensson PA, Stahlman M, Boren J, Carlsson LM, Sjoholm K. Establishment of a transgenic mouse model specifically expressing human serum amyloid A in adipose tissue. PLoS One. 2011; 6:e19609. [PubMed: 21611116]

Paret C, Schon Z, Szponar A, Kovacs G. Inflammatory protein serum amyloid A1 marks a subset of conventional renal cell carcinomas with fatal outcome. Eur Urol. 2010; 57:859–66. [PubMed: 19747761]

Patel H, Fellowes R, Coade S, Woo P. Human serum amyloid A has cytokine-like properties. Scand J Immunol. 1998; 48:410–8. [PubMed: 9790312]

Patke S, Maheshwari R, Litt J, Srinivasan S, Aguilera JJ, Colon W, Kane RS. Influence of the carboxy terminus of serum amyloid A on protein oligomerization, misfolding, and fibril formation. Biochemistry. 2012; 51:3092–9. [PubMed: 22448726]

Prelli F, Pras M, Frangione B. Degradation and deposition of amyloid AA fibrils are tissue specific. Biochemistry. 1987; 26:8251–6. [PubMed: 3442653]

Quinton LJ, Blahna MT, Jones MR, Allen E, Ferrari JD, Hilliard KL, Zhang X, Sabharwal V, Algul H, Akira S, Schmid RM, Pelton SI, Spira A, Mizgerd JP. Hepatocyte-specific mutation of both NF-kappaB RelA and STAT3 abrogates the acute phase response in mice. J Clin Invest. 2014; 122:1758–63.

Ren Y, Wang H, Lu D, Xie X, Chen X, Peng J, Hu Q, Shi G, Liu S. Expression of serum amyloid A in uterine cervical cancer. Diagn Pathol. 2014; 9:16. [PubMed: 24447576]

Rosenthal CJ, Franklin EC. Variation with age and disease of an amyloid A protein-related serum component. J Clin Invest. 1975; 55:746–53. [PubMed: 47333]

Rosenthal CJ, Franklin EC, Frangione B, Greenspan J. Isolation and partial characterization of SAA-an amyloid-related protein from human serum. J Immunol. 1976; 116:1415–8. [PubMed: 1270801]

Sandri S, Rodriguez D, Gomes E, Monteiro HP, Russo M, Campa A. Is serum amyloid A an endogenous TLR4 agonist? J Leukoc Biol. 2008; 83:1174–80. [PubMed: 18252871]

Sano T, Huang W, Hall JA, Yang Y, Chen A, Gavzy SJ, Lee JY, Ziel JW, Miraldi ER, Domingos AI, Bonneau R, Littman DR. An IL-23R/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses. Cell. 2015; 163:381–93. [PubMed: 26411290]

Sellar GC, Jordan SA, Bickmore WA, Fantes JA, van Heyningen V, Whitehead AS. The human serum amyloid A protein (SAA) superfamily gene cluster: mapping to chromosome 11p15.1 by physical and genetic linkage analysis. Genomics. 1994; 19:221–7. [PubMed: 8188252]

Shah C, Hari-Dass R, Raynes JG. Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. Blood. 2006; 108:1751–7. [PubMed: 16735604]

Simons JP, Al-Shawi R, Ellmerich S, Speck I, Aslam S, Hutchinson WL, Mangione PP, Disterer P, Gilbertson JA, Hunt T, Millar DJ, Minogue S, Bodin K, Pepys MB, Hawkins PN. Pathogenetic mechanisms of amyloid A amyloidosis. Proc Natl Acad Sci U S A. 2013; 110:16115–20. [PubMed: 23959890]

Sipe J. Revised nomenclature for serum amyloid A (SAA). Nomenclature Committee of the International Society of Amyloidosis. Part 2. Amyloid. 1999; 6:67–70. [PubMed: 10211414]

