JLB

Review

Emerging functions of serum amyloid A in inflammation

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RECEIVED MARCH 2, 2015; REVISED MAY 31, 2015; ACCEPTED JUNE 2, 2015. DOI: 10.1189/jlb.3VMR0315-080R

ABSTRACT

SAA is a major acute-phase protein produced in large quantity during APR. The rise of SAA concentration in blood circulation during APR has been a clinical marker for active inflammation. In the past decade, research has been conducted to determine whether SAA plays an active role during inflammation and if so, how it influences the course of inflammation. These efforts have led to the discovery of cytokine-like activities of rhSAA, which is commercially available and widely used in most of the published studies. SAA activates multiple receptors, including the FPR2, the TLRs TLR2 and TLR4, the scavenger receptor SR-BI, and the ATP receptor P2X7. More recent studies have shown that SAA not only activates transcription factors, such as NF-κB, but also plays a role in epigenetic regulation through a MyD88-IRF4-Jmjd3 pathway. It is postulated that the activation of these pathways leads to induced expression of proinflammatory factors and a subset of proteins expressed by the M2 macrophages. These functional properties set SAA apart from well-characterized inflammatory factors, such as LPS and TNF-α, suggesting that it may play a homeostatic role during the course of inflammation. Ongoing and future studies are directed to addressing unresolved issues, including the difference between rSAA and native SAA isoforms and the exact functions of SAA in physiologic and pathologic settings.
***J. Leukoc. Biol.* 98: 923–929; 2015.**

Introduction

The APR is a systemic reaction to environmental insults that are harmful to the host, including infection, trauma, severe stress, and late-stage neoplasia [1]. APR is conserved across all mammals and is highly coordinated, involving numerous changes in the expression level of plasma proteins, as well as metabolic, physiologic, and behavioral changes. Central to these changes is the hepatic production of acute-phase proteins, including SAA, C-reactive protein, and complement proteins [2]. It is widely believed that APR serves to isolate pathogens for eradication and to reduce tissue damage. However, how APR protects host against environmental insults remains largely unknown. During APR, the plasma level of SAA rises by up to 1000-fold [2]. In inflammatory tissues, macrophages serve as a major source of SAA [3]. Elevated SAA production is seen in rheumatoid arthritis [4, 5], atherosclerosis [6–8], Crohn's disease [9], and Type 2 diabetes [10, 11]. These findings suggest that SAA may play an active role in inflammatory disorders.

SAA GENES AND SUBTYPES

To understand the potential functions of SAA in physiologic and pathologic conditions, it is necessary to first examine the SAA genes and subtypes. SAA is the name of a collection of small proteins with 104 aa in their mature forms [12]. These proteins are encoded by 4 separate but closely related genes located on chromosome 11 [13]. In humans, the expression of SAA1 and SAA2 is induced by inflammatory cues, including IL-1β, IL-6, and LPS. The induction of these genes involves the transcription factors NF-κB, AP-1, and Yin Yang 1, which are activated during APR [14]. Induced expression of SAA1 and SAA2 by hepatocytes contributes significantly to the rise of SAA in plasma during APR. Human *SAA3* is a pseudogene, and human *SAA4* encodes a protein that is constitutively produced [15]. As a result, the inducible SAA isoforms (SAA1 and SAA2) are termed acute-phase SAAs. Both SAA1 and SAA2 are also produced in inflammatory tissues by several types of cells, including macrophages. In mice, Saa1 and Saa2 are major forms of SAA proteins produced by hepatocytes, but mouse *Saa3* encodes a functional SAA protein and is the major form of SAA in inflammatory tissues. Mouse *Saa4*, like the human counterpart, is constitutively expressed [16–19].

The SAA isoforms produced by hepatocytes during APR are rapidly released into blood circulation, where they associate with HDL [20]. With a marked increase in plasma SAA level, acute-phase SAA can displace Apo A-1 and becomes tightly

Abbreviations: Apo A-1 = apolipoprotein A1, APR = acute-phase response, CLA-1 = CD36 and lysosomal integral membrane protein type II analogous-1, FPR2 = formyl peptide receptor 2, IL-1ra/rn = IL-1R antagonist, IRF = IFN regulatory factor, Jmjd3 = Jumonji domain containing-3, MMP = matrix metalloproteinase, NLRP3 = nucleotide-binding oligomerization domain-like receptor 3, rhSAA = recombinant human serum amyloid A, SAA = serum amyloid A, SR-BI = scavenger receptor class B type I

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associated with HDL [21]. The exact role for SAA in lipid metabolism has been the subject of intense research. It has led to the findings of SAA modulation of cholesterol efflux and of SAA binding to the SR-BI (mouse) and CLA-1 (human). The HDL-associated SAA proteins display different physical properties than their free form, and it is believed that lipid-associated SAA is functionally different from lipid-free SAA. In blood circulation, SAA may serve as an immune opsonin for Gram-negative bacteria, including *Escherichia coli* and *P. Pseudomonas aeruginosa* [22]. Both human and mouse SAA proteins have been found to bind retinol with nanomolar affinity that limits bacterial burden in tissues after acute infection [23].

In contrast to the hepatocyte-derived SAA that enters blood circulation, tissue-derived SAA proteins interact with nearby cells through a number of SAA receptors (**Fig. 1**). High levels of SAA proteins are found in the joints of rheumatoid arthritis patients, in blood vessels with atherosclerotic lesions, and in many types of solid tumors. Published studies have associated tissue-derived SAA with the progression of rheumatoid arthritis, atherosclerosis, and tumor metastasis. However, whether SAA serves to accelerate the progression of these disorders or deter their development remains to be investigated.

