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The cytokine-serum amyloid A-chemokine network

Mieke De Buck[a], **Mieke Gouwy**[a], **Ji Ming Wang**[b], **Jacques Van Snick**[c,d], **Paul Proost**[a], **Sofie Struyf**[a], **Jo Van Damme**[a,*]

[a]KU Leuven, University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Immunology, Minderbroedersstraat 10, 3000 Leuven, Belgium

[b]Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702, USA

[c]Ludwig Cancer Research, Brussels Branch, Brussels, Belgium

[d]de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

Abstract

Levels of serum amyloid A (SAA), a major acute phase protein in humans, are increased up to 1000-fold upon infection, trauma, cancer or other inflammatory events. However, the exact role of SAA in host defense is yet not fully understood. Several pro- and anti-inflammatory properties have been ascribed to SAA. Here, the regulated production of SAA by cytokines and glucocorticoids is discussed first. Secondly, the cytokine and chemokine inducing capacity of SAA and its receptor usage are reviewed. Thirdly, the direct (via FPR2) and indirect (via TLR2) chemotactic effects of SAA and its synergy with chemokines are unraveled. Altogether, a complex cytokine–SAA–chemokine network is established, in which SAA plays a key role in regulating the inflammatory response.

Keywords

SAA; FPR2; Chemotaxis; Cytokines; TLR2

1. Introduction

During inflammatory events, such as infection, trauma and neoplasia, the acute phase response is initiated to eliminate pathogens and to restore homeostasis without causing too much damage to the organism. This acute phase response is characterized by a wide range of systemic and metabolic changes (e.g. fever, leukocytosis, altered plasma levels of minerals and vitamins), including the induction of acute phase proteins (e.g. serum amyloid A, C-reactive protein) in the liver [1,2]. In humans, serum amyloid A (SAA) is one of the major acute phase proteins and consists of SAA1, SAA2, SAA3 and SAA4. SAA1 (with the

*Corresponding author. Fax: +32 16 3 37340. jo.vandamme@rega.kuleuven.be (J. Van Damme).

Conflict of interest

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isoforms SAA1α, −β and −γ) and SAA2 (with the isoforms SAA2α and −β) are also designated “acute phase SAA” (A-SAA), since the serum concentration of these SAA types increases up to 1000-fold during the acute phase response. Once secreted in the blood circulation, A-SAA binds to high density lipoprotein (HDL), thereby displacing apolipoprotein (Apo)-A-I. Although SAA3 is predominantly expressed extrahepatically in other mammals [3], it was detected only at very low concentrations in humans [4]. In contrast to A-SAA, SAA4 is constitutively present in the blood circulation [5]. For that reason, SAA4 is also denominated “constitutive SAA” (C-SAA).

Until now, several functions have been attributed to SAA, such as antimicrobial activities and the induction of matrix degrading enzymes (e.g., MMP-9) [6–10]. However, the complete role of this highly conserved acute phase protein has not yet been elucidated. Part of its pro-inflammatory capacities resides in its ability to induce cytokines and chemokines and to exert direct chemotactic activity via its receptors, predominantly TLR2 and FPR2, respectively.

In this survey, the regulated production of A-SAA by (anti-) inflammatory agents, its cytokine and chemokine inducing activity, as well as its chemotactic effect will be reviewed. Since the liver is the primary source of this acute phase protein [2,11,12], the induction of A-SAA in human hepatocytes by cytokines and glucocorticoids will be discussed first.

2. Regulated production of acute phase SAA in the liver

2.1. Induction of A-SAA by cytokines

The induction of SAA mRNA and/or protein in primary hepatocytes and hepatoma cell lines in vitro is summarized in Table 1. Some of these data are contra-intuitive, for instance, although known to be major inducers of acute phase proteins in the liver, IL-1β or IL-6 alone did not always induce SAA expression in vitro [13–20]. In general, in hepatoma cell lines, IL-6 stimulates the production of SAA to a lesser extent than IL-1 [20–23]. TNF-α is a weak SAA-inducer in hepatocytes (Table 1). Only Thorn et al. [18] detected production of SAA when HepG2 cells were stimulated with TNF-α alone. IL-1 is the strongest inducer of SAA mRNA and protein, with IL-1β being more potent than IL-1α [22,24].

In contrast to the sometimes weak in vitro induction rate of SAA when IL-1, IL-6 and TNF-α were used separately to stimulate hepatocytes or hepatoma cells, these cytokines were able to cooperate or even synergize to greatly enhance the production of SAA [14,15,18–21,23,25]. In fact, in vivo, IL-1 stimulates the production of IL-6 in several cell types, thereby directly and indirectly amplifying the synthesis of acute phase proteins in the liver [26]. In all the studies, where IL-6 alone did not induce SAA, this cytokine synergized with IL-1 to increase the expression of the SAA gene in hepatoma cells [14,20,23,25]. Moreover TNF-α, which is often a poor inducer of SAA, potentiated the effect of IL-6 [14,18,19]. However, Vreugdenhil et al. [25] reported that TNF-α inhibited the IL-1β- or IL-1β plus IL-6-induced SAA production in HuH7 hepatoma cells. Nonetheless, no other data are available about the effect of TNF-α on the induction of SAA in HuH7 cells; additional studies are required to draw firm conclusions.

The kinetics of SAA induction seem to depend on the cytokine stimulating the synthesis of SAA. IL-6 induced a maximal expression of SAA2 mRNA in HepG2 hepatoma cells as early as 3 h post induction, whereas maximal amounts of SAA2 mRNA were only induced after 12–24 h when the cells were treated with IL-1β or TNF-α [19]. Secondly, Thorn and White head [16] and Thorn et al. [17] stipulated that the cytokine type determines which A-SAA form is preferably induced. These authors showed that, besides SAA1 mRNA, particularly SAA2 mRNA was induced in HepG2 cells upon stimulation with IL-1 and/or IL-6 or with a combination of IL-6 and TNF-α. Furthermore, the production level of SAA after stimulation of Hep3B and HepG2 cells with a combination of IL-1 and IL-6 depended on the cytokine concentration. SAA synthesis dose-dependently increased with increasing concentrations of one of both cytokines, as long as the other cytokine was present at minimal concentrations [14,22]. Finally, the order of addition of the cytokines to the cells is important for the level of SAA synthesis, since Thorn et al. [18] found that there was a better induction of A-SAA mRNA when HepG2 cells were treated with TNF-α and subsequently with IL-6, rather than vice versa.

2.2. Induction of A-SAA by glucocorticoids

Glucocorticoids down-regulate the synthesis of IL-1, IL-6 and TNF-α [12,22,27]. On the other hand, these anti-inflammatory agents also directly stimulate the production of some acute phase proteins, including SAA. The glucocorticoid receptor–ligand complex translocates to the nucleus, where it functions as a transcription factor, up-regulating the transcription of acute phase genes [12,27].

In contrast to their effects on some non-hepatic cell lines [17,18,28–33], glucocorticoids (mostly studied using the synthetic glucocorticoid dexamethasone) on their own did not induce SAA production in hepatocytes or hepatoma cell lines [14,20–22,33–35]. However, glucocorticoids were shown to be potent enhancers of SAA induction in hepatocytes and hepatoma cells when combined with IL-1 and/or IL-6 [14,17,20–22,33,36]. No cooperation between TNF-α and dexamethasone was found in HepG2 hepatoma cells, but this combination has so far been studied poorly [22]. Synergy between glucocorticoids and TNF-α to induce SAA, as well as between glucocorticoids and the other cytokines, was nonetheless already evidenced in several non-hepatic cell lines [17,18,25,28,31]. Even more, dexamethasone was found to be necessary to induce SAA in smooth muscle cells, multipotent adipose-derived stem cells and monocytic THP-1 cells, stimulated with inflammatory agents such as IL-1, IL-6 or lipopolysaccharide (LPS) [32,37,38]. This effect was not observed in hepatocytes.

When glucocorticoids associate with their receptor, this receptor-ligand complex binds as a transcription factor to a glucocorticoid response element (GRE; GGCACATCTTGTTCC) in the promotor of certain genes. Concerning the different *A-SAA* genes, a GRE is present only in the *SAA1* promotor, but not in the promotor of the *SAA2* gene. This explains why only the transcription of the *SAA1* gene massively increased when hepatoma cells or cells of the oral epidermal carcinoma cell line KB were treated with a combination of dexamethasone and cytokines (alone or in combination). As a consequence, the SAA1 level was specifically augmented in contrast to the amount of SAA2, whereas the equilibrium between the

transcription of both *A-SAA* genes was opposite when the cells were stimulated with cytokines without dexamethasone [16–18].