Soler L, Luyten T, Stinckens A, Buys N, Ceron JJ, Niewold TA. Serum amyloid A3 (SAA3), not SAA1 appears to be the major acute phase SAA isoform in the pig. Vet Immunol Immunopathol. 2011; 141:109–15. [PubMed: 21439655]

Soler L, Molenaar A, Merola N, Eckersall PD, Gutierrez A, Ceron JJ, Mulero V, Niewold TA. Why working with porcine circulating serum amyloid A is a pig of a job. J Theor Biol. 2013; 317:119–25. [PubMed: 23073471]

Srinivasan S, Patke S, Wang Y, Ye Z, Litt J, Srivastava SK, Lopez MM, Kurouski D, Lednev IK, Kane RS, Colon W. Pathogenic serum amyloid A 1.1 shows a long oligomer-rich fibrillation lag phase contrary to the highly amyloidogenic non-pathogenic SAA2.2. J Biol Chem. 2013; 288:2744–55. [PubMed: 23223242]

Stevens FJ. Hypothetical structure of human serum amyloid A protein. Amyloid. 2004; 11:71–80. [PubMed: 15478462]

Stonik JA, Remaley AT, Demosky SJ, Neufeld EB, Bocharov A, Brewer HB. Serum amyloid A promotes ABCA1-dependent and ABCA1-independent lipid efflux from cells. Biochem Biophys Res Commun. 2004; 321:936–41. [PubMed: 15358117]

Su SB, Gong W, Gao JL, Shen W, Murphy PM, Oppenheim JJ, Wang JM. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. J Exp Med. 1999; 189:395–402. [PubMed: 9892621]

Sun L, Zhou H, Zhu Z, Yan Q, Wang L, Liang Q, Ye RD. Ex vivo and in vitro effect of serum amyloid a in the induction of macrophage m2 markers and efferocytosis of apoptotic neutrophils. J Immunol. 2015; 194:4891–900. [PubMed: 25870242]

Takase H, Tanaka M, Miyagawa S, Yamada T, Mukai T. Effect of amino acid variations in the central region of human serum amyloid A on the amyloidogenic properties. Biochem Biophys Res Commun. 2014; 444:92–7. [PubMed: 24440699]

Tape C, Tan R, Nesheim M, Kisilevsky R. Direct evidence for circulating apoSAA as the precursor of tissue AA amyloid deposits. Scand J Immunol. 1988; 28:317–24. [PubMed: 3194701]

Thaler R, Sturmlechner I, Spitzer S, Riester SM, Rumpler M, Zwerina J, Klaushofer K, van Wijnen AJ, Varga F. Acute-phase protein serum amyloid A3 is a novel paracrine coupling factor that controls bone homeostasis. FASEB J. 2015; 29:1344–59. [PubMed: 25491310]

Thompson JC, Jayne C, Thompson J, Wilson PG, Yoder MH, Webb N, Tannock LR. A brief elevation of serum amyloid A is sufficient to increase atherosclerosis. J Lipid Res. 2015; 56:286–93. [PubMed: 25429103]

Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem. 1999; 265:501–23. [PubMed: 10504381]

van den Brand B, Waterborg C, van den Berg W, van de Loo F. Is the serum amyloid A we use really serum amyloid A? Comment on the artible by Connolly et al. Arthritis Rheum. 2013; 65:283–90. [PubMed: 23044993]

van der Hilst JC, Yamada T, Op den Camp HJ, van der Meer JW, Drenth JP, Simon A. Increased susceptibility of serum amyloid A 1.1 to degradation by MMP-1: potential explanation for higher risk of type AA amyloidosis. Rheumatology (Oxford). 2008; 47:1651–4. [PubMed: 18815155]

van der Westhuyzen DR, Cai L, de Beer MC, de Beer FC. Serum amyloid A promotes cholesterol efflux mediated by scavenger receptor B-I. J Biol Chem. 2005; 280:35890–5. [PubMed: 16120612]