THE CYTOKINE-LIKE PROPERTIES OF SAA

With the use of rhSAA, Badolato et al. [24] first identified its chemotactic activity for phagocytes. The study was conducted in vitro with freshly isolated blood neutrophils. SAA was found to induce the migration of these cells at 0.8 μM, a concentration easily attainable during APR. A subsequent study shows that this activity of SAA is mediated through the FPR2 that is present on neutrophils and other phagocytes [25].

Patel et al. [26] reported that rhSAA could induce the transcripts of IL-1β, IL-1rn (also termed IL-1ra), and TNFR-II in the human monocytic cell line THP-1. Subsequently, other studies have shown that rhSAA can induce the expression of TNFα, IL-6, and IL-8 in stimulated blood neutrophils [27, 28]. In rheumatoid arthritis patients, elevation of the local SAA level is associated with increased expression of MMP, which contributes to joint damage [29]. Peripheral blood monocytes exposed to rhSAA secrete the tissue factor, which has proinflammatory functions [30]. In vitro studies have also shown that rhSAA induces the expression of IL-12p40, as well as IL-23p19, but not IL-12p35. As a result, rhSAA is an agonist that stimulates monocyte production of IL-23 (dimer of p40 and p19) but not IL-12 (dimer of p40 and p35) [31]. Subcutaneous injection of rhSAA results in elevated *Csf3* (mouse G-CSF gene) expression, accompanied with increased neutrophil count in peripheral blood [32].

Collectively, these findings suggest that rhSAA may serve as an inflammatory cytokine. Whereas rhSAA is chemotactic to phagocytes, its structure differs from that of the chemokines [33]. As a result, SAA does not belong to any family of known chemokines and inflammatory cytokines. Unlike most inflammatory cytokines, rhSAA has pleotropic effects in vitro, as it not only induces the expression of proinflammatory cytokines but also those exhibiting anti-inflammatory properties, such as IL-1rn and IL-10 [34]. Systemic SAA1 induces IL-10-secreting neutrophils and promotes their interaction with invariant NK T cells, which in turn, limits the suppressive activity of these neutrophils by diminishing IL-10 production and promoting IL-12 production [35]. More recent studies have shown that rhSAA stimulates the expression of IL-33 that modulates different functions of immune cells [36]. As reported at the 2014 Joint Meeting of Society for Leukocyte Biology & International Endotoxin and Innate Immunity Society, human SAA1 are able to skew macrophages toward the M2 phenotype, including an enhanced ability to clear apoptotic neutrophils (efferocytosis), and induced expression of a set of secreted and cell-surface proteins, collectively termed the M2 markers [37]. These findings challenge the traditional view that SAA promotes the inflammatory process through its cytokine-like activities, suggesting that the primary role for SAA may be homeostatic rather than proinflammatory or anti-inflammatory.

SAA RECEPTORS AND DOWNSTREAM SIGNALING PATHWAYS

The pleotropic functions of rhSAA imply that this acute-phase protein may interact with >1 receptor and activate multiple signaling pathways. To date, at least 6 receptors have been identified for SAA (**Fig. 1**). FPR2 is a G protein-coupled chemoattractant receptor with multiple agonists [38, 39]. Exogenous expression of FPR2 in cell lines that lack the native receptor confers the ability of these cells to migrate toward SAA [25] and to respond to SAA stimulation with other cellular activities, such as NF-κB activation and production of the chemokine IL-8 [28, 40]. The expression of FPR2 is elevated in arthritic synovium along with the production of MMP [29]. The SAA-FPR2 pair also contributes to inflammation-mediated neovascularization in cornea [41]. Interestingly, SAA appears to exert an effect opposite to that of lipoxin A4 in several studies measuring induced production of proinflammatory cytokines and chemokines [28, 40] and the delay of neutrophil apoptosis [42]. Whether the opposite effects are mediated entirely through the same receptor, remains to be examined.

Two independent studies have shown that SAA can activate the TLRs TLR2 [34] and TLR4 [43], despite the fact that SAA has little structural resemblance to the bacteria-derived ligands of either receptor. One of these studies identified the TLR2/1 heterodimer as the preferred receptor for SAA [34]. SAA stimulation of these 2 receptors results in MyD88-dependent signaling, including phosphorylation of ERK and p38 MAPK, as well as transcriptional activation that leads to increased expression of IL-1β, TNF-α, IL-12p40, IL-1rn, and IL-10 [34] and elevated production of NO [43]. TLR2 also mediates SAA-induced expression of IL-23p19 [31] and G-CSF [32]. More recently, it has been shown that activation of TLR2 contributes to SAA-induced expression of IL-33 and the M2 macrophage markers [36, 37]. The SAA-TLR2 pair is believed to play a role in tumorigenesis through Stat3-dependent expansion of the immune-suppressive effect of myeloid-derived suppressive cells [44].

SR-BI and CLA-1 have been shown to interact with SAA [45, 46]. The functional consequence of this interaction is primarily

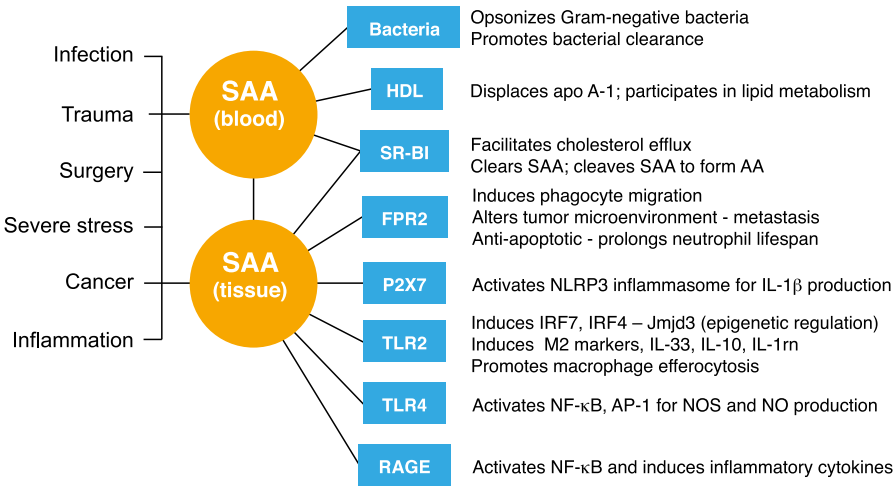

Figure 1. Schematic drawing depicting SAA synthesis and interaction with its receptors. Note that plasma SAA and tissue SAA are separated to illustrate their different functions, although nonhepatic production of SAA may also contribute to its plasma level. Many of the SAA receptors share common properties in activating MAPK and transcription factors, whereas induction of chemotaxis and promotion of cholesterol efflux use specific receptors. Not all activities are shown with all SAA receptors. AA, Amyloid A; RAGE, receptor for advanced glycation endproducts.

