

Effect of serum amyloid A1 treatment on global gene expression in THP-1-derived macrophages

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Abstract

Objective To investigate the effect of serum amyloid A1 (SAA1) on global gene expression in macrophages derived from THP-1 monocytes.

Materials and methods Global genetic expression in THP-1-derived macrophages was determined using Illumina HT-12 microarray chips and the results were validated by real-time PCR. Cytokine levels in cellular supernatant were quantified by ELISA.

Results In total, 55 genes were upregulated with fold difference greater than two when THP-1-derived macrophages were incubated with SAA1 for 8 h. SAA1 is a strong cytokine inducer with significant upregulation of chemokines CCL1, CCL3, and CCL4 and this was confirmed by both real-time PCR and ELISA quantification. SAA1 also promotes the upregulation of genes involved in phagocytosis, anti-apoptosis, and tissue remodeling.

Conclusions SAA1 appears to play an important role during the immune response and in chronic inflammatory diseases through the stimulation of genes involved in cytokine production, phagocytosis, and anti-apoptosis.

Keywords Serum amyloid A1 · Atherosclerosis · Acute-phase protein · Coronary artery disease · Rheumatoid arthritis

Introduction

The acute phase response constitutes part of the innate immunity in humans and serves to counteract infection or injury. Acute-phase serum amyloid A (A-SAA), consisting of (SAA1 and SAA2, are major components of acute phase proteins and constitute 2.5% of the hepatic protein produced during the acute phase response [1]. During an acute systemic inflammation, the plasma concentration of A-SAA can increase by 500- to 1,000-fold [2]. Although the exact role of A-SAA is not known, its well-conserved nature and its production during systemic inflammation probably indicate its importance in immune activation and protection from infection. While the liver is the main source of A-SAA production during an acute-phase response, the adipose tissue is the dominant source of A-SAA production in healthy and obese individuals [3, 4]. Its role in immune activation is also supported by clinical studies which reported that A-SAA was upregulated in patients with acute coronary syndrome, stable coronary artery disease (CAD), cancer, rheumatoid arthritis (RA), and metabolic syndrome [5–9].

A-SAA has been associated with chronic inflammatory diseases such as CAD and RA. Studies have shown that it plays both atherogenic and athero-protective roles. Several lines of evidence support the atherogenic role of A-SAA.

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In mice and rabbits fed on a cholesterol-rich diet, there is a positive correlation between developed lesion size and serum A-SAA level [10, 11]. This is further elaborated in another study, where atherosclerosis-susceptible mice (C57BL/6) demonstrated a fivefold increase in A-SAA level over atherosclerosis-resistance mice (C3H/HeJ) when both received high fat diets [12]. There also exists circumstantial evidence: A-SAA transcripts are found in important components of the atherosclerotic lesion including endothelial cells lining the lumen of the coronary artery and the vaso vasorum as well as in newly formed vessels. In addition, the expression is especially high in macrophage foam cells and in adventitial adipocytes [13]. A-SAA stimulates the hydrolytic activity of secretory group IIA phospholipase A2 (sPLA2), which is responsible for the production of atherogenic oxygenated and non-oxygenated fatty acids [14]. Other evidence includes its ability to induce secretion of extracellular matrix degrading enzymes such as collagenase, matrix metalloproteinases 2 and 3 from synovial fibroblasts [15, 16], and inflammatory cytokines from monocytes and macrophages [17].

On the other hand, evidence supporting the atheroprotective roles of A-SAA includes its ability to inhibit the activation and aggregation of platelets [18] as well as in facilitating reverse cholesterol transport. A-SAA-enriched high-density lipoprotein (HDL) was reported to stimulate a threefold increase in cholesterol efflux activity in cholesterol-loaded macrophages [19].

The direct contribution of SAA to both atherogenesis and other chronic inflammatory diseases is not well understood, other than its ability to induce the release of certain inflammatory mediators. Macrophages play an important role in atherogenesis and RA as they are involved in critical processes of atherosclerosis. In this study, we aim to investigate the global gene expression changes induced in macrophages upon treatment with SAA1, as this is the predominant form of A-SAA found in plasma and a precursor of fibrillar deposits in reactive amyloidosis [20]. We believe that this study will provide insight into the role of SAA1 in atherosclerosis and other chronic inflammatory diseases.