2.3. Induction of A-SAA by toll-like receptor ligands

Direct induction of A-SAA by toll-like receptor (TLR) ligands in humans has until now only been shown for LPS, originating from the outer membrane of gram-negative bacteria. In contrast to mice [12], LPS-mediated induction of A-SAA has not yet been intensively studied in humans, except for some human cell lines in which LPS has been used as an SAA inducer. Ray and Ray [39] and Bozinovski et al. [28] demonstrated the expression of SAA mRNA in undifferentiated THP-1 cells treated with LPS. In addition, LPS and dexamethasone synergized strongly for inducing SAA mRNA [28,38].

2.4. (Post-)transcriptional and (post-)translational regulation of A-SAA synthesis

Under inflammatory conditions, macrophages and other leukocytes produce large amounts of IL-1, IL-6 and TNF-α. Binding of these cytokines to their proper receptors triggers the activation of different intracellular signal transduction pathways, leading eventually to the activation of several transcription factors in the cytoplasm, mostly via phosphorylation events. For cytokine-induced production of A-SAA in humans, four transcription factors are important: NF-κB, activated by IL-1 and TNF, NF-IL6 [human orthologue of C/enhancer binding protein (EBP) in other species], activated by IL-1 and IL-6, NF-IL6β, activated by phosphorylated (i.e. activated) NF-IL6, and SAA activating sequence (SAS) activating factor (SAF), activated by IL-6 and LPS [12,27,39–46]. Binding of these transcription factors to regulatory elements in the A-SAA promotor results in transcription of A-SAA mRNA. In rats, an additional transcription factor, YY1, which inhibits the transcription of the *SAA1* gene, was found. Two potential YY1-binding sites have been found in the promotor of the human *SAA2* gene, but the significance of this negative regulator has not yet been elucidated in humans [27]. Nonetheless, Edbrooke et al. [47] detected a negative NF-κB-binding element in the human *A-SAA* promotor, i.e. SAAκB2. Mutation of this binding site, resulting in disabled binding of NF-κB, caused a higher transcription rate of C-SAA and of IL-1-induced SAA. Further research about these negative regulating binding sites is required.

The production of A-SAA is not only regulated at the transcriptional level, but also at the post-transcriptional stage. This was demonstrated in cytokine-stimulated hepatoma Hep3B cells by Jiang et al. [15,48], who found a discrepancy between transcriptional activity of the *SAA* gene(s) and the actual SAA mRNA levels detected, the latter being relatively high. Moreover, treatment of hepatoma PLC/PRF/5 cells, KB (epithelial) cells or ECV304 (endothelial) cells with the transcription inhibitor actinomycin D did not diminish A-SAA mRNA levels, suggesting that the mRNA becomes stabilized. However, this phenomenon does not occur via inhibition of poly(A) tail shortening, since the stability of SAA mRNA with different lengths of the poly(A) tail was identical in PLC/PRF/5 cells [33,35].

Besides transcriptional and post-transcriptional control of *A-SAA* gene expression, there is also evidence of translational regulation of this gene. Despite high A-SAA mRNA levels in PLC/PRF/5 cells, secretion of the protein itself was relatively low [35]. No explanation for

this discrepancy was found. In fact, there was neither a change in A-SAA mRNA polyribosome association, nor a decline in export time of the protein. More studies are needed to unravel the post-transcriptional and potential (post-)translational regulation of the SAA genes. In contrast, several post-translationally modified SAA forms have been detected in deposits of amyloid A fibrils, causing amyloid A amyloidosis [49].

3. The SAA-chemokine cascade

3.1. SAA as a cytokine inducer

3.1.1. Induction of cytokines by SAA—As illustrated in Table 2, many cytokines are produced by SAA-stimulated cells. Members of different cytokine families can be induced. Induction of cytokines belonging to the chemokine family is discussed in a separate section (vide infra).

IL-1β, IL-6 and TNF-α are the most extensively studied cytokines to be induced by SAA. Maximal production of IL-1β after 18–24h stimulation of cells with SAA did generally reach not more than 1 ng/ml [50–54]. Often, relatively high concentrations (1–10 μg/ml) of SAA (in comparison to IL-1β induction by LPS) were needed to significantly induce this cytokine in monocytes, macrophages, synoviocytes and keratinocytes [51,52,54–57]. IL-1β was only induced by high concentrations (≥100 μg/ml) of SAA in neutrophils and HMC-1 mast cells [50,58]. Niemi et al. [58] and Yu et al. [54] evidenced that SAA stimulated IL-1β production by binding to TLR2 and TLR4 expressed on macrophages and keratinocytes, since incubation of these cells with anti-TLR2 or anti-TLR4 antibodies significantly reduced the expression of IL-1β mRNA stimulated by SAA (Table 2). Moreover, when the cells were treated with a combination of both antibodies, the IL-1β mRNA expression further decreased. In contrast, treatment of the cells with pertussis toxin, which binds Ga and prevents $G_{\alpha i}$ protein subunit dissociation, or with anti-cluster of differentiation (CD) 36 antibody did not affect mRNA expression induced by SAA. This indicates that the GPCR formyl peptide receptor like 1/formyl peptide receptor 2 (FPRL1/FPR2) and CD36 are not involved in IL-1β induction by SAA in these cells. CD36 is a member of the class B scavenger receptor family binding LDL and has been shown to mediate induction of chemokines by SAA (vide infra). In contrast to IL-1β, induction of IL-33 involves binding to FPR2 in addition to TLR2. Besides significant inhibition of IL-33 production in peritoneal macrophages from TLR2 knockout mice, IL-33 production in peritoneal macrophages from FPR2-depleted mice was also inhibited for 50%, compared to normal mice [59].

SAA also stimulated the production of IL-6. Levels ranging from less than 1 ng/ml in primary dermal fibroblasts [60] and 3–5 ng/ml in endothelial cells [61,62] up to 25–70 ng/ml in synovial fibroblasts and chondrocytes [29,42] were detected after 24 h stimulation. Like for IL-1β induction, a relatively high (about 0.5–10 μg/ml) SAA concentration (in comparison to IL-6 induction by LPS and IL-1β) was required to induce IL-6. Different receptors have been proposed for the induction of the production of IL-6 by SAA: TLR2, TLR4 and CLA-1 (CD36 and LIMP II analogous-1). CLA-1 is the human orthologue of the murine scavenger receptor class B type I (SR-BI) and binds, besides lipoproteins, a variety of ligands [63,64]. Both anti-TLR2 antibody and small interfering (si)RNA, knocking down

TLR2, significantly inhibited IL-6 induction in SAA-stimulated fibroblasts [60]. Lakota et al. [61] also observed an inhibition of IL-6 induction by TLR2-targeted siRNA in endothelial cells, but this decline in IL-6 production was not significant. Mullan et al. [62] showed that the induction of IL-6 diminished clearly in synovial fibroblasts from three out of four rheumatoid arthritis (RA) patients upon treatment of the cells with anti-(murine) SR-BI antibody that also inhibited (human) CLA-1, suggesting the involvement of CLA-1 in cytokine induction by SAA in certain cases. Nonetheless, de Seny et al. [29] did not see any effect on SAA-induced IL-6 production in synovial fibroblasts or in human primary chondrocytes from osteoarthritis patients neither using an anti-SR-BI antibody, nor using the irreversible CD36 inhibitor SSO or an antibody against receptor for advanced glycation end products (RAGE), shown to mediate the SAA-induced production of tissue factor in monocytes [65]. Furthermore, competition with lipoxin A4 for binding to FPR2 did not reduce IL-6 production. On the other hand, these authors demonstrated that the IL-6 induction in synovial fibroblasts and primary chondrocytes from osteoarthritis patients was triggered by binding of SAA to TLR4 [29].

In contrast to the SAA-induced production of IL-1β, that of TNF-α seemed to be highest at 18 h post stimulation and was already declining at 24h. At 18 h, supernatants from cell cultures of neutrophils, monocytes or macrophages contained 5–20 ng/ml TNF-α [51,52], whereas there was only 2–5 ng/ml left at 24 h [50,66–69]. Just as for IL-1β and IL-6, TNF-α was significantly induced in these cells by SAA at relatively high concentrations (from 1.25–10 μg/ml onwards). Mast cells needed to be stimulated with a high concentration (120 μg/ml) of SAA to produce TNF-α [58]. Furthermore, monocytes and macrophages tended to produce more TNF-α than neutrophils in response to SAA. With regard to the receptor mediating the induction of TNF-α by SAA, Chen et al. [69] found a slight, but significant inhibition of TNF-α production when macrophages were preincubated with anti-TLR2 antibody. Nonetheless, the involvement of other putative receptors has still to be investigated.