the efflux of cholesterol, which is known to be facilitated by an elevated plasma level of SAA [21]. Despite having a very short intracellular domain, SR-BI/CLA-1 has been shown to transduce signals upon binding to SAA, resulting in ERK and p38 MAPK phosphorylation [45]. The SR-Bs may also mediate uptake of SAA, which is cleaved inside macrophages to generate extracellular amyloid A [47].

Additional receptors for SAA continue to emerge. The ability of SAA to stimulate IL-1β production is thought to result from SAA-induced NLRP3 inflammasome activation, which is TLR2 dependent [48]. However, another study suggests that the ATP receptor P2X7 is responsible for the activation of the NLRP3 inflammasome [49]. The P2X7 receptor is also involved in the antiapoptotic effect of neutrophils [50]. As the cells used in these studies (monocytes) express most, if not all, SAA receptors, it is difficult to isolate a particular receptor for SAA with use of the traditional loss-of-function approaches.

With its ability to activate more than 1 receptor, SAA is likely to trigger multiple signaling pathways simultaneously. In fact, this has already been shown in published studies. Activation of these receptors and pathways leads to distinct functions (e.g., chemotaxis) and common cellular activities (e.g., phosphorylation of ERK and p38 MAPK and activation of NF-κB) that are also stimulated by inflammatory cytokines and chemokines. SAA-induced chemotaxis involves activation of the Gi proteins, which can be disrupted by pertussis toxin [24, 25]. Likewise, FPR2-dependent activation of NF-κB is subject to inhibition by pertussis toxin [28]. The MAPKs ERK and p38 MAPK are activated by SAA through >1 signaling mechanism, possibly involving a Giα-Gβγ pathway downstream of FPR2, a MyD88-dependent pathway downstream of TLR2 and TLR4, and an unidentified pathway downstream of SR-BI/CLA-1 [28, 34, 43, 45]. More recent studies have identified additional pathways and signaling molecules that are activated by SAA. For instance, SAA has been shown to induce IL-33 expression through IRF7 [36], whereas it induces the expression of M2 macrophage markers through IRF4 [37]. Preliminary results have shown that SAA preferentially activates the histone H3 demethylase Jmjd3 [51] rather than the established Stat6 pathway downstream of IL-4R [52]. As a result, SAA not only triggers transcription factor activation but also influences gene expression through epigenetic regulation [52].

POTENTIAL INVOLVEMENT OF SAA IN INFLAMMATION AND UNRESOLVED ISSUES

Given the potent effects of rhSAA on inflammatory cells, SAA may be considered a mediator of "danger signal" that influences the inflammation process [53]. A major acute-phase reactant, SAA is produced when mammals sense potentially harmful environmental cues, including trauma, infection, tumor growth, surgery, and severe stress. Its low basal level and high inducibility also match to the standard of danger signal molecules [54]. SAA appears at the site of inflammation and is highly expressed in all inflammatory disorders, including sterile inflammation. Published studies suggest that SAA may have profound effects on innate immunity as a result of its chemotactic and cytokine-inducing activities. Its ability to activate TLR2 and TLR4 is of particular interest given that these TLRs have important functions in innate immunity and inflammation and that their genetic variants are closely related to the disposition of several inflammatory and metabolic diseases featuring elevated SAA production [55, 56].

The majority of published studies that use rhSAA has shown its proinflammatory activities, including the abilities to attract phagocytes, to elicit proinflammatory cytokine expression, and to prolong the lifespan of neutrophils through an antiapoptotic effect. More recent studies demonstrate that the functions of SAA in inflammation may be more regulatory and homeostatic than proinflammatory. As discussed above, rhSAA induces not only proinflammatory cytokines but also anti-inflammatory cytokines, such as IL-10 and IL-1rn. Moreover, the induction of an M2 macrophage phenotype, including the expression of M2 markers and potentiation of macrophage efferocytosis of apoptotic neutrophils, points to the possibility that SAA may be involved in the resolution of inflammation [37].