Materials and methods

Plasmid construction

Wild-type SAA1 cDNA was synthesized using a custom gene synthesis service (Genscript, Piscataway, NJ, United States). The synthesized sequence contained the sequence 5'-CATGGATCCGATGATGATGATAAG-3' at the 5' end which incorporates the *Bam*HI restriction enzyme recognition site (underlined) and the enterokinase recognition site (in bold). The 3' end contained the sequence

5'-CTGAGAAATACTGAGCTTCCTCGAATTCTGTCCGACG-3' with the *Eco*RI recognition site. The cDNA was subcloned into pET21-a(+) vector (Novagen, Madison, WI, USA) and subsequently transformed into *E. coli* strain BL21(DE3)pLysS competent cells (Novagen). Successful transformants were verified by sequencing the plasmid DNA extracted from the transformants.

Production of recombinant human SAA1

A successful clone of wild-type SAA1 was grown in LB medium supplemented with 500 µg/ml carbenicillin and 34 µg/ml chloramphenicol at 37°C. Expression of the recombinant protein was induced by the addition of 1 mM iso-propylthio-β-D-galactoside (IPTG) and 200 µg/ml rifampicin (Novagen) 30 min after the addition of IPTG. The culture was then incubated for a further 3 h before the cells were collected by centrifugation. The cell pellet was lysed and the lysate was purified by immunoaffinity purification using a T7-tag antibody agarose resin (Novagen, Darmstadt, Germany). Protein was ascertained for purity using Coomassie Blue staining and a functional test. Endotoxin was removed from the purified protein using an endotoxin removal kit (Norgen Biotek, Thorold, ON, Canada). The wild-type recombinant SAA1 was verified to have an endotoxin level of less than 0.0625 EU/µg of protein.

Cell culture

Human monocytic leukemia cell line, THP-1, was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were grown in RPMI medium supplemented with 10% FBS, 0.05 mM 2-mercaptoethanol, 100 u/ml penicillin and 100 µg/ml streptomycin in a 5% CO₂ humidified atmosphere. To induce differentiation, 2.5 × 10⁶ cells/well were cultured in a 6-well plate in the presence of 1 ml growth medium supplemented with 0.1 µg/ml phorbol 12-myristate 13-acetate (PMA) for 7 days. On the 8th day, the medium was replaced with serum-free RPMI medium and the cells were treated with 1 µg/ml recombinant SAA1 for either 8 or 24 h. At the end of the incubation, cell pellets were used for RNA isolation while the supernatants were used for the quantification of chemokines through ELISA.

RNA isolation and cRNA synthesis

RNA was extracted from cell pellets using a RNA extraction kit (Qiagen, Valencia, CA, USA). Prior to RNA amplification, the integrity of RNA was verified using formaldehyde gel electrophoresis. RNA amplification was carried out using Illumina TotalPrep RNA amplification kit (Life Technologies, Carlsbad, CA, USA). The amplification

procedure consists of first and second strand cDNA synthesis, following which the double-stranded cDNA was purified and used for cRNA synthesis using biotinylated nucleotides. The concentration and integrity of the cRNA was determined using Bioanalyser (Agilent, Santa Clara, CA, USA). cRNA was stored at -20°C prior to hybridization onto a microarray chip.

Transcriptomic analysis by microarrays

cRNA was loaded onto Illumina human HT-12 microarray chips (Illumina, San Diego, CA, USA). The chips were incubated for 16 h in a hybridization oven. After hybridization, they were washed using the supplied proprietary buffer and stained with streptavidin Cy3. The stained chips were dried by centrifugation and the chip was read using a BeadArray reader.

Real-time PCR

The sequences of the primers used are shown in Table 1. Real-time PCR was carried out using a Roche Light Cycler 480 (Roche, Indianapolis, IA, USA). The reaction mixture consisted of $2\times$ reaction master mix, $0.2\ \mu\text{M}$ of forward and reverse primers, and 30 ng of cDNA in a 10- μl reaction mix. Amplification consisted of an initial denaturation at 95°C for 10 min and 45 cycles of 10 s of denaturation at 95°C , 30 s of annealing at 60°C , and 8 s of extension at 72°C . The threshold cycle (C_T) value and the efficiency of PCR amplification for each set of primers were determined using the accompanying software.

Measurement of chemokines released from macrophages

The concentration of chemokines CCL1, CCL3, and CCL4 in the supernatant was assayed using ELISA kits (Ray-Biotech, Norcross, GA, USA). The procedures for the assays were in accordance with the product manual.