Induction of cytokines other than IL-1β, IL-6 or TNF-α by SAA was less studied. It is worth mentioning that SAA stimulates the induction of active IL-23 (consisting of the IL-12p40 and the IL-23p19 subunits), but not of IL-12 (consisting of the IL-12p40 and the IL-12p35 subunits), since it induces only the IL-12p40 [56,70] and the IL-23p19 [55,56,70] subunits, but not the IL-12p35 subunit [70]. TLR2 has regularly been mentioned as a signaling receptor for SAA-induced production of cytokines (Table 2). Induction of GM-CSF and the anti-inflammatory cytokine IL-10 in endothelial cells and macrophages, respectively, diminished significantly upon treatment of cells with anti-TLR2 antibody or with siRNA targeting TLR2 [61,69]. FPR2 was shown to be involved in the SAA-mediated induction of GM-CSF, M-CSF and IL-10 in A549, HepG2 and U937 cells, respectively [28,55]. Since knocking down FPR2 via treatment of cells with the FPR2 antagonist WRW_4 or with short hairpin RNA partially inhibited M-CSF and IL-10 induction (40% and 60% inhibition, respectively) [55], one must indeed conclude that multiple receptors are involved in SAA-mediated cytokine induction.

3.1.2. Induction of chemokines by SAA—SAA induces the production of CC and CXC chemokines in different cell types (Table 3). The production of the inflammatory

chemokines CCL2 and CXCL8 has been investigated in detail and will be discussed hereafter.

The induction of CCL2 after stimulation with SAA has mostly been investigated in monocytes and endothelial cells. In monocytes, the highest CCL2 production (20–25 ng/ml) was seen 24 h after stimulation with SAA [53,71]. At a concentration as low as 25 ng/ml, SAA was already able to induce a significant amount of CCL2 in these cells [71]. CCL2 production in SAA-treated (6–25 μg/ ml) endothelial cells reached levels of 20–60ng/ml 24h post stimulation [72–74]. Significant levels of CCL2 (10–35 ng/ml) were already induced at 4–8 h after treatment of endothelial cells with SAA [71,74,75].

Although for the induction of cytokines SAA has been reported to signal mostly via TLRs and less via FPR2, the induction of CCL2 was found to be mainly mediated by FPR2 [28,55,71,73,74]. Bozinovski and co-workers [28] found that human FPR2-transfected A549 lung epithelial cells produced three times more CCL2 (90 ng/ml) upon stimulation with SAA (1 mg/ml) than when cells were treated with vehicle (30 ng/ml). Moreover, the induction of CCL2 was completely inhibited in monocytes and endothelial cells using the FPR2 antagonist WRW_4 [71,74]. Partial (but significant) inhibition (about 50%) of CCL2 production was observed in endothelial cells transfected with FPR2 siRNA [73], as well as in HepG2 cells transfected with FPR2 short hairpin RNA (35% inhibition) [55]. Connolly et al. [72] demonstrated that CCL2 production under influence of endogenously produced SAA in synovial explant cultures minimally diminished (from 9 to 6.5 ng/ml) upon competition for binding to FPR2 with lipoxin A4. These data and related results on the induction of some cytokines suggest that other receptors are also involved in SAA-mediated CCL2 production. Indeed, a minimal but significant inhibition of CCL2 production (from 8.5 to 6ng/ml after 24h stimulation) was detected when synovial explant cultures were treated with mouse anti-SR-BI antibody (which also inhibits binding of ligands to human CLA-1) [72]. Likewise, CCL2 production in SAA-stimulated synovial fibroblasts from osteoarthritis patients was significantly, but not completely, inhibited by treatment of cells with the TLR4 inhibitor TAK242, whereas this production also (not significantly) diminished upon treatment of cells with anti-RAGE antibody. CCL2 production in SAA-stimulated primary chondrocytes from the same patients was also reduced after preincubation of cells with TAK242 (significantly) or with RAGE or anti-SR-BI antibody (not significantly). Treatment of synoviocytes or chondrocytes with the irreversible CD36 inhibitor SSO or with the FPR2 agonist lipoxin A4 did not alter the CCL2-inducing capacity of SAA [29].

Several reports described CXCL8 production in SAA-stimulated cells 24 h post induction. Neutrophils, monocytes and, to a lesser extent, endothelial cells were studied. Monocytes produced greater amounts of chemokine than neutrophils [51,76,77], as already shown by other investigators in response to other stimuli [78–80]. Moreover, 17–125μg/ml SAA was required to significantly induce CXCL8 in neutrophils [50,51,76,77,81,82], whereas, in monocytes, 10–100 ng/ml of SAA already induced about 10 ng/ml of CXCL8 3 h after stimulation [76,83]. In a 24 h period, monocytes and macrophages produced 10–50 ng/ml of CXCL8 in response to 10–25ng/ml SAA [53]. Endothelial cells more potently produced CXCL8 than CCL2, since treatment with only 0.5–1.25 μg/ml SAA during 8 or 24h significantly induced 10–20ng/ml of CXCL8 [61,62,75], whereas generally higher

concentrations of SAA were necessary to induce a substantial amount of CCL2 (vide supra). Maximal production of CXCL8 was reached 20–24 h after SAA stimulation of monocytes (50–400 ng/ml) [53,76,77,83], neutrophils (10–45 ng/ml) [50,77,84] or immature dendritic cells (30 ng/ ml) [83]. SAA induced, from 3–4h post stimulation onwards, significant amounts of CXCL8 in monocytes (about 10 ng/ml) [76,83], neutrophils (about 1 ng/ml) [82] and immature dendritic cells (about 15 ng/ml) [83]. In our hands, SAA did not induce significant amounts of CXCL8 in neutrophils [76].

Furthermore, it has to be noted that Song et al. [53] explored cytokine production in SAA-stimulated lymphocytes. They found that T lymphocytes produced a very low, but significant amount of CXCL8 (50 pg/ml), but not of IL-1β, IL-6, GM-CSF, IL-10, TNF, CCL2 or CCL3, after stimulation with a very low concentration of SAA (25 ng/ml). A ten-fold higher dose of SAA induced less but still significant amounts of CXCL8 (about 20 pg/ml).

Similar to the induction of some cytokines, several receptors have been reported to mediate CXCL8 induction in SAA-stimulated cells: TLR2, TLR4, FPR2, CLA-1 and CD36 (Table 3). Receptor transfected cell lines were often used to show receptor-specific production of CXCL8 upon stimulation with SAA [28,63,85,86]. In our hands, SAA-induced production of CXCL8 was mediated through TLR2, as the production of CXCL8 in monocytes diminished significantly (62% inhibition) upon treatment of cells with anti-TLR2 antibody [76]. In contrast, the FPR2 antagonist WRW_4 did not influence CXCL8 induction, nor could we detect any significant CXCL8 production in human FPR2-transfected human embryonic kidney 293 (HEK293) cells stimulated with SAA, indicating that not FPR2, but TLR2 mediates CXCL8 induction in SAA-stimulated cells. This is not in agreement with the finding that preincubating neutrophils with pertussis toxin, which blocks signaling through $G_{\alpha i}$ proteins, strongly reduced (about 80% inhibition) SAA-induced CXCL8 production [82]. Further, human FPR2-transfected Chinese hamster ovary (CHO) cells and human FPR2-transfected A549 lung epithelial cells were able to produce CXCL8 upon stimulation with SAA [28,86]. In contrast, treatment of synovial fibroblasts and primary chondrocytes from osteoarthritis patients with lipoxin A4 did not inhibit CXCL8 induction when these cells were stimulated with SAA [29]. Treating these cells with anti-SR-BI or anti-RAGE antibody or with the CD36 inhibitor SSO did not alter CXCL8 production either. On the other hand, just as described for IL-6, CCL2 and CXCL1 production, a significant inhibition of SAA-induced CXCL8 production was obtained when the synoviocytes or chondrocytes were preincubated with the TLR4 inhibitor TAK242. CD36 was also stipulated as a receptor mediating SAA-induced CXCL8 production in CD36-transfected HEK293 cells [85], but this finding is controversial [29]. Another putative receptor mediating SAA-induced CXCL8 production is CLA-1. Treatment of endothelial cells with anti-SR-BI antibody potently reduced CXCL8 production after stimulation with SAA. The production of this chemokine in two out of three SAA-stimulated cultures of synovial fibroblasts from RA patients was also diminished using this antibody [62]. This last finding illustrates once more that probably, depending on the cell type, different receptors are involved in SAA-induced cytokine production and that there might even be an interplay between receptors of different classes.

