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Review

Extrahepatic production of acute phase serum amyloid A

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Summary. Amyloidosis is a group of diseases characterized by the extracellular deposition of protein that contains non-branching, straight fibrils on electron microscopy (amyloid fibrils) that have a high content of ß-pleated sheet conformation. Various biochemically distinct proteins can undergo transformation into amyloid fibrils. The precursor protein of amyloid