Evaluation of the physiologic functions of SAA has met with challenges, including the presence of multiple SAA isoforms, as well as receptors, and the fact that most studies published thus far use an rhSAA that differs from the native SAA isoforms (SAA1 and SAA2) by 2 aa. There is also skepticism on the proinflammatory activity of the *E. coli*-derived rSAA, suspecting that LPS contamination may be a major contributing factor to the observed cytokine-like activities. These concerns have to be addressed to advance SAA research further. rhSAA (apo-SAA, as listed in the catalog of PeproTech, Rocky Hill, NJ, USA) contains 2 aa substitutions and an additional methionine at the N terminus compared with the mature form of human SAA1. An aspartic acid in SAA1 is substituted with an asparagine, as in SAA2 (D61N), and a histidine in SAA1 is substituted with an arginine, as in SAA2 (H72R). The exact reason for substituting these amino acids is unclear, but the resulting rhSAA becomes a hybrid of SAA1 and SAA2. In a letter to the editor of *Arthritis & Rheumatism*, van den Brand et al. [57] compared this rhSAA hybrid with SAA1, which is also an *E. coli*-produced recombinant protein from the same commercial source. Their results indicate that the rSAA1 is much less effective in the induction of IL-8 transcripts and NF-κB activation. In a separate report, Bjorkman et al. [58] reported that endogenous SAA (presumably, SAA1 and SAA2) lacks the ability to induce L-selectin shedding when compared with rhSAA. This is in contrast to the original report by Patel et al. [26] that shows the ability of both rSAA and native SAA-enriched HDL complex to induce the transcripts of IL-1β, IL-1rn, and TNFR-II. Kim et al. [59] have found induction of the *csf3* (mouse G-CSF gene) transcript in J774 macrophages by SAA purified from human plasma and cardiac surgery patients. The induction is TLR2 dependent but is seen only with the lipid-poor SAA. Therefore, it is possible that the commercially available SAA (apo-SAA) and SAA1 have different properties, but this may not lead to the generalized conclusion that rhSAA has cytokine-like activity because of the substituted amino acids. In a better controlled study, Chen et al. [60] compared rSAA1 isoforms (SAA1.1, SAA1.3, and SAA1.5) with rSAA2.2 for their use of 2 different receptors. This work shows that SAA1.1 and SAA2.2 are equally efficacious at FPR2 and TLR2 when chemotaxis, calcium mobilization, and ERK phosphorylation are measured, but small differences exist between different SAA1 isoforms [60]. Several other studies have shown that mouse Saa3 is biologically active both in vitro and in vivo [61–63]. Therefore, it appears that the specific biologic assays and the form of the SAA protein (lipid-rich or lipid-poor) are more important factors than the sequence difference between SAA isoforms in the determination of their functional properties.

Another concern over the interpretation of data derived from studies that use rhSAA is the contribution of contaminating LPS. Both rhSAA (apo-SAA) and rhSAA1 from PeproTech are derived from *E. coli* expression. Given the lipid-binding propensity of SAA, LPS may be difficult to separate from the rSAA proteins. As indicated by the manufacturer, the LPS content is below 1 ng/mg of SAA protein (<1/10,000 w/w). Nevertheless, an effect of contaminating LPS cannot be excluded, considering that TLR4 is one of the SAA receptors [64]. Therefore, it is important to incorporate into the experimental plan assays and controls for this effect with the use of rhSAA, including determination of the LPS content, incubation of the SAA preparation with polymyxin B before the experiment, heat inactivation of SAA, and the use of TLR4-deficient cells, as done in one of the recent studies [60]. Studies that use 1 or more of these controls have shown that rhSAA still possesses biologic activities in the induction of cytokines and chemotaxis [25, 28, 31, 43], the latter being less likely affected by LPS contamination as a result of the use of a G protein-coupled receptor, FPR2. In addition to LPS, other bacterial products may contaminate the SAA preparation when expressed in *E. coli*. Whether these other contaminants contribute significantly to the biologic functions of SAA has been examined in some of the published studies. It was shown that rSAA, in the serum-free medium of cultured Chinese hamster ovary cells, retains its TLR2-activating property [34]. Moreover, endogenous human SAA, purified from cardiac surgery patients, is able to induce G-CSF expression in J774 cells [59]. Of note, rhSAA1, prepared by the same manufacturer, has very low biologic activity in some of the functional assays [57], suggesting that it is possible to minimize LPS contamination in rSAA preparations to levels that do not affect significantly evaluation of SAA in functional assays. In a recently published study, *E. coli*-expressed rSAA1 isoforms have been shown to stimulate the expression of cytokines (TNF-α, IL-1β, IL-1rn, and IL-10) by macrophages from the C57BL/10ScN mice, which lack functional TLR4 [60]. These findings suggest that the cytokine-inducing activity of rSAA is a property of the SAA protein.

CONCLUDING REMARKS

Accumulating evidence suggests that the acute-phase protein SAA plays an active role in inflammation. As a result of page limitation, it is not possible to discuss all published results in this brief review, but some of the recent findings on SAA involvement in inflammation are summarized (see **Table 1**). Because of the presence of multiple isoforms and receptors, the effects of SAA are pleotropic and may not be categorized simply as being proinflammatory or anti-inflammatory. SAA has been shown to attract migrating phagocytes to site of inflammation; to activate transcription factors, such as NF-κB and exert an effect on epigenetic regulation; to induce the expression of inflammatory cytokines and M2 macrophage markers; and to influence the lifespan of neutrophils through inhibition of apoptosis and enhanced efferocytosis. These properties of SAA and the related signaling pathways may be explored to benefit human health. To advance SAA research to a higher level, it is important to examine the functions of SAA in physiologic settings and in disease models. To this end, it is encouraging that new methods and reagents have been developed for in vivo expression of SAA. These include adenoviral vector-mediated systemic expression of SAA [65, 66] and transgenic expression of SAA in an inducible fashion [67] or in particular tissues [68]. More recently, mice lacking specific SAA genes have been generated [69] (also see Table 1). It is predicted that these approaches will enable us to examine functions of SAA in specific tissues and disease models, thereby advancing our understanding of the APR for its role in inflammation and immunity.