3.1.3. Influence of HDL on the cytokine-like properties of SAA—Researchers studying the cytokine-inducing properties of SAA mostly used a recombinant SAA form under serum-free conditions. However, in the blood circulation, SAA is preferably bound to HDL. Therefore, a HDL-conjugated SAA form was used in some studies to investigate whether this HDL-binding could influence its cytokine-and chemokine-inducing capacity. Baranova et al. [85] showed that binding of SAA to HDL reduced CXCL8 induction in CD36-transfected HEK293 cells up to 70% after 20 h of stimulation. This inhibition diminished as the SAA/HDL molar ratio increased (SAA from 10 to 1000 μg/ml, bound to 2μg/ml HDL, corresponding to a SAA/HDL molar ratio of 1:20 to 1:0.2), like is the case during the acute phase response. Thus, during such acute phase response, SAA (partly) bound to HDL should still be able to induce a substantial amount of CXCL8. In our study, we deliberately used a relatively low SAA/HDL ratio (1 μg/ml SAA bound to 2 mg/ml HDL) to induce CXCL8 in monocytes. SAA at 1 μg/ml clearly induced CXCL8 (about 450 ng/ml) in monocytes 24 h post stimulation. In line with the results of Baranova et al. [85], CXCL8 induction was completely (96%) inhibited at this low SAA/HDL ratio [76]. Furthermore, Furlaneto and Campa [50] also demonstrated an inhibition of the induction of IL-1β, TNF-α and CXCL8 in neutrophils treated with HDL-SAA. Stimulation of cells with 100 μg/ml of SAA strongly induced these cytokines, whereas their production was completely blocked when the cells were stimulated with HDL (containing SAA) from patients with an infection and thus undergoing an acute phase response.

On the other hand, Patel et al. [87] reported that purified HDL, containing 30 μg/ml SAA, induced (after 6 h) practically the same amount of IL-1β in THP-1 cells as pure synthetic HDL-free SAA1α. When another 30 μg/ml of SAA was added to the HDL-SAA preparation (without preincubation), even more IL-1β was induced. Moreover, Lee et al. [71] showed that induction of CCL2 in monocytes (24 h) was not affected by HDL-conjugated SAA. However, in the latter study a relatively high SAA/HDL molar ratio was used, which was probably the reason for the lack of inhibition.

3.2. Chemokine induction by endogenous (cytokines) and exogenous (TLR ligands) inflammatory mediators

The previously mentioned data in this overview illustrate that the acute phase protein SAA induces production of higher amounts of chemokines than of cytokines. Moreover, compared to the amount of SAA needed for induction of cytokines, lower SAA concentrations are required to induce chemokines. Other well-known and potent chemokine inducers are the TLR4 agonist LPS and the SAA-inducing cytokine IL-1β (Table 4). In fact, SAA is as potent as LPS and IL-1β in terms of maximal amounts of chemokines produced. Depending on the cell type, SAA, LPS or IL-1β induced respectively (after 24h) 2–400 ng/ml [53,61, 74,76,83], 3–400 ng/ml [83,88–90] or 7–400 ng/ml [83,89,91] of CXCL8, CCL2 and CCL3. Furthermore, the concentration of SAA required to induce chemokines is comparable with that of LPS. SAA induces chemokines already at concentrations in the ng/ml range, i.e. 10 ng/ml of SAA being sufficient to significantly induce CXCL8 (8 ng/ml) in monocytes 3 h post stimulation [83]. IL-1β is even able to induce significant amounts of chemokine at still lower concentrations (≤1 ng/ml) than SAA and LPS [83,89–94]. Gouwy

et al. [83] showed that IL-1β at 1 ng/ml induced already 5–10 ng/ml CXCL8 and CCL3 in immature dendritic cells after 4 h.

The other two SAA-inducing cytokines, TNF-α and IL-6, are also able to induce chemokines (Table 4). Cells treated with TNF-α during maximally 24h produce 5–600 ng/ml chemokine [91,95,96], which is comparable with chemokine production in SAA-stimulated cells. However, just as for IL-1β, lower concentrations of TNF-α (1–20 ng/ml) are generally needed to induce chemokines [91,92,94,96–99]. On the other hand, a limited number of reports is available on chemokine induction by IL-6. IL-6 in general is a less potent inducer of the inflammatory chemokines CXCL8, CCL2 and CCL3 than SAA, IL-1β, TNF-α and LPS.

Not all cytokines induce the same type of chemokines. For instance, the class II cytokine family member IFN-γ is generally a poor CXCL8, CCL2 and CCL3 inducer (Table 4). Highly inducible chemokines by IFN-γ are the angiostatic CXCR3 ligands CXCL9, CXCL10 and CXCL11 [98,100,101]. Two to 20 ng/ml of IFN-γ is required to induce up to 2 μg/ml CXCL10 in endothelial cells after 72 h stimulation [98,100]. In one study, IFN-γ (5 ng/ml) was reported to induce 15 ng/ml CCL3 in monocytes [102], but in other studies, IFN-γ (2–2000 ng/ml) did not induce chemokines (except for CXCR3 ligands) in monocytes [80,89,98,103,104]. On the contrary, it was found that this cytokine inhibited CXCL8 production, since low concentrations of IFN-γ (5–10 ng/ml) reduced CXCL8 expression in LPS- or TNF-α-stimulated neutrophils and fibroblasts [103,105]. The same inhibitory effect of IFN-γ on CXCL6 production was seen in fibroblasts treated with IL-1β, TNF-α, LPS or double-stranded RNA (dsRNA) [94].

Exogenous dsRNA, which is mostly mimicked by synthetic polyriboinosinic:polyribocytidylic acid (polyrI:rC) in induction experiments, is also a chemokine inducer via binding to a TLR, namely to TLR3 (Table 4). About 10 μg/mlof polyrI:rC is generally needed to induce chemokines, which is modestly higher than the amount of SAA required for chemokine induction in monocytes and endothelial cells. However, polyrI:rC- and SAA-stimulated cells produce comparable amounts of the inflammatory chemokines CXCL8 and CCL2, i.e. 10–175 ng/ml being produced after 24 to 72 h stimulation with polyrI:rC [89,92–94,101,104].

Taken together, cytokines which induce the chemokine-inducing SAA (IL-1β, TNF-α, IL-6) are also able to directly induce chemokines. Furthermore, concerning the concentration required to induce chemokines, we can state the following: dsRNA ≥ SAA = LPS > IL-1β = TNF-α, reflecting their levels during infection. In contrast to the different amounts of inducers needed for chemokine induction, the maximal production level of chemokines is comparable between these SAA inducers. Both endogenous (i.e. IL-1β, TNF-α, IL-6, SAA) and exogenous (i.e. LPS, dsRNA) inflammatory mediators thus create a massive chemokine network, which amplifies the migratory potency of leukocytes during the inflammatory response (Fig. 1).

3.3. SAA as a chemoattractant

3.3.1. Human SAA target cells for chemotaxis—In comparison to the cytokine-inducing capacity of SAA, its chemotactic potency is relatively poorly studied. Cells responsive to SAA in chemotaxis assays are shown in Table 5. In Boyden chamber chemotaxis assays, the chemotactic potency of a chemoattractant can be expressed as a chemotactic index (CI), calculated by dividing the number of migrated cells to the chemoattractant by the number of migrated cells to the negative buffer control. Although its CI is mostly low, SAA at 10 μg/ml has been reported to stimulate the directed migration of human monocytes, neutrophils, immature dendritic cells, T cells, mast cells, endothelial cells, smooth muscle cells and synovial fibroblasts (Table 5). However, monocytes and immature dendritic cells are more sensitive to SAA as a chemoattractant, since already 12.5 and 100 ng/ml of SAA, respectively, provoked significant chemotaxis [71,83]. In contrast, neutrophils significantly migrated toward SAA from a concentration of 1 μg/ml onwards [76]. The chemotactic potency of SAA on synovial fibroblasts from patients with RA was assessed by a wound repair assay [72]. After 24–48 h, clearly more cells repopulated an artificial wound made in a confluent cell layer upon stimulation of the cells with SAA than when cells were stimulated with vehicle (i.e.1% DMSO). Moreover, SAA was shown to induce ruffling of the membrane of synovial fibroblasts and to mediate its chemotactic effect by active remodeling of the actin cytoskeleton, creating lamellopodia and philopodia.

Since SAA is a very weak chemoattractant, the question may rise whether the migration of cells toward this acute phase protein is caused by a chemokinetic effect, i.e. a random, undirected movement of the cells. To test this possibility, SAA was added together with the cells to the upper instead of the lower compartment of a chemotaxis chamber. Xu et al. [106] and Olsson et al. [107] demonstrated that SAA did not induce chemokinesis in T cells and mast cells, respectively. On the contrary, on neutrophils [108] and monocytes [83] a mild chemokinetic effect was noticed upon stimulation with SAA, but the chemokinetic effect was weaker than its chemotactic effect.