Webb NR, de Beer MC, van der Westhuyzen DR, Kindy MS, Banka CL, Tsukamoto K, Rader DL, de Beer FC. Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice. J Lipid Res. 1997; 38:1583–90. [PubMed: 9300780]

Webb NR, De Beer MC, Wroblewski JM, Ji A, Bailey W, Shridas P, Charnigo RJ, Noffsinger VP, Witta J, Howatt DA, Balakrishnan A, Rateri DL, Daugherty A, De Beer FC. Deficiency of Endogenous Acute-Phase Serum Amyloid A Protects apoE−/− Mice From Angiotensin II-Induced Abdominal Aortic Aneurysm Formation. Arterioscler Thromb Vasc Biol. 2015; 35:1156–65. [PubMed: 25745063]

Wilson PG, Thompson JC, Webb NR, de Beer FC, King VL, Tannock LR. Serum amyloid A, but not C-reactive protein, stimulates vascular proteoglycan synthesis in a pro-atherogenic manner. Am J Pathol. 2008; 173:1902–10. [PubMed: 18974302]

Xie X, Ma YT, Yang YN, Fu ZY, Li XM, Ma X, Huang D, Liu F, Chen BD, Xiang Y, Huang Y. Association of genetic polymorphisms of serum amyloid protein A1 with plasma high density lipoproteins cholesterol. Zhonghua Yi Xue Za Zhi. 2010; 90:1824–6. [PubMed: 20979827]

Xie X, Ma YT, Yang YN, Fu ZY, Li XM, Zheng YY, Huang D, Ma X, Chen BD, Liu F. Polymorphisms in the SAA1 gene are associated with ankle-to-brachial index in Han Chinese healthy subjects. Blood Press. 2011; 20:232–8. [PubMed: 21449704]

Xie X, Ma YT, Yang YN, Li XM, Fu ZY, Zheng YY, Ma X, Chen BD, Liu F, Huang Y, Yu ZX, Chen Y. Serum uric acid levels are associated with polymorphism in the SAA1 gene in Chinese subjects. PLoS One. 2012; 7:e40263. [PubMed: 22768267]

Xie X, Ma YT, Yang YN, Li XM, Zheng YY, Liu F, Ma X, Fu ZY, Yu ZX, Chen Y, Chen BD, Huang Y. Genetic polymorphisms of serum amyloid A1 and coronary artery disease risk. Tissue Antigens. 2015; 85:168–76. [PubMed: 25656165]

Yamada T. Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin Chem Lab Med. 1999; 37:381–8. [PubMed: 10369107]

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. Cell Signal. 2014; 26:1783–91. [PubMed: 24703936]

Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN, Murphy PM. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. Pharmacol Rev. 2009; 61:119–61. [PubMed: 19498085]

Ye RD, Sun L. Emerging functions of serum amyloid A in inflammation. J Leukoc Biol. 2015 jlb. 3VMR0315-080R. Epub ahead of print.

Zhang LJ, Yuan B, Li HH, Tao SB, Yan HQ, Chang L, Zhao JH. Associations of genetic polymorphisms of SAA1 with cerebral infarction. Lipids Health Dis. 2013a; 12:130. [PubMed: 23987125]

Zhang X, Tang QZ, Wan AY, Zhang HJ, Wei L. SAA1 gene variants and childhood obesity in China. Lipids Health Dis. 2013b; 12:161. [PubMed: 24171731]

Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann KJ, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, et al. Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity. 1995; 3:9–19. [PubMed: 7621081]

Highlights

- SAA1 is a major acute-phase serum amyloid A in humans
- Polymorphism of the SAA1 gene is associated with disease disposition
- The use of a chimeric recombinant SAA in some studies has raised concerns
- SAA1 transgenic and knockout mice have been generated for in vivo studies
- Recent studies have shown regulatory and homeostatic functions of SAA1