Data and statistical analysis

The quality of the microarray data and normalization was determined using Genome Studio (Illumina). For each treatment group, the average of the two readings was determined and fold changes between SAA1 and untreated at both 8 and 24 h were determined. To identify significantly enriched pathways, upregulated genes were mapped onto PathwayAPI [21], which comprises pathway information extracted from KEGG [22], WikiPathways [23, 24], and Ingenuity Pathways (IPA) [25]. PathwayAPI is available at <http://pathwayapi.com/>. Currently, there are 4,268 genes and 35,307 unique interactions, corresponding to 544

Table 1 Sequences of the primers used for real-time PCR

Gene	Forward primer/ reverse primer
Baculoviral IAP repeat-containing protein 3 (<i>BIRC3</i>)	5'-ATGCTTTTGCTGTGATGGTG-3' 5'-CGGATGAACTCCTGTCTTT-3'
Chemokine (C-C motif) ligand 3 (<i>CCL3</i>)	5'-GGCTCTCTACAACCAGTTCT-3' 5'-CTGGCTGCTCGTCTCAAAG-3'
Chemokine (C-C motif) ligand 4-like 1 (<i>CCL4</i>)	5'-GCTGCCTTCTGCTCTAGC-3' 5'-ACCACAAAGTTGCGAGGAAG-3'
Interleukin 23A (<i>IL23A</i>)	5'-ACAGAAGCTCTGCACACTGG-3' 5'-CCACACTGGATATGGGGAAC-3'
Integrin, alpha 1 (<i>ITGA1</i>)	5'-GTCCAGTTGGGAGAGGTGAA-3' 5'-CTCCATTTGGGTTGGTGACT-3'
Myristoylated alanine-rich C-kinase substrate (<i>MARCKS</i>)	5'-ATGAGTGCCCAGTTCTCC-3' 5'-AGTGTGCGCCGTTACCTT-3'
Oxidised low density lipoprotein receptor 1 (<i>OLR1</i>)	5'-AAGGACCAGCCTGATGAGAA-3' 5'-AGGCAAAGGACCCCTAGAGT-3'
Plasminogen activator inhibitor 2 precursor (<i>SERPIN2</i>)	5'-CATTATCCTTCCGCTCTCT-3' 5'-CTACTGCCTGGGGTTCTGAG-3'
β -2 microglobulin (<i>B2M</i>)	5'-GTGCTCACGCTACTCTCTCT-3' 5'-TCAATGTCGGATGGATGAAA-3'
FXYD domain-containing ion transport regulator 5 (<i>FXYD5</i>)	5'-CCTGTGTCTCTCACCATCG-3' 5'-GGAACCTGAATGTCCATGAT-3'

The annealing temperature for all the primers was 60°C and the concentration of primers used was $0.2\ \mu\text{M}$

pathways. Statistical significance was determined using the hypergeometric test ($P < 0.05$).

Enrichment for significant Gene Ontology (GO) terms among differentially expressed genes was determined using GOEAST (<http://omicslab.genetics.ac.cn/GOEAST/>).

For real-time PCR, statistical differences between two treatment groups were determined using the randomization and bootstrapping technique incorporated into the REST 2009 software (Qiagen). Readings from three independent experiments were used for the statistical analysis. Statistical differences of chemokine levels were determined using Student's *t*-test with significance set at $P < 0.05$.

Results

Effects of SAA1 on global gene expression in THP-1-derived macrophages at 8 and 24 h

In total, 55 genes were upregulated with a fold difference of ≥ 2 (Electronic Supplementary Material Table S1). The top ten most-upregulated genes when human macrophages

Table 2 Top ten upregulated genes when THP-1-derived macrophages were incubated with SAA1 for 8 h

Gene	Fold difference
C-C motif chemokine 4 precursor (<i>CCL4</i>)	19.1
Tumor necrosis factor-inducible gene 6 protein (<i>TNFAIP6</i>)	15.5
C-C motif chemokine 1 precursor (<i>CCL1</i>)	14.5
C-C motif chemokine 3 precursor (<i>CCL3</i>)	9.1
Superoxide dismutase 2 (<i>SOD2</i>)	7.4
Lysosome-associated membrane glycoprotein 3 (<i>LAMP3</i>)	7.3
Interleukin-23 subunit alpha precursor (<i>IL23A</i>)	6.9
Interleukin-8 precursor (<i>IL8</i>)	5.8
Solute carrier family 2 (facilitated glucose transporter), member 6 (<i>SLC2A6</i>)	5.2
Mucolipin-2 (<i>MCOLN2</i>)	4.7