SAA has also been tested as a chemoattractant in vivo. Subcutaneous (SC) injection of mice with SAA (10 or 50 μg daily) provoked a clear recruitment of neutrophils and monocytes toward the injection site after 72 h [87,108]. The same effect was seen with T cells 24 h after a simple SC injection of 10 μg SAA [106]. SC injection of mice with 120 μg/kg (about 3 μg/mouse) SAA once a day caused a significant neutrophilia after 48 h, with a maximal neutrophilia after 5 days [109]. Furthermore, intranasal administration of 2 μg SAA to mice weekly for five weeks resulted in recruitment of significantly higher numbers of neutrophils into the lungs and bronchoalveolar lavages (BAL) than in saline-treated mice [110]. Moreover, we recently found that intraperitoneal (IP) injection of only 1 μg SAA in mice also caused a rapid and significant influx of neutrophils into the peritoneal cavity 2 h post injection [76].

3.3.2. The role of SAA-induced chemokines and cytokines in the chemotactic response to SAA—In contrast to the chemotactic activity of human CXCL8 or mouse CXCL6, which is the murine counterpart of CXCL8, SAA proved to be a more potent neutrophil chemoattractant when injected IP into mice than when it was applied in in vitro

Boyden chemotaxis assays. Indeed, a 5000-fold higher concentration of SAA was needed to acquire the same in vitro chemotactic effect as CXCL8, whereas only a ten-fold higher concentration of SAA was required to recruit even more neutrophils to the peritoneal cavity than mouse CXCL6 [76]. In fact, we demonstrated that this phenomenonwas due to a rapid (within 2–3h) induction of CXCL8 (or equivalent mouse CXCL6) in monocytes, which subsequently synergized with SAA to recruit an even greater number of neutrophils than the sum of neutrophil migration toward SAA and the induced chemokine tested separately. Lymphocytes and monocyte-derived macrophages are the main leukocyte cell populations in the peritoneal cavity. Since monocytes and macrophages are very good producers of chemokines when stimulated with SAA [53,76,83] and since lymphocytes are poor chemokine producers [53], we can assume that the former cells, possibly together with mesothelial cells delineating the peritoneal cavity, are contributing to the induction of chemokines. In line with these results, the recruitment of monocytes, neutrophils and T cells to the injection site 4–72h after SC administration of SAA [87,106,108] is probably also enforced by the local induction of cytokines and chemokines in fibroblasts, macrophages and endothelial cells (vide supra), which mediate the accumulation of supplementary leukocytes at the injection site. Indeed, He et al. [109] showed that the neutrophilia in SAA-treated (SC) mice was caused by induction of G-CSF, which mobilizes neutrophils from the bone marrow to the blood.

The induction of chemokines by SAA did not only influence its in vivo chemotactic potency, it was also found to affect in vitro cell migration to SAA. Indeed, in a previous study we pointed out that within the Boyden chamber the migration of monocytes and immature dendritic cells toward SAA was mediated by rapid (within 2–3 h) induction of CCL3 and CXCL8, which subsequently synergized with each other and possibly also with SAA to chemoattract even more cells than when only the effect of SAA would be taken into account [83]. This was evidenced by a significantly higher concentration of CCL3 and CXCL8 produced by the migrated cells in the lower wells of the chemotaxis assay upon stimulation of cells with SAA, compared to the concentration of chemokine in the upper wells. In addition, treatment of cells with antagonists of the CCL3 receptors CCR1 and CCR5 or with antibodies against CCL3 and/or CXCL8 significantly inhibited the chemotactic response of monocytes toward SAA [83].

In contrast to monocytes, neutrophils stimulated with SAA were not able to express CXCL8 or CCL3 within the duration of the chemotaxis assay [76]. Hence, the weak chemotactic activity of SAA for neutrophils in the Boyden microchamber assay (duration of 45 min) is a direct effect of SAA. This is in agreement with the study of Connolly et al. [72], in which they found that the trans-endothelial migration of neutrophils (24 h assay duration) toward 10 or 50 μg/ml SAA was (majorly) elicited by induction of CXCL8 in the endothelial cells. In addition, Ather et al. [111] proved that, compared to saline-treated mice, the higher amount of neutrophils recruited into BAL fluid of mice treated with 10 μgSAA during 4h via oropharyngeal aspiration was the result of the induction of IL-1β. Indeed, BAL fluids of SAA-treated mice contained significantly higher amounts of IL-1β after 4 h stimulation than BAL fluids of saline-treated mice and the IL-1R antagonist Anakinra significantly reduced the number of neutrophils in BAL fluid of mice treated with SAA for 24h. Probably, an SAA-IL-1β/IL-6-IL-17A-CXCL1 induction cascade is responsible for the influx of

neutrophils (migrating to CXCL1) into the BAL fluid of SAA-treated mice. Evidence for this statement was given by the fact that the expression of IL-1β, IL-6 (both inducers of IL-17A) and IL-17A in BAL fluid after intranasal administration of SAA was significantly higher than in BAL fluid of saline-treated mice [110]. Moreover, treatment of mice with anti-IL-17 antibody prior to treatment with SAA decreased neutrophil recruitment to lungs and BAL fluid and significantly reduced the expression of CXCL1, but not of CXCL2 [110].

3.3.3. Influence of HDL on the chemotactic properties of SAA—Although Lee et al. [71] stated that HDL did not have any effect on the monocyte chemotactic activity of SAA, four other papers reported the contrary. Badolato et al. [108] and Xu et al. [106] demonstrated that HDL dose-dependently inhibited the migration of monocytes, neutrophils and T cells toward SAA. In contrast to the SAA-induced production of cytokines, which was not affected by HDL at high SAA/HDL molar ratios, the chemotactic activity of HDL-conjugated SAA was already greatly impaired at relatively high SAA/HDL molar ratios, whereas the effect of HDL on the chemotactic activity toward other chemoattractants was minimal [106,108]. Furthermore, conjugation of HDL (1 mg/ml) with SAA (10 μg/ml), almost completely inhibited chemotaxis of FPR2-transfected HEK293 cells to SAA [112]. Moreover, it was evidenced by Patel et al. [87] that HDL also markedly reduced the in vivo chemotactic properties of SAA. After SC injection of HDL-conjugated SAA or HDL co-injected with 30 mg SAA, these authors found that there was macroscopically no swelling or signs of inflammation at the injection site, which was indeed the case when only SAA was injected. In addition, clearly less monocytes and neutrophils were infiltrated at the injection site in the presence of HDL. Taken together, HDL inhibits SAA-mediated chemotactic responses in a more pronounced way than the SAA-mediated induction of cytokines (vide supra).

3.3.4. Receptors mediating the SAA-induced chemotaxis—Until now, only FPR2 has been reported to mediate the chemotactic activity of SAA [112,113]. This GPCR is also a low binding affinity receptor for the bacterial *N*-formyl-methionylleucyl-phenylalanine (fMLF). Besides fMLF, FPR2 also interacts with other chemotactic pathogen-associated molecular patterns (PAMPs) and with chemotactic damage-associated molecular patterns (DAMPs; e.g. SAA) [114]. In the early studies reporting the chemotactic activity of SAA, the use of a GPCR on monocytes, T cells and mast cells was already evidenced [106,107,115]. In 1999, the group of Su et al. [112] was able to identify human FPR2 as the receptor responsible for SAA-mediated chemotaxis. These researchers evidenced this by multiple findings. Firstly, SAA induced migration of human FPR2-transfected HEK293 cells from 125 ng/ml onwards to reach a maximal CI at 12.5 μg/ml, whereas fMLF at these concentrations was not able to provoke a chemotactic response. Secondly, SAA was able to induce a rise in intracellular Ca^{2+} concentration in the same cells, which is a typical chemoattractant-related intracellular signal. This Ca^{2+} response was inhibited by pertussis toxin, suggesting that it was mediated by binding to a GPCR. Thirdly, SAA binding experiments showed that SAA specifically bound to human FPR2-transfected HEK293 cells. fMLF competed only at high concentrations with SAA for binding to the cells, again suggesting that binding of SAA to the cells was FPR2-, but not FPR1-mediated.