TABLE 1. Some recent findings of SAA involvement in inflammation

Disease models and cell processes	Experiments and major findings	Refs.
Cytokine gene expression in dermal fibroblasts	Healthy dermal fibroblasts respond to SAA stimulation with induced expression of IL-6 and activation of NF-κB, which are blocked by anti-TLR2 antibody and small interfering RNA for TLR2.	[70]
Tumor metastasis models	S100A4 stimulates Saa1 and Saa3 expression via TLR4, which leads to induced expression of RANTES, G-CSF, MMP2, MMP3, MMP9, and MMP13. Ectopic expression of Saa1 and Saa3 in tumor cells promotes widespread metastasis and immune cell infiltration.	[71]
Chemotaxis of immature DC	SAA1 not only stimulates chemotaxis but also induces the expression of CCL3 and CXCL8, which contribute to chemotaxis of immature DC and prolong leukocyte recruitment.	[72]
Proinflammatory heterotypic macrophage differentiation	SAA induces the development of a CD11c (high)/CD11b (high) macrophage population in a CSF-1R-dependent manner. These SAA-treated macrophages express concurrently the M2 markers CD163 and IL-10. SAA-differentiated macrophages respond to LPS with higher levels of IL-6 and IL-1b expression.	[73]
Macrophage M2 marker expression and efferocytosis	Stimulation of mouse bone marrow and peritoneal macrophages results in a MyD88- and IRF4-dependent expression of M2 markers, including IL-10, Ym1, Fizz-1, Mrc1, IL-1rn, and CCL17. SAA also improves macrophage efferocytosis of apoptotic neutrophils.	[37]
Atherosclerosis in apoE$^{-/-}$ mice	Deletion of *Saa1.1* and *Saa2.1* does not affect atherosclerosis-related inflammatory marker expression nor does it affect atherosclerotic lesion or cholesterol distribution.	[74]
Atherosclerosis in apoE$^{-/-}$ mice	Mice receiving only a single injection of SAA-expressing adenovirus show increased atherosclerosis compared with controls. SAA increases LDL retention and vascular biglycan content in a TGF-β signaling-dependent manner.	[75]
Mouse obesity model	In obese mice fed with a high-fat, high-sucrose diet with added cholesterol, the absence of Saa3 attenuates liver-specific SAA secretion into plasma and reduces weight gain and macrophage accumulation.	[69]
Abdominal aortic aneurysm formation	Plasma SAA level increases by 60-fold in apoE$^{-/-}$ mice 24 h after receiving intraperitoneal injection of angiotensin II. Deletion of Saa1.1 and Saa2.1 protects against experimental AAA, in part, through reduced MMP2 activity.	[76]

DC, Dendritic cell; Ym1, chitinase 3-like 3; Fizz-1, found in inflammatory zone 1; Mrc1, mannose receptor 1; apoE$^{-/-}$, apolipoprotein E deficient.

AUTHORSHIP

R.D.Y. and L.S. reviewed the literature and wrote the manuscript.

ACKNOWLEDGMENTS

This work was supported, in part, by grants from the National Natural Science Foundation of China (Grants 31270941 and 81202316), National Basic Research Program of China (973 Program, Grant 2012CB518000), Specialized Research Fund for the Doctoral Program of Higher Education of China (Grants 20120073110069 and 20120073120092), and U.S. National Institutes of Health National Institute of Allergy and Infectious Diseases (Grant R56 AI040176).

DISCLOSURES

The authors declare that they have no competing interest.

REFERENCES

1. Kushner, I., Rzewnicki, D. (1999) Acute phase response. In *Inflammation: Basic Principles and Clinical Correlates* (J. I. Gallin, R. Snyderman, eds.), Lippincott Willams & Wilkins, Philadelphia, 317–329.
2. Gabay, C., Kushner, I. (1999) Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340,** 448–454.
3. Meek, R. L., Eriksen, N., Benditt, E. P. (1992) Murine serum amyloid A3 is a high density apolipoprotein and is secreted by macrophages. *Proc. Natl. Acad. Sci. USA* **89,** 7949–7952.
4. O'Hara, R., Murphy, E. P., Whitehead, A. S., FitzGerald, O., Bresnihan, B. (2000) Acute-phase serum amyloid A production by rheumatoid arthritis synovial tissue. *Arthritis Res.* **2,** 142–144.
5. Vallon, R., Freuler, F., Desta-Tsedu, N., Robeva, A., Dawson, J., Wenner, P., Engelhardt, P., Boes, L., Schnyder, J., Tschopp, C., Urfer, R., Baumann, G. (2001) Serum amyloid A (apoSAA) expression is up-regulated in rheumatoid arthritis and induces transcription of matrix metalloproteinases. *J. Immunol.* **166,** 2801–2807.
6. Meek, R. L., Urieli-Shoval, S., Benditt, E. P. (1994) Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: implications for serum amyloid A function. *Proc. Natl. Acad. Sci. USA* **91,** 3186–3190.
7. Wilson, P. G., Thompson, J. C., Webb, N. R., de Beer, F. C., King, V. L., Tannock, L. R. (2008) Serum amyloid A, but not C-reactive protein, stimulates vascular proteoglycan synthesis in a pro-atherogenic manner. *Am. J. Pathol.* **173,** 1902–1910.
8. Dong, Z., Wu, T., Qin, W., An, C., Wang, Z., Zhang, M., Zhang, Y., Zhang, C., An, F. (2011) Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Mol. Med.* **17,** 1357–1364.
9. Niederau, C., Backmerhoff, F., Schumacher, B., Niederau, C. (1997) Inflammatory mediators and acute phase proteins in patients with Crohn's disease and ulcerative colitis. *Hepatogastroenterology* **44,** 90–107.
10. Marzi, C., Albrecht, E., Hysi, P. G., Lagou, V., Waldenberger, M., Tönjes, A., Prokopenko, I., Heim, K., Blackburn, H., Ried, J. S., Kleber, M. E., Mangino, M., Thorand, B., Peters, A., Hammond, C. J., Grallert,