The average fluorescence level of samples obtained from macrophages treated with SAA1 was compared with the fluorescence level from those obtained from untreated macrophages ($n = 2$). All readings were normalized using Genome Studio

were treated with SAA1 are shown in Table 2. Six of the genes, *CCL1*, *CCL3*, *CCL4*, *IL8*, *IL23A*, and *TNFAIP6*, are associated with roles in immune processes such as inflammation and regulation of immune cells activation and proliferation. The chemokines *CCL1*, *CCL3*, *CCL4*, and *IL8* were highly upregulated with a 19.1-fold increase for *CCL4* and a 5.8-fold increase for *IL8*. Both *TNFAIP6* and *LAMP3* have roles in matrix reorganization as they encode for proteins that have hyaluronic acid binding properties.

SAA1 appears to have a more subtle effect on the downregulation of genes in macrophages. There were only three downregulated genes with a fold change of less than -2 . *LOXLA* is an amine oxidase with associated role in matrix organization, *GJAI* is a gap junction protein, while *VCL* is a cytoskeletal protein.

The effects of SAA1 on macrophages upon treatment with SAA1 for 24 h were also determined. However, the subset of genes that were influenced by SAA1 treatment did not differ between 8 and 24 h. However, there was a noticeably reduced level of upregulation of gene expression at 24 h.

Enriched pathways upon treatment with SAA1 for 8 h

The top ten pathways upon treatment with SAA1 are shown in Table 3. SAA1 stimulates an inflammatory response and regulates the response through the activation of anti-inflammatory pathways involving IL-10 and IL-6. Pathways that contribute to inflammatory and anti-inflammatory

Table 3 Enriched pathways upon treatment of THP-1 macrophages with SAA1 for 8 h

Pathway	P value
Cytokine-cytokine receptor interaction	2.41×10^{-22}
Hematopoietic cell lineage	5.19×10^{-11}
Toll-like receptor signaling	5.31×10^{-8}
Inflammatory response	1.21×10^{-6}
Myometrial relaxation and contraction	2.29×10^{-5}
GnRH signaling pathway	3.51×10^{-5}
IL-10 signaling	4.08×10^{-5}
Mitochondrial LC-fatty acid β -oxidation	4.62×10^{-5}
TNF- α /NF- κ B signaling pathway	1.11×10^{-4}
IL-6 signaling	1.71×10^{-4}

The enriched pathways were obtained by mapping upregulated genes on PathwayAPI, which comprises pathway information extracted from KEGG, WikiPathways, and Ingenuity Pathways (IPA). Statistical significance was determined using the hypergeometric test ($P < 0.05$)

responses made up more than half of the top ten most-enriched pathways, suggesting that SAA1 is an important activator and modulator of immune response.

SAA1 increases expression of genes involved in immune regulation, anti-apoptosis, and phagocytosis at 8 h

SAA1-upregulated genes can be clustered into several functional groups based on their associated function roles. These include angiogenesis, anti-apoptosis, anti-inflammatory, pro-inflammatory, lipid homeostasis, phagocytosis, and tissue remodeling (Table 4). Fourteen of the upregulated genes are involved in pro-inflammatory process. The highest upregulated genes in this category are the chemokines *CCL1*, *CCL3*, and *CCL4*, which had fold changes ranging from 9.1 to 19.1. Upregulated genes associated with phagocytosis include *MARCKS*, *ADORA2A*, *HCK*, *NCF1*, and *SRC* with fold changes ranging from 4.1 for *MARCKS* to 2.4 for *SRC*. Upregulated genes with functional roles in tissue remodeling include *TNFAIP6*, *LAMP3*, *IL23A*, *LEPREL1*, and *PTGS2*. The full list of genes and their associated fold differences can be found in Electronic Supplementary Material Table S2.

Validation of microarray result by real-time PCR

The microarray result was validated using real-time PCR. The fold changes and 95% confidence interval were obtained from three independent experiments (Table 5). The results were in agreement with those from microarray, and upregulation was significant for all tested genes.