In line with the findings of the group of Su et al., we found that FPR2 also mediated the synergy between SAA and CXCL8 to chemoattract neutrophils [76], confirming that FPR2 is the receptor mediating the chemotactic activity of SAA. This was also supported by the finding of Chen et al. [116] that SAA-stimulated human FPR2-transfected rat basophilic leukemia RBL-2H3 cells showed a rise in intracellular Ca^{2+} concentration and exhibited a chemotactic response toward the acute phase protein. In fact, these authors demonstrated that the SAA isoforms have a different mode of action by preferably binding specific receptors, since human SAA1a and SAA2b preferably bound to mouse FPR2, but not to TLR2 or TLR4, to provoke a chemotactic response of the cells. With regard to the synergy between SAA and SAA-induced chemokines in the chemotactic response, we have shown that both FPR2 and the receptors of the induced chemokines are involved [76].

SAA equally signals through FPR2 to chemoattract mouse cells. HEK293 cells transfected with mouse FPR2, like mouse neutrophils, were weakly chemoattracted by SAA at a concentration of 12.5–25 μg/ml onwards. Moreover, a rise in intracellular Ca^{2+} concentration in the mouse FPR2-transfected HEK293 cells was also observed [117].

4. Remarks and conclusions

The SAA family of acute phase proteins consists mainly of the A-SAA proteins SAA1 and SAA2 and the C-SAA protein SAA4. Like inflammatory chemokines, A-SAA has inflammatory properties at higher concentrations (100 ng/ml), but its serum levels can rise up to 1000-fold during inflammation. In fact, several pro- and antiinflammatory functions have been ascribed to this highly conserved acute phase protein. SAA was found to be chemotactic for neutrophils, monocytes, T cells and mast cells, but the mode of action of its chemotactic activity was until recently poorly studied. We found that the monocyte chemotactic activity of SAA1α is due to rapid induction of the inflammatory chemokines CXCL8 and CCL3, which subsequently cooperate with each other to enhance monocyte migration. Moreover, we showed that the SAA-induced CXCL8 in mononuclear cells can synergize with SAA1a to attract neutrophils in vitro and in vivo (Fig. 2). Our observations are in line with the work of Connolly and co-workers [72] who demonstrated that CXCL8, induced in endothelial cells by SAA, is involved in trans-endothelial migration of neutrophils toward SAA. SAA-mediated induction of IL-1β and IL-6, leading to further induction of chemokines, was also found to be responsible for the higher neutrophil counts in BAL fluids from mice treated with SAA [110,111]. Hence, besides having a direct chemotactic effect, SAA also acts as a leukocyte chemoattractant in an indirect way, via induction of chemokines and cytokines. Indeed, a variety of primary mediators (e.g. SAA) is produced simultaneously at an inflammatory site and an array of secondary effector molecules (including cytokines and chemokines) is secreted by the stimulated cells. These effector molecules can act in concert (e.g. SAA with chemokines) to attract leukocytes to the inflammatory site.

Other functions described for SAA may also be exerted in an indirect way. SAA induces autocrine and paracrine cytokine and chemokine production in a variety of cell types (Tables 2 and 3). These cytokines and chemokines are communication molecules which are eventually responsible for very diverse effects and which can mediate the outcome of SAA

functions via their fine-tuned network [118]. Actually, it was demonstrated that monocyte adhesion in response to SAA was mediated by induction of CCL2 [72]. Investigating the involvement of cytokine and/or chemokine induction in the various described effects mediated by SAA will thus be of great interest in future studies.

Since the commercially available SAA forms are recombinant proteins expressed in *Escherichia coli*, the question rises whether contamination with LPS may be responsible for some of its biological effects. In the past, several studies included the Limulus amebocyte lysate assay or other tests to determine the role of LPS in the observed biological effects of recombinant SAA preparations [71,73,82,109,112,119]. In none of these studies, low LPS contamination (<100 pg/ml) was found to influence the biological properties of SAA. Just like observed by He et al. [82], we showed that LPS at concentrations equal to those present in the recombinant SAA preparation did not result in the production of chemokines [83]. Similarly, antibodies against TLR4 did not reduce the SAA-mediated G-CSF production in murine macrophages, nor did macrophages of TLR4-deficient mice, stimulated with recombinant SAA, produce less G-CSF than macrophages of wild type mice [109]. Several other cytokines were equally well produced by SAA-stimulated macrophages from TLR4-deficient mice, indicating that the TLR4 ligand LPS is not responsible for this biological effect [116].

It has been reported that SAA elicits its functions via interaction with various receptors. Despite numerous experimental efforts to identify the receptor(s) mediating the SAA-induced cytokine and chemokine production, there is no consensus about a single receptor being responsible for this SAA-mediated biological activity. The toll-like receptors TLR2 and TLR4 and the formylpeptide receptor FPR2, which is also the receptor mediating the chemotactic response of leukocytes toward SAA, were all shown to mediate cytokine and chemokine induction [28,29,52,54,55, 59–61,69,71,73,74,76,82,86]. Other receptors reported to be used by SAA to induce cytokines and chemokines are the scavenger receptors CD36 and CLA-1. CD36 is a membrane protein belonging to the class B scavenger receptor family. This receptor is present on various cell types and recognizes a variety of ligands. Since CD36 exerts its functions by interacting with other receptors, including TLRs, one can assume that this scavenger receptor functions as a co-receptor for TLRs, just like CD14 is for TLR4, and that a combination of TLR2 and CD36 is thus needed to induce cytokines and chemokines [120]. CLA-1 is the human homologue of murine scavenger receptor class B type I (SR-BI) and is genetically related to CD36 and rat LIMPII, another member of the scavenger receptor class B family [121]. Nevertheless, the use of CD36 and CLA-1 by SAA to induce cytokines and chemokines is poorly studied and needs more investigation. We found that SAA induced CXCL8 in monocytes through interaction with TLR2, but not through binding to FPR2. Therefore, we postulate that SAA predominantly activates TLR2 for cytokine and chemokine induction, whereas FPR2 is used for SAA-mediated cell chemotaxis. FPR2 belongs to the class of GPCRs, which include the classical chemokine receptors. Such receptors interact with chemotactic PAMPS (e.g. FPR1 for fMLF) and DAMPS (e.g. FPR2 for SAA), which are structurally very diverse [114]. Peptides from *Listeria* function as early chemotactic PAMPs for neutrophils through binding to FPR2. Just like SAA, these bacterial peptides also bind to TLR2 on hepatocytes and leukocytes, thereby inducing chemokine ligands for CXCR2, which provoke a late wave of neutrophil influx into

the infected liver [122]. Another finding supporting the use of TLR2 for SAA-mediated cytokine and chemokine induction is the fact that SAA upregulates the expression of TLR2 on endothelial cells, which may amplify its cytokine and chemokine inducing capacity [61]. Stimulation of TLR2 or TLR4 also increases the expression levels of FPR2 in mice, facilitating the SAA-mediated leukocyte chemotactic activity [123,124].

Taken together, when TLR2 or TLR4 on leukocytes interacts with PAMPs from gram-positive (peptidoglycan) or gram-negative (LPS) bacteria, respectively, these cells start producing cytokines. The cytokines greatly augment the synthesis of acute phase proteins (i.e. SAA) in the liver. In turn, SAA functions as a weak chemotactic DAMP for leukocytes, acting via interaction with FPR2, and induces chemokines through binding to TLR2. SAA-induced chemokines cooperate and act synergistically with SAA itself to attract leukocytes. Simultaneously, these chemokines function in a signal relay, propagating the chemotactic message to a wider extent. Subsequently, this highly coordinated chemotactic cascade leads to an optimization of the recruitment of leukocytes to sites of inflammation.

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Biography

Mieke De Buck graduated as a veterinarian in 2009 at the University of Ghent and worked for two years as a veterinarian. In 2011, she started a PhD at the Laboratory of Molecular Immunology at the Rega Institute under the promotorship of Prof. Jo Van Damme, which she completed in December 2015. Her research focuses on the biochemical identification and biological characterization of novel chemoattractants in serum, in particular on the mode of action of different forms of the chemokine inducing acute phase protein serum amyloid A.

Mieke Gouwy (1978) graduated as biologist in 2000 and obtained a PhD in Medical Sciences (2005), under the promotership of Prof. Jo Van Damme at the Rega Institute, University of Leuven, Belgium. Her postdoctoral research focused on the synergy between chemoattractants in leukocyte migration. Currently, she is working in the Laboratory of

Molecular Immunology as a Research Expert in chemotaxis. Her current research involves the biochemical identification and biological characterization of novel chemoattractants present in serum.

Ji Ming Wang obtained his medical degree in 1983 at the Shanghai 2nd Medical University, (currently School of Medicine, Shanghai Jiaotong University) Shanghai, China. He finished his PhD in 1987 at the Mario Negri Pharmacological Research Institute, Milan, Italy, where he continued his study as a postdoctoral fellow (1987–1990). He then worked as a visiting scientist (1990–1996) at the Laboratory of Molecular Immunoregulation, Center for Cancer research, NCI, Frederick, USA. After a tenure track positon as a Principal Investigator (1996–2004) in the Cancer and Inflammation Program at the Center for Cancer Research, NCI, Frederick, he became a tenured Senior Principal Investigator in 2004. Ji Ming Wang has significantly contributed to the field of cell migration with focus on chemoattractrants (including chemokines and serum amyloid A) and their G protein-coupled receptors.