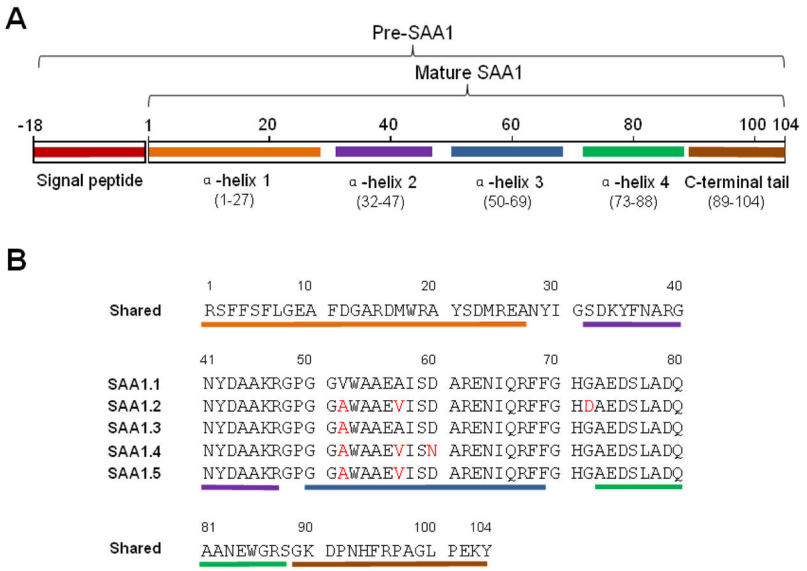

Figure 1. Schematic depiction of SAA1 and its protein sequence variations

A, linear representation of human SAA1 showing its N-terminal signal peptide of 18 amino acids and the mature SAA1 protein. The 4 α-helices and the C-terminal tail reported recently based on a SAA1.1 crystal structure (Lu et al., 2014) are marked with different colors and their relative positions are indicated. *B*, comparison of the amino acid sequence encoded by the human SAA1 allelic variants. Mature protein sequences of the 5 human SAA1 variants are shown (based on Sipe et al., 1999). Different amino acids in each of the variants are highlighted in red. Amino acids corresponding to the α-helices and the C-terminal tail are underlined.

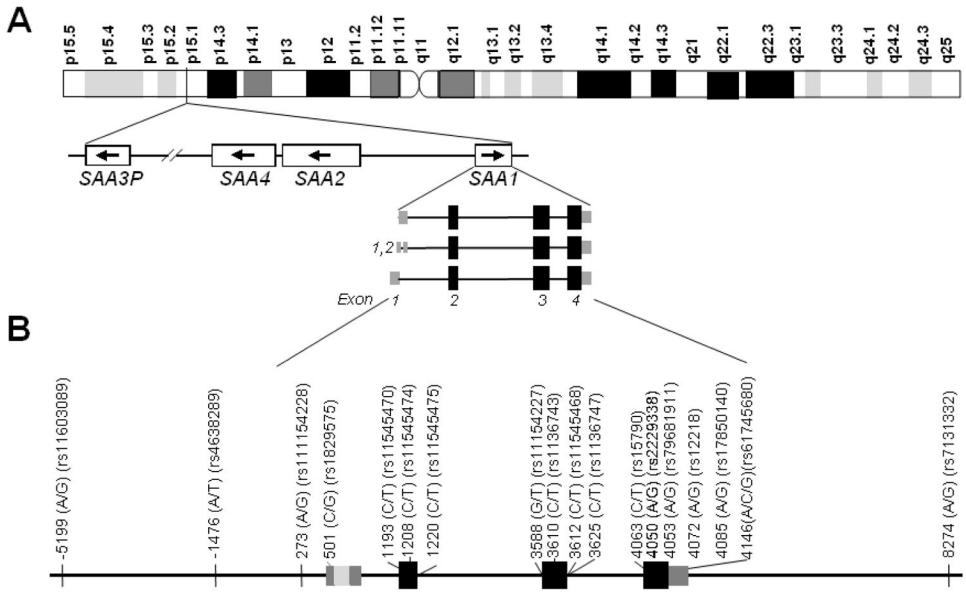

Figure 2. Organization of the human SAA gene cluster and single nucleotide polymorphisms in the human SAA1 gene