H., Boehm, B. O., Kovacs, P., Geistlinger, L., Prokisch, H., Winkelmann, B. R., Spector, T. D., Wichmann, H. E., Stumvoll, M., Soranzo, N., März, W., Koenig, W., Illig, T., Gieger, C. (2010) Genome-wide association study identifies two novel regions at 11p15.5-p13 and 1p31 with major impact on acute-phase serum amyloid A. *PLoS Genet.* **6,** e1001213.
11. Marzi, C., Huth, C., Herder, C., Baumert, J., Thorand, B., Rathmann, W., Meisinger, C., Wichmann, H. E., Roden, M., Peters, A., Grallert, H., Koenig, W., Illig, T. (2013) Acute-phase serum amyloid A protein and its implication in the development of type 2 diabetes in the KORA S4/F4 study. *Diabetes Care* **36,** 1321–1326.
12. Uhlar, C. M., Whitehead, A. S. (1999) Serum amyloid A, the major vertebrate acute-phase reactant. *Eur. J. Biochem.* **265,** 501–523.
13. Sellar, G. C., Whitehead, A. S. (1993) Localization of four human serum amyloid A (SAA) protein superfamily genes to chromosome 11p: characterization of a fifth SAA-related gene sequence. *Genomics* **16,** 774–776.
14. Jensen, L. E., Whitehead, A. S. (1998) Regulation of serum amyloid A protein expression during the acute-phase response. *Biochem. J.* **334,** 489–503.
15. Uhlar, C. M., Burgess, C. J., Sharp, P. M., Whitehead, A. S. (1994) Evolution of the serum amyloid A (SAA) protein superfamily. *Genomics* **19,** 228–235.
16. Lowell, C. A., Potter, D. A., Stearman, R. S., Morrow, J. F. (1986) Structure of the murine serum amyloid A gene family. Gene conversion. *J. Biol. Chem.* **261,** 8442–8452.
17. Meek, R. L., Benditt, E. P. (1986) Amyloid A gene family expression in different mouse tissues. *J. Exp. Med.* **164,** 2006–2017.
18. Butler, A., Whitehead, A. S. (1996) Mapping of the mouse serum amyloid A gene cluster by long-range polymerase chain reaction. *Immunogenetics* **44,** 468–474.
19. Chiba, T., Han, C. Y., Vaisar, T., Shimokado, K., Kargi, A., Chen, M. H., Wang, S., McDonald, T. O., O'Brien, K. D., Heinecke, J. W., Chait, A. (2009) Serum amyloid A3 does not contribute to circulating SAA levels. *J. Lipid Res.* **50,** 1353–1362.
20. Benditt, E. P., Eriksen, N. (1977) Amyloid protein SAA is associated with high density lipoprotein from human serum. *Proc. Natl. Acad. Sci. USA* **74,** 4025–4028.
21. Banka, C. L., Yuan, T., de Beer, M. C., Kindy, M., Curtiss, L. K., de Beer, F. C. (1995) Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux. *J. Lipid Res.* **36,** 1058–1065.
22. Shah, C., Hari-Dass, R., Raynes, J. G. (2006) Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. *Blood* **108,** 1751–1757.
23. Derebe, M. G., Zlatkov, C. M., Gattu, S., Ruhn, K. A., Vaishnava, S., Diehl, G. E., MacMillan, J. B., Williams, N. S., Hooper, L. V. (2014) Serum amyloid A is a retinol binding protein that transports retinol during bacterial infection. *eLife* **3,** e03206.
24. Badolato, R., Wang, J. M., Murphy, W. J., Lloyd, A. R., Michiel, D. F., Bausserman, L. L., Kelvin, D. J., Oppenheim, J. J. (1994) Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J. Exp. Med.* **180,** 203–209.
25. Su, S. B., Gong, W., Gao, J. L., Shen, W., Murphy, P. M., Oppenheim, J. J., Wang, J. M. (1999) A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J. Exp. Med.* **189,** 395–402.
26. Patel, H., Fellowes, R., Coade, S., Woo, P. (1998) Human serum amyloid A has cytokine-like properties. *Scand. J. Immunol.* **48,** 410–418.
27. Furlaneto, C. J., Campa, A. (2000) 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.* **268,** 405–408.
28. He, R., Sang, H., Ye, R. D. (2003) Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. *Blood* **101,** 1572–1581.
29. O'Hara, R., Murphy, E. P., Whitehead, A. S., FitzGerald, O., Bresnihan, B. (2004) 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.* **50,** 1788–1799.
30. Cai, H., Song, C., Endoh, I., Goyette, J., Jessup, W., Freedman, S. B., McNeil, H. P., Geczy, C. L. (2007) Serum amyloid A induces monocyte tissue factor. *J. Immunol.* **178,** 1852–1860.
31. He, R., Shepard, L. W., Chen, J., Pan, Z. K., Ye, R. D. (2006) Serum amyloid A is an endogenous ligand that differentially induces IL-12 and IL-23. *J. Immunol.* **177,** 4072–4079.
32. He, R. L., Zhou, J., Hanson, C. Z., Chen, J., Cheng, N., Ye, R. D. (2009) Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. *Blood* **113,** 429–437.
33. Lu, J., Yu, Y., Zhu, I., Cheng, Y., Sun, P. D. (2014) Structural mechanism of serum amyloid A-mediated inflammatory amyloidosis. *Proc. Natl. Acad. Sci. USA* **111,** 5189–5194.