Table 4 SAA1 upregulates the expression of genes that are involved in inflammation, angiogenesis, phagocytosis, tissue remodeling, and anti-apoptosis

Functional role	Gene	Fold changes
Angiogenesis	Interleukin-8 precursor (<i>IL8</i>)	5.8
	Tumor necrosis factor-inducible gene 2 protein (<i>TNFAIP2</i>)	3.2
	Interleukin 1, beta (<i>IL1B</i>)	2.6
	Chemokine (C–C motif) ligand 2 (<i>CCL2</i>)	2.5
	Zinc finger CCCH-type containing 12A (<i>ZC3H12A</i>)	2.0
Anti-apoptosis	Plasminogen activator inhibitor 2 (<i>SERPINB2</i>)	3.9
	Adenosine A2A receptor (<i>ADORA2A</i>)	2.8
	Immediate early response 3 (<i>IER3</i>)	2.4
	Baculoviral IAP repeat-containing protein 3 (<i>BIRC3</i>)	2.1
Anti-inflammatory	Tumor necrosis factor-inducible gene 6 protein (<i>TNFAIP6</i>)	15.5
	Plasminogen activator inhibitor 2 (<i>SERPINB2</i>)	3.9
	Zinc finger CCCH-type containing 12A (<i>ZC3H12A</i>)	2.0
Lipid homeostasis	Oxidised low density lipoprotein receptor 1 (<i>OLRI</i>)	3.0
Phagocytosis	Myristoylated alanine-rich protein kinase C substrate (<i>MARCKS</i>)	4.1
	Adenosine A2A receptor (<i>ADORA2A</i>)	2.8
	Tyrosine-protein kinase (<i>HCK</i>)	2.4
	Neutrophil cytosol factor 1 (<i>NCF1</i>)	2.4
	Proto-oncogene tyrosine-protein kinase Src (<i>SRC</i>)	2.4
Pro-inflammatory	C–C motif chemokine 4 precursor (<i>CCL4</i>)	19.1
	C–C motif chemokine 1 precursor (<i>CCL1</i>)	14.5
	C–C motif chemokine 3 precursor (<i>CCL3</i>)	9.1
	Interleukin-23, alpha subunit p19 (<i>IL23A</i>)	6.9
	Interleukin-8 precursor (<i>IL8</i>)	5.8
Tissue remodeling/ wound healing	Tumor necrosis factor-inducible gene 6 protein (<i>TNFAIP6</i>)	15.5
	Lysosome-associated membrane glycoprotein 3 (<i>LAMP3</i>)	7.3
	Interleukin 23, alpha subunit p19 (<i>IL23A</i>)	6.9
	Leprecan-like 1 (<i>LEPRELI</i>)	3.2
	Prostaglandin-endoperoxide synthase 2 (<i>PTGS2</i>)	2.4

Upregulated genes were classified according to their functional roles, which include inflammation, angiogenesis, phagocytosis, tissue remodeling, and anti-apoptosis. A gene ontology program, GOEAST, was used to aid in the functional classification of upregulated genes. For each functional role, a maximum of five genes is shown. The full list is shown in Table S2

SAA1 is a strong inducer of chemokines

There was an increased secretion of chemokines CCL1, CCL3, and CCL4 upon incubation of macrophages with SAA1 (Fig. 1). SAA1 is a potent inducer of chemokines; production of CCL1, CCL3, and CCL4 were increased by 14, 20, and 27-fold, respectively, compared to the untreated control when treated with 1 µg/ml SAA1. The release of cytokines from the macrophages was dose-dependent, consistent with the microarray result.

Discussion

SAA1 is a major acute phase protein although its exact role during an acute phase response is not known. In recent years, SAA1 has been increasingly associated with chronic inflammatory diseases, in particular atherosclerosis and RA. It is, however, unclear whether SAA1 is proatherogenic or

atheroprotective. To gain insight into the role of SAA1 in inflammatory diseases, we performed a microarray analysis to determine the global changes in gene expression when THP-1-derived macrophages were treated with SAA1. Macrophages were used for the study as these cells are central to the pathology of both atherosclerosis and RA [26]. The study was conducted using RNA extracted from macrophages that were subjected to either 8 or 24 h of incubation with SAA1 to ensure that any lags in induction of gene expression could be detected. The circulating concentration of A-SAA in healthy individuals, consisting of SAA1 and SAA2, has a wide range with a mean of 10 µg/ml [27]. However, since the lesion development occurs within in the arterial walls where less SAA1 is found, a lower concentration of 1 µg/ml SAA1 was used.