Jacques Van Snick obtained his Medical degree in 1975 at the Catholic University of Louvain, Belgium, and finished his PhD in 1982. Initially affiliated with the Belgian Science Foundation, he joined the Ludwig Institute for Cancer Research in 1986. He has been involved in cytokine research ever since and identified mouse IL-6 in 1986 and mouse IL-9 in 1988, both in collaboration with Dr. Catherine Uyttenhove. It is in his lab that mouse and human IL-9 receptors were cloned and that the first IL-9 transgenic mice were developed, in collaboration with Dr. Jean-Christophe Renauld. His present interest is focused on in vivo studies of Th17 and Treg cytokines using auto-vaccines.

Paul Proost (1964, engineer in industrial sciences—biochemistry, University of Antwerp, Belgium) is research professor at the Faculty of Medicine of the University of Leuven and Head of the Laboratory of Molecular Immunology, Rega Institute for Medical Research. After finishing his PhD in 1996 on the identification and characterization of human CCL7 and CCL8 and postdoctoral research as fellow of the Belgian Science Foundation on

CXCR2 and CXCR3 ligands, his research in the Laboratory of Molecular Immunology has focused on the identification and biochemical and biological characterization of natural post-translationally modified chemokines, their interaction with proteases, peptidylarginine deiminases and glycosaminoglycans and their role in inflammation.

Sofie Struyf graduated as bio-engineer in 1996 at the University of Leuven, Belgium. She obtained her PhD degree (2002) in applied biological sciences at the Rega Institute (Laboratory of Molecular Immunology, University of Leuven, Belgium) on post-translational modifications of chemokines. She is holding a position of professor at the Rega Institute. Her research is currently focused on the role of chemokines in angiogenesis and cancer.

Jo Van Damme (1950, bio-engineer, PhD, University of Ghent, Belgium) is professor emeritus at the Laboratory of Molecular Immunology, Rega Institute for Medical Research, Medical Faculty of the University of Leuven, Belgium. He was president of the European Cytokine Society (2001–2007). He has done pioneer work in cytokine research (in collaboration with Prof. G. Opdenakker) and was involved in the identification of several human interleukins (IL-1, IL-6, IL-8) and chemokines (CXCL6, CXCL8, CCL7, CCL8). His current research is dealing with the role of chemoattractants and their receptors in infection, inflammation and cancer. He has published more than 400 scientific papers on cytokine research in peer-reviewed journals.

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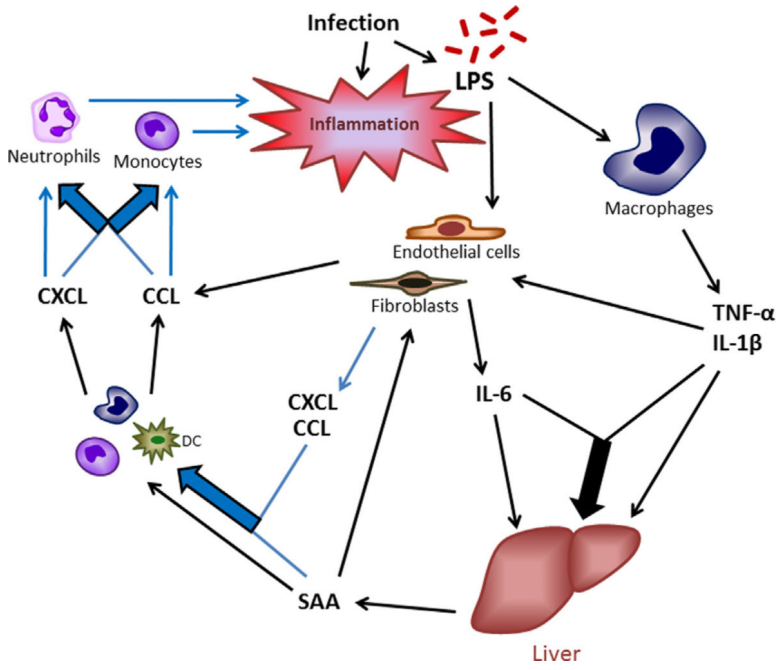

Fig. 1.

The cytokine-SAA-chemokine network.

Exogenous inflammatory mediators, such as LPS, stimulate macrophages to produce TNF-α and IL-1β, which in turn induce the production of the acute phase protein SAA in the liver. At the same time, TNF-α and IL-1β stimulate the production of IL-6 in endothelial cells and fibroblasts. IL-6 on its own or in synergy with TNF-α and/or IL-1β (bold black arrow) also triggers the production of SAA in the liver. Endogenous TNF-α, IL-1β and SAA induce CXC and CC chemokines in various cell types [e.g. macrophages, dendritic cells (DC), monocytes, endothelial cells, fibroblasts]. The induced chemokines subsequently stimulate the migration of leukocytes (e.g. monocytes, neutrophils, DC) to the site of inflammation (blue arrows). Furthermore, CXC and CC chemokines can synergize (bold blue arrows) with each other, but also with SAA, to enhance leukocyte migration to inflammatory foci.

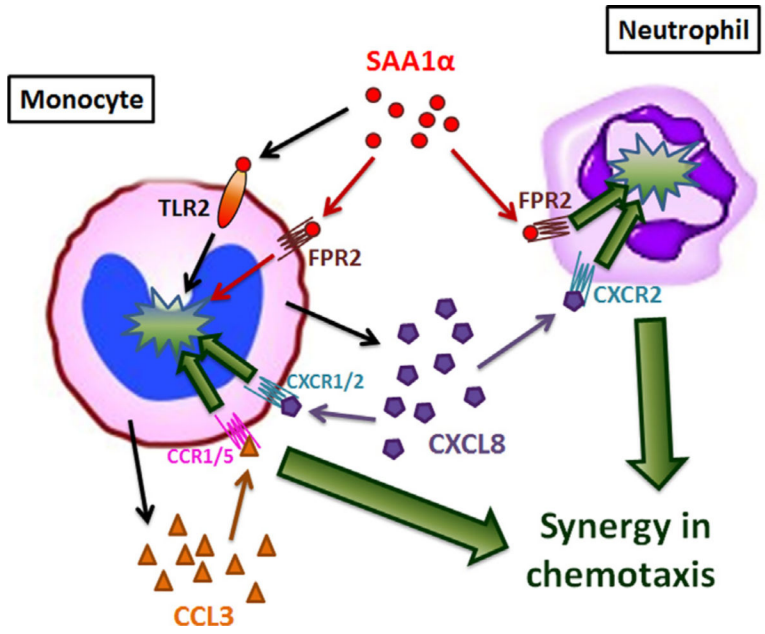

Fig. 2.

Mechanism of chemotactic activity of SAA1α on monocytes and neutrophils. SAA1α chemoattracts monocytes and neutrophils through binding to FPR2 (red arrows). At the same time, this acute phase protein activates TLR2 on monocytes, leading to induction of chemokines in monocytes, such as CCL3 and CXCL8 (black arrows). CCL3 and CXCL8 cooperate to enhance monocyte recruitment (large green arrows) via binding to their corresponding receptors, which are CCR1/5 (orange arrow) and CXCR1/2 (purple arrow), respectively. On neutrophils, SAA1α and CXCL8 synergize by binding to FPR2 and CXCR2, respectively (large green arrows).

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Table 1

In vitro induction of SAA in hepatocytes.