34. Cheng, N., He, R., Tian, J., Ye, P. P., Ye, R. D. (2008) Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J. Immunol.* **181,** 22–26.
35. De Santo, C., Arscott, R., Booth, S., Karydis, I., Jones, M., Asher, R., Salio, M., Middleton, M., Cerundolo, V. (2010) Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. *Nat. Immunol.* **11,** 1039–1046.
36. Sun, L., Zhu, Z., Cheng, N., Yan, Q., Ye, R. D. (2014) Serum amyloid A induces interleukin-33 expression through an IRF7-dependent pathway. *Eur. J. Immunol.* **44,** 2153–2164.
37. Sun, L., Zhou, H., Zhu, Z., Yan, Q., Wang, L., Liang, Q., Ye, R. D. (2015) Ex vivo and in vitro effect of serum amyloid a in the induction of macrophage m2 markers and efferocytosis of apoptotic neutrophils. *J. Immunol.* **194,** 4891–4900.
38. Migeotte, I., Communi, D., Parmentier, M. (2006) Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev.* **17,** 501–519.
39. Ye, R. D., Boulay, F., Wang, J. M., Dahlgren, C., Gerard, C., Parmentier, M., Serhan, C. N., Murphy, P. M. (2009) International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* **61,** 119–161.
40. Sodin-Semrl, S., Spagnolo, A., Mikus, R., Barbaro, B., Varga, J., Fiore, S. (2004) Opposing regulation of interleukin-8 and NF-kappaB responses by lipoxin A4 and serum amyloid A via the common lipoxin A receptor. *Int. J. Immunopathol. Pharmacol.* **17,** 145–156.
41. Ren, S. W., Qi, X., Jia, C. K., Wang, Y. Q. (2014) Serum amyloid A and pairing formyl peptide receptor 2 are expressed in corneas and involved in inflammation-mediated neovascularization. *Int. J. Ophthalmol.* **7,** 187–193.
42. El Kebir, D., József, L., Khreiss, T., Pan, W., Petasis, N. A., Serhan, C. N., Filep, J. G. (2007) Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. *J. Immunol.* **179,** 616–622.
43. Sandri, S., Rodriguez, D., Gomes, E., Monteiro, H. P., Russo, M., Campa, A. (2008) Is serum amyloid A an endogenous TLR4 agonist? *J. Leukoc. Biol.* **83,** 1174–1180.
44. Lee, J. M., Kim, E. K., Seo, H., Jeon, I., Chae, M. J., Park, Y. J., Song, B., Kim, Y. S., Kim, Y. J., Ko, H. J., Kang, C. Y. (2014) Serum amyloid A3 exacerbates cancer by enhancing the suppressive capacity of myeloid-derived suppressor cells via TLR2-dependent STAT3 activation. *Eur. J. Immunol.* **44,** 1672–1684.
45. Baranova, I. N., Vishnyakova, T. G., Bocharov, A. V., Kurlander, R., Chen, Z., Kimelman, M. L., Remaley, A. T., Csako, G., Thomas, F., Eggerman, T. L., Patterson, A. P. (2005) 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.* **280,** 8031–8040.
46. Cai, L., de Beer, M. C., de Beer, F. C., van der Westhuyzen, D. R. (2005) 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.* **280,** 2954–2961.
47. Kluve-Beckerman, B., Manaloor, J. J., Liepnieks, J. J. (2002) A pulse-chase study tracking the conversion of macrophage-endocytosed serum amyloid A into extracellular amyloid. *Arthritis Rheum.* **46,** 1905–1913.
48. Ather, J. L., Ckless, K., Martin, R., Foley, K. L., Suratt, B. T., Boyson, J. E., Fitzgerald, K. A., Flavell, R. A., Eisenbarth, S. C., Poynter, M. E. (2011) Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. *J. Immunol.* **187,** 64–73.
49. Niemi, K., Teirilä, L., Lappalainen, J., Rajamäki, K., Baumann, M. H., Öörni, K., Wolff, H., Kovanen, P. T., Matikainen, S., Eklund, K. K. (2011) Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a cathepsin B-sensitive pathway. *J. Immunol.* **186,** 6119–6128.
50. Christenson, K., Björkman, L., Tängemo, C., Bylund, J. (2008) Serum amyloid A inhibits apoptosis of human neutrophils via a P2X7-sensitive pathway independent of formyl peptide receptor-like 1. *J. Leukoc. Biol.* **83,** 139–148.
51. Satoh, T., Takeuchi, O., Vandenbon, A., Yasuda, K., Tanaka, Y., Kumagai, Y., Miyake, T., Matsushita, K., Okazaki, T., Saitoh, T., Honma, K., Matsuyama, T., Yui, K., Tsujimura, T., Standley, D. M., Nakanishi, K., Nakai, K., Akira, S. (2010) The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* **11,** 936–944.
52. Yan, Q., Sun, L., Zhu, Z., Wang, L., Li, S., Ye, R. D. (2014) Jmjd3-mediated epigenetic regulation of inflammatory cytokine gene expression in serum amyloid A-stimulated macrophages. *Cell. Signal.* **26,** 1783–1791.
53. Matzinger, P. (2007) Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* **8,** 11–13.
54. Seong, S. Y., Matzinger, P. (2004) Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* **4,** 469–478.