SAA1 is an acute phase protein and plays a role in innate immunity. Treatment of macrophages with SAA1 induced an upregulation of a number of genes involved with immune regulation, inflammation, and phagocytosis.

Table 5 Validation of microarray results using real-time PCR

Gene	Fold changes	95% Confidence interval	<i>P</i> value
<i>CCL3</i>	9.8	8.6–10.8	<0.0001
<i>IL23A</i>	24.4	20.6–29.5	<0.0001
<i>ITGA1</i>	2.3	1.8–3.3	<0.0001
<i>MARCKS</i>	6.0	5.1–7.1	<0.0001
<i>OLR1</i>	2.6	2.2–3.1	<0.0001
<i>CCL4</i>	13.4	11.5–16.5	<0.0001
<i>SERPINB2</i>	14.6	8.9–20.8	0.024

Fold changes between samples obtained from macrophages treated with SAA1 and untreated macrophages were determined using *B2M* as an internal control for all genes except *SERPINB2*. *FXVD5* was used as an internal control for *SERPINB2* ($n = 3$). All genes were found to be upregulated compared to untreated controls ($P < 0.05$).

These include seven chemokines (*CCL1*, *CCL3*, *CCL4*, *CXCL6*, *CXCL10*, and *CCL20*) and five genes with roles in phagocytosis (*HCK*, *MARCKS*, *NCF1*, *SRC*, and *ADORA2A*). As the CCL family chemokines (*CCL1*, *CCL3*, and *CCL4*) showed high levels of upregulation in both microarray and real-time PCR studies, ELISA was performed to ascertain whether a high level of these proteins exists. The inflammatory response is probably modulated with the induction of anti-inflammatory *TNFAIP6* and *CD83*. Induction of anti-apoptotic genes by SAA1 might help to promote the survival of the macrophage as it performs phagocytosis of foreign particles, a process which might be injurious to the cell. SAA1 also stimulates the upregulation of *OLR1*, a receptor for oxidized LDL, which helps protect the endothelium from the oxidative stress induced by oxidized lipids [28]. Lastly, SAA1 promotes healing through the upregulation of a number of genes such as *TNFAIP6*, *LAMP3*, and *CD44* [29]. Hence, SAA1 carries out its function in innate immunity through stimulating the migration of monocytes and macrophages to the site of injury, promoting clearance of foreign particles and facilitating the healing of injured tissue through stimulation of factors that promote healing.

The production of SAA1 by the liver is tightly regulated. However, SAA1 levels can increase 1,000-fold during an acute-phase response. Aberrant production is possibly detrimental due to its association with chronic inflammatory diseases such as atherosclerosis and RA. Genes that are upregulated by SAA1 treatment such as cytokines, chemokines, *CD40* [30], *CD44*, and *OLR1* are potentially atherogenic. *CCL1* and *CCL4* are both stimulators of macrophage migration into tissues [31, 32] and are found in the majority of samples from atherosclerotic plaques of human coronary arteries [33]. Increased production of *OLR1* can facilitate the formation of foam cells [34]. Genes that promote wound healing might also result in