A, linear map of human *SAA1* relative to other human SAA genes including the pseudogene *SAA3P*, on chromosome 11p15.1 (Betts et al., 1991; Sellar et al., 1994). Arrows depict the orientation of the genes. The organization of exons in *SAA1* is based on GenBank entries (from top) NM_199161, NM_001178006, and NM_000331, respectively. The human *SAA1* gene has the typical 4-exon and 3-intron organization seen in other species. The coding regions in the exons are marked in black and non-coding regions are marked in gray. *B,* the confirmed human *SAA1* SNPs (Leow et al., 2013) are marked in the SAA1 gene. The numbers in each name indicate nucleotide positions of the SNPs, and substitutions in nucleotides are shown in the first set of parentheses. The second set of parentheses contain the names of the SNPs.

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hSAA1.1   RSFFSFLGEA FDGARDMWRA YSDMREANYI GSDKYFHARG NYDAAKRGPG GVWAAEAISD
mSAA1.1   -GFFSFIGEA FQGAGDMWRA YTDMKEAGWK DGDKYFHARG NYDAAQRGPG GVWAAEKISD
mSAA2.1   -GFFSFVHEA FQGAGDMWRA YTDMKEANWK NSDKYFHARG NYDAAQRGPG GVWAAEKISD
mSAA2.2   -GFFSFVHEA FLGAGDMWRA YTDMKEAGWK DGDKYFHARG NYDAAQRGPG GVWAAEKISD
mSAA3     -RWVQFMKEA GQGSRDMWRA YSDMKKANWK NSDKYFHARG NYDAARRGPG GAWAAKVISD
pSAA2     QRWASFLKEA GQGAKDMWRA YSDMREANYK NSDKYFHARG NYDAAQRGPG GAWAAKVISD
               *  **      ***** * **  *      ******** ***** **** * ***  ***

hSAA1.1   ARENIQRFF- -------GHG AEDSLADQAA NEWGRSGKDP NHFRPAGLPE KY
mSAA1.1   ARESFQEFF- -------GRG HEDTMADQEA NRHGRSGKDP NYYRPPGLPA KY
mSAA2.1   GREAFQEFF- -------GRG HEDTIADQEA NRHGRSGKDP NYYRPPGLPD KY
mSAA2.2   GREAFQEFF- -------GRG HEDTMADQEA NRHGRSGKDP NYYRPPGLPD KY
mSAA3     AREAVQKFT- -------GHG AEDSRADQFA NEWGRSGKDP NHFRPAGLPK RY
pSAA2     ARENVQRVTD LFKHGDSGHG VEDSRADQAA NAWGRSGKDP NHFRPRGLPD KY
           **  *           * *  *  *** * *  ******* *  ** ***   *
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Figure 3. Comparison of the amino acid sequence of human SAA1.1 with SAA isoforms from mice and pigs

Mature protein sequences of the known SAA isoforms are shown, with identical amino acids marked with asterisks (*) and unmatched gaps marked with dashes (−). The sequences shown are derived from NCBI (http://www.ncbi.nlm.nih.gov) and include human SAA1.1 (NP_000322.2), mouse Saa1.1 (NP_035444.1), mouse Saa2.1 (NP_033143.1), mouse Saa2.2 (AAA19818.1), and porcine SAA2 (NP_001038017.1). Porcine SAA-like (not shown) is available at XP_003122986.2 of NCBI. These SAA proteins are listed according to the nomenclature of J. Sipe (1999). Note that the pre-1999 nomenclature is still in use by NCBI, such that the mouse Saa1.1 shown in this figure is marked as Saa2, and the mouse Saa2.1 shown is labeled as Saa1, in the NCBI protein database.