55. Mullick, A. E., Tobias, P. S., Curtiss, L. K. (2005) Modulation of atherosclerosis in mice by Toll-like receptor 2. *J. Clin. Invest.* **115,** 3149–3156.
56. Tobias, P. S., Curtiss, L. K. (2008) TLR2 in murine atherosclerosis. *Semin. Immunopathol.* **30,** 23–27.
57. Van den Brand, B., Waterborg, C., van den Berg, W., van de Loo, F. (2013) Is the serum amyloid A we use really serum amyloid A? Comment on the article by Connolly et al. *Arthritis Rheum.* **65,** 283–284.
58. Björkman, L., Raynes, J. G., Shah, C., Karlsson, A., Dahlgren, C., Bylund, J. (2010) The proinflammatory activity of recombinant serum amyloid A is not shared by the endogenous protein in the circulation. *Arthritis Rheum.* **62,** 1660–1665.
59. Kim, M. H., de Beer, M. C., Wroblewski, J. M., Webb, N. R., de Beer, F. C. (2013) SAA does not induce cytokine production in physiological conditions. *Cytokine* **61,** 506–512.
60. Chen, M., Zhou, H., Cheng, N., Qian, F., Ye, R. D. (2014) Serum amyloid A1 isoforms display different efficacy at Toll-like receptor 2 and formyl peptide receptor 2. *Immunobiology* **219,** 916–923.
61. Hiratsuka, S., Watanabe, A., Sakurai, Y., Akashi-Takamura, S., Ishibashi, S., Miyake, K., Shibuya, M., Akira, S., Aburatani, H., Maru, Y. (2008) The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. *Nat. Cell Biol.* **10,** 1349–1355.
62. Deguchi, A., Tomita, T., Omori, T., Komatsu, A., Ohto, U., Takahashi, S., Tanimura, N., Akashi-Takamura, S., Miyake, K., Maru, Y. (2013) Serum amyloid A3 binds MD-2 to activate p38 and NF-κB pathways in a MyD88-dependent manner. *J. Immunol.* **191,** 1856–1864.
63. Shigemura, H., Ishiguro, N., Inoshima, Y. (2014) Up-regulation of MUC2 mucin expression by serum amyloid A3 protein in mouse colonic epithelial cells. *J. Vet. Med. Sci.* **76,** 985–991.
64. Tsan, M. F., Gao, B. (2004) Endogenous ligands of Toll-like receptors. *J. Leukoc. Biol.* **76,** 514–519.
65. Webb, N. R., de Beer, M. C., van der Westhuyzen, D. R., Kindy, M. S., Banka, C. L., Tsukamoto, K., Rader, D. L., de Beer, F. C. (1997) Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice. *J. Lipid Res.* **38,** 1583–1590.
66. Kindy, M. S., King, A. R., Yu, J., Gerardot, C., Whitley, J., de Beer, F. C. (1998) Adenoviral expression of murine serum amyloid A proteins to study amyloid fibrillogenesis. *Biochem. J.* **332,** 721–728.
67. Kindy, M. S., de Beer, M. C., Yu, J., de Beer, F. C. (2000) Expression of mouse acute-phase (SAA1.1) and constitutive (SAA4) serum amyloid A isotypes: influence on lipoprotein profiles. *Arterioscler. Thromb. Vasc. Biol.* **20,** 1543–1550.
68. Olsson, M., Ahlin, S., Olsson, B., Svensson, P. A., Ståhlman, M., Borén, J., Carlsson, L. M., Sjöholm, K. (2011) Establishment of a transgenic mouse model specifically expressing human serum amyloid A in adipose tissue. *PLoS ONE* **6,** e19609.
69. Den Hartigh, L. J., Wang, S., Goodspeed, L., Ding, Y., Averill, M., Subramanian, S., Wietecha, T., O'Brien, K. D., Chait, A. (2014) Deletion of serum amyloid A3 improves high fat high sucrose diet-induced adipose tissue inflammation and hyperlipidemia in female mice. *PLoS ONE* **9,** e108564.
70. O'Reilly, S., Cant, R., Ciechomska, M., Finnigan, J., Oakley, F., Hambleton, S., van Laar, J. M. (2014) Serum amyloid A induces interleukin-6 in dermal fibroblasts via Toll-like receptor 2, interleukin-1 receptor-associated kinase 4 and nuclear factor-κB. *Immunology* **143,** 331–340.
71. Hansen, M. T., Forst, B., Cremers, N., Quagliata, L., Ambartsumian, N., Grum-Schwensen, B., Klingelhöfer, J., Abdul-Al, A., Herrmann, P., Osterland, M., Stein, U., Nielsen, G. H., Scherer, P. E., Lukanidin, E., Sleeman, J. P., Grigorian, M. (2015) A link between inflammation and metastasis: serum amyloid A1 and A3 induce metastasis, and are targets of metastasis-inducing S100A4. *Oncogene* **34,** 424–435.
72. Gouwy, M., De Buck, M., Pörtner, N., Opdenakker, G., Proost, P., Struyf, S., Van Damme, J. (2015) Serum amyloid A chemoattracts immature dendritic cells and indirectly provokes monocyte chemotaxis by induction of cooperating CC and CXC chemokines. *Eur. J. Immunol.* **45,** 101–112.
73. Anthony, D., McQualter, J. L., Bishara, M., Lim, E. X., Yatmaz, S., Seow, H. J., Hansen, M., Thompson, M., Hamilton, J. A., Irving, L. B., Levy, B. D., Vlahos, R., Anderson, G. P., Bozinovski, S. (2014) SAA drives proinflammatory heterotypic macrophage differentiation in the lung via CSF-1R-dependent signaling. *FASEB J.* **28,** 3867–3877.
74. De Beer, M. C., Wroblewski, J. M., Noffsinger, V. P., Rateri, D. L., Howatt, D. A., Balakrishnan, A., Ji, A., Shridas, P., Thompson, J. C., van der Westhuyzen, D. R., Tannock, L. R., Daugherty, A., Webb, N. R., De Beer, F. C. (2014) Deficiency of endogenous acute phase serum amyloid A does not affect atherosclerotic lesions in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **34,** 255–261.
75. Thompson, J. C., Jayne, C., Thompson, J., Wilson, P. G., Yoder, M. H., Webb, N., Tannock, L. R. (2015) A brief elevation of serum amyloid A is sufficient to increase atherosclerosis. *J. Lipid Res.* **56,** 286–293.
76. Webb, N. R., De Beer, M. C., Wroblewski, J. M., Ji, A., Bailey, W., Shridas, P., Charnigo, R. J., Noffsinger, V. P., Witta, J., Howatt, D. A., Balakrishnan, A., Rateri, D. L., Daugherty, A., De Beer, F. C. (2015) Deficiency of endogenous acute-phase serum amyloid A protects apoE−/− mice from angiotensin II-induced abdominal aortic aneurysm formation. *Arterioscler. Thromb. Vasc. Biol.* **35,** 1156–1165.

KEY WORDS:
acute-phase reactants · stress response · cytokines · epigenetic regulation

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J Leukoc Biol 2015 98: 923-929 originally published online June 30, 2015
Access the most recent version at doi:10.1189/jlb.3VMR0315-080R