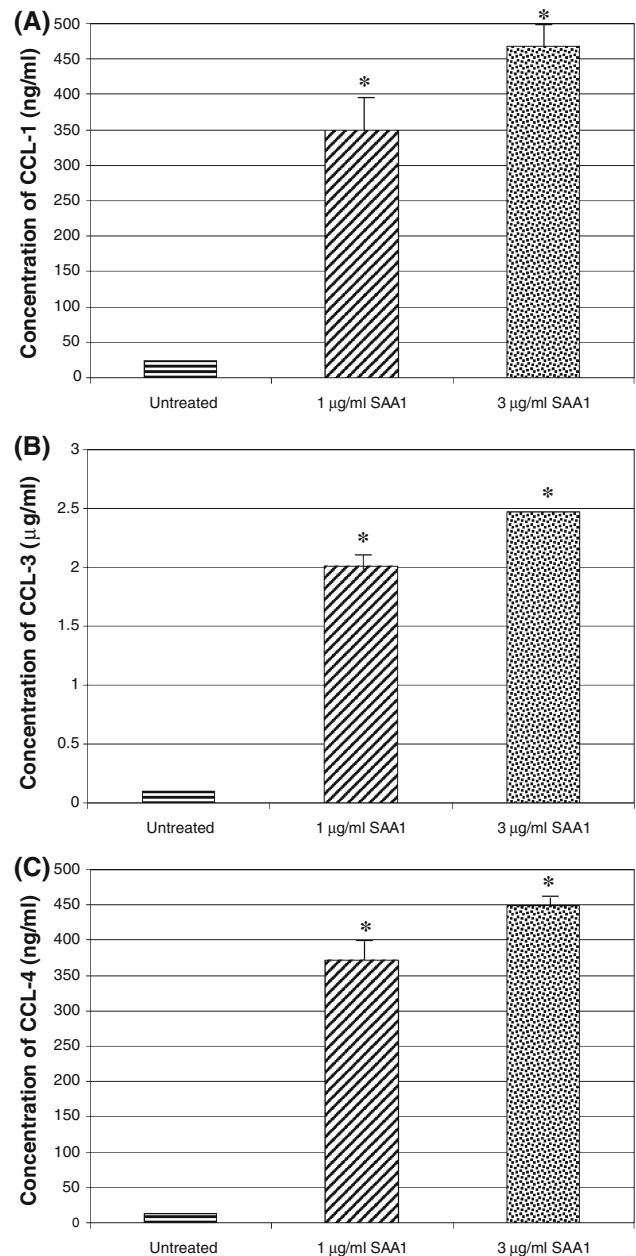


Fig. 1 Effects of varying concentrations of recombinant human SAA1 on the production of **a** CCL-1, **b** CCL-3, and **c** CCL-4 from THP-1-derived macrophages. 2.5×10^6 cells were incubated with recombinant SAA1 for 24 h and the supernatants were assayed for cytokines using ELISA. Error bars represent standard deviations ($n = 3$). Asterisk indicates $P < 0.05$ for recombinant human SAA1 vs. untreated

tissue remodeling, which plays a role in the pathogenesis of both atherosclerosis and RA. In addition, *CD44*, which is involved in wound healing, might have other atherogenic roles. *CD44* is reported to be involved in the recruitment of macrophages and T cells to atherosclerotic lesions [35], and augmented levels of *CD44* are found in macrophages from atherosclerotic subjects [36]. In addition, *CD44* also

increases the expression level of genes that are involved in processes crucial to atherogenesis [37].

It was reported in some studies that SAA1 might facilitate cholesterol efflux from macrophages. In our study, we found no significant upregulation of ABCA1 and ABCG1 transporters that are essential for cholesterol efflux. Thus, SAA1-mediated cholesterol efflux may be due to either SAA1 functioning as a lipid acceptor [38] or facilitating in the remodeling of HDL [19].

The utilization of SAA as a clinical biomarker for RA and CAD implies that both local and low-grade systemic inflammation plays a role in the pathogenesis of chronic inflammatory disease. Besides the liver, the adipose tissue is another major source of SAA, especially under non-acute phase conditions. The SAA that is produced by the adipose tissue is similar to that produced by the liver. Both visceral adipose tissue [39] and human white adipocytes [40] were shown to produce significant amounts of SAA. Since adipose tissue is a source of SAA, the perivascular adipocytes might be an important local source of SAA as well as other adipokines and cytokines that might contribute to atherosclerosis. Perivascular adipose tissue is found in the vicinity of the aorta and is not separated from the blood vessel wall by an anatomical barrier. The perivascular adipocytes thus provide a local source of SAA production to the macrophages residing in the atherosclerotic lesion. Indeed, segments of coronary arteries that were not surrounded by adipose tissue were found to be free from atherosclerosis while those adjacent to the adipocytes were more prone to atherosclerosis [41, 42]. Given that SAA upregulates a significant number of genes and pathways in macrophages that are atherogenic, a chronic production of SAA in the coronary artery might facilitate atherosclerosis.

Through this study, we gained insight into the role of SAA in innate immunity and its contribution to chronic inflammatory disease. During an acute phase response, SAA1 stimulates the rapid clearance of foreign agents through its ability to induce the migration of immune cells to the site of injury and enhance phagocytosis of the foreign agents. In addition, it also promotes healing of the damaged tissue. Stimulation of SAA1 is, however, tightly regulated and increased production only occurs during the acute phase response. Chronic production of SAA1 is, however, atherogenic and this might be contributed by the adipocytes as its production does not appear to be associated with an acute phase response which is tightly regulated. The local production of SAA1 by adipocytes could result in the migration of immune cells and ultimately local inflammation of the tissue. Atherosclerosis or rheumatoid arthritis might occur if the local inflammation occurs adjacent to the artery or in the joint, respectively.

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