Cells	Inducer	SAA[a]		A-SAA[b]		SAA4 (C-SAA)		References
		mRNA	Protein	mRNA	Protein	mRNA	Protein	
Primary human hepatocytes	dex		−					[34]
	mu IL-1α + dex/IL-6 ± dex/mu IL-1α + IL-6 + dex		+					[34,36]
Hep3B hepatoma cells	None/dex			−		+		[33]
	MoCM ± dex			+		+		[33]
	IL-1α/IL-1β/IL-6/TNF-α/dex	−	−					[13,14,23]
	IL-1β/IL-6/IL-1β + IL-6	+						[15]
	IL-1α + IL-6 ± dex/IL-1β + IL-6/IL-6 + TNF-α	+	+					[14,23]
HepG2 hepatoma cells	None/dex			−		+		[33]
	MoCM ± dex			+		+		[33]
	IL-1β ± dex		+	+				[17,20]
	IL-6/dex/IL6 + dex		−					[20]
	IL-1/IL-6/IL-1β ± IL-6/IL-1 + IL-6 ± dex/TNF-α ± IL-6		+	+				[16,19,20]
	IL-1α ± pred/IL-1β ± pred/IL-6 ± pred/TNF-α/pred/IL-6 + TNF-α + pred		−					[22]
	IL-6/TNF-α/IL-6 + TNF-α			+				[18]
	IL-1α + IL-6 ± pred/IL-1β + IL-6 ± pred		+					[22]
HuH7 hepatoma cells	None			+		+		[30,125]
	None/dex			−		+		[33]
	MoCM ± dex			+		+		[33]
	IL-6/TNF-α		−					[25]
	IL-1β ± IL-6/IL-6 + TNF-α		+					[25]
PLC/PRF/5 hepatoma cells	None/dex			−	−	+		[21,33,35]
	MoCM ± dex			+		+		[33]
	IL-6		+	+				[21,23]
	IL-1β ± IL-6/IL-1β + dex ± IL-6			+				[21]
	IL-1β + IL-6 + dex			+	+			[35]

Abbreviations: dex: dexamethasone; MoCM: monocyte conditioned medium; mu: murine; pred: prednisolone.

The presence or absence of SAA, A-SAA (SAA1 and SAA2), and/or SAA4 (C-SAA) mRNA and/or protein in hepatocytes or hepatoma cell lines is indicated by + or −, respectively.

[a] Not specified which SAA type produced.

[b] A-SAA expression without discrimination between SAA1 and SAA2.

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Table 2

SAA-mediated cytokine production by human cell types.

Cytokine family	Cytokine induced	Producer cells[a]	SAA receptor	References
IL-1	IL-1β	Macrophages, keratinocytes	TLR2 + TLR4	[54,58]
		Monocytes, macrophages, neutrophils, THP-1 cells, U937 cells, HMC-1 mast cells; synoviocytes (mRNA)	ND[b]	[50,51,53,55–58,87]
	IL-1RA	Monocytes (mRNA), THP-1 cells	ND	[87,126]
	IL-33	THP-1 cells	TLR2 + FPR2	[59]
Class I cytokine	IL-6	Endothelial cells, fibroblasts	TLR2	[60,61]
		Synovial fibroblasts, chondrocytes	TLR4	[29]
		Endothelial cells, synovial fibroblasts	CLA-1	[62]
		Monocytes, macrophages, endothelial cells, U937 cells, synovial fibroblasts, synoviocytes	ND	[42,44,53,55,75,127]
	IL-23	Monocytes, U937 cells, synoviocytes	ND	[55,56,70]
	GM-CSF	Endothelial cells	TLR2	[61]
		hu FPR2-transfected A549 cells	FPR2	[28]
		Monocytes, macrophages	ND	[53]
	G-CSF	Monocytes	ND	[109]
	M-CSF	HepG2 cells	FPR2	[55]
Class II cytokine	IL-10	Macrophages	TLR2	[69]
		U937 cells	FPR2	[55]
		Monocytes, macrophages, synoviocytes	ND	[53,68,126,127]
TNF	TNF-α	Macrophages	TLR2	[69]
		Monocytes, macrophages, neutrophils, U937 cells, HMC-1 mast cells, synoviocytes	ND	[50–52,55,58,66–68,82]
IL-17	IL-17A	hu FPR2-transfected A549 cells	FPR2	[110]

[a] Unless indicated, cytokine protein was measured.

[b] ND: not determined.

Table 3

SAA-mediated chemokine production by human cell types.

Chemokine induced	Producer cells[a]	SAA receptor	References
CCL1	U937 cells	ND[b]	[55]
	THP-1 cells		[128]
CCL2	Monocytes	FPR2	[71]
	Endothelial cells		[73,74]
	HepG2 cells		[55]
	hu FPR2-transfected A549 cells		[28]
	Synovial fibroblasts, chondrocytes	TLR4	[29]
	Synoviocytes	CLA-1	[72]
	Monocytes, macrophages	ND	[53]
	Endothelial cells		[72,75]
	Synoviocytes		[127]
	THP-1 cells (mRNA)		[128]
CCL3	Monocytes	ND	[53,83]
	Macrophages		[53]
	Dendritic cells		[83]
	THP-1 cells		[128]
CCL4	Synoviocytes	ND	[127]
	THP-1 cells		[128]
CCL5	Endothelial cells	ND	[61]
CCL17	Monocytes (mRNA)	ND	[126]
	U937 cells		[55]
CCL20	Synoviocytes	ND	[127]
CXCL1/2/3	Synovial fibroblasts, chondrocytes	TLR4	[29]

Table 4

Induction of chemokines by pro-inflammatory mediators.

Inducer	Receptor	Cell type	Chemokine[a]				References
			CXCL8[b]	CXCL10	CCL2	CCL3	
SAA	FPR2	Neutrophils	+				[82]
		Endothelial cells			+		[74]
	TLR2	Monocytes	+				[76]
	Not determined	Monocytes, macrophages	+		+	+	[53,83]
		Neutrophils	−			−	[76]
		Dendritic cells	+			+	[83]
		Endothelial cells	+	+			[61]
dsRNA	TLR3	Monocytes	+	+	+		[98,104]
		Macrophages	+			+	[93,131]
		Endothelial cells	+	+	+		[89,92,94,100]
		Fibroblasts	+	+			[94,98,104,132]
		Chondrocytes	+		+		[89,94,132]
		Epithelial cells	+		+		[133]
		MRC-5 cells, A549 cells		+			[134]
LPS	TLR4	Monocytes	+	−	+	+	[80,83,90,98,104,135]
		Macrophages	+				[93,99]
		Neutrophils	−		−	−	[76,80]
		Neutrophils				+	[88]
		Dendritic cells	+	+ (mRNA)		+	[83,95]
		Endothelial cells	+	−	+		[89,92,94,100]
		Fibroblasts	+	−			[98]
		Chondrocytes	+		+		[89,94]
IL-1β	Immunoglobulin family receptors	Monocytes	+		+	+	[80,90,135]
		Monocytes	+		−	−	[83,104]

Inducer	Receptor	Cell type	Chemokine[a]				References
			CXCL8[b]	CXCL10	CCL2	CCL3	
		Macrophages	+				[93,99]
		Neutrophils	−		−	−	[76,80]
		Dendritic cells	+			+	[83]
		Endothelial cells	+		+		[89,91,92,94]
		Fibroblasts	+		+		[89,94,104,132]
		Chondrocytes	+		+		[89,94,132]
		Smooth muscle cells	+				[91]
IL-6	Class I cytokine receptors	Monocytes			+		[136]
		Endothelial cells	−		−		[137]
		U937 cells	−		+	−	[136]
IFN-γ	Class II cytokine receptors	Monocytes	−	+	−	+	[80,89,98,102,104]
		Neutrophils	−		−		[80,103]
		Endothelial cells	−	+	−	−	[91,92,94,100,138,139]
		Fibroblasts	−	+			[94,98,104]
		Smooth muscle cells	−				[91]
TNF-α	TNF receptor family	Monocytes		−		−	[98,135]
		Macrophages	+ (mRNA)				[99]
		Dendritic cells				+	[95]
		Endothelial cells	+		+	−	[91,92]
		Fibroblasts	+	+			[94,98]
		Chondrocytes	+				[94]
		Smooth muscle cells	+			−	[91]
		HT-29 cells, HCT-8 cells	+		+	−	[96,97]

[a] Unless indicated, chemokine protein was measured.

[b] The presence or absence of CXCL8, CXCL10, CCL2 and CCL3 mRNA or protein in cells is indicated by + or −, respectively.

Table 5

Cell types responsive to SAA in in vitro (human) and in vivo (mouse) cell migration assays.

Target cells	SAA concentration (μg/ml) or dose (μg)[a]	References
Monocytes	1–100	[108]
	50	[115]
	0.0125–0.125	[71]
	0.03–1	[83]
	10 (SC, mice)	[108]
	50 (SC, mice)	[87]
Immature dendritic cells	0.1–1	[83]
Neutrophils	10–100	[108]
	10–50	[72]
	1–3	[76]
	10 (SC, mice)	[108]
	50 (SC, mice)	[87]
	3 (SC, mice)	[109]
	2 (intranasal, mice)	[110]
	1 (IP, mice)	[76]
T cells	10–100	[106]
	10 (SC, mice)	[106]
Mast cells	12.5	[107]
Endothelial cells	50	[140]
Smooth muscle cells	10–100	[141]
Synovial fibroblasts	10	[142]

[a]Refers to the SAA concentrations that were chemotactic for the indicated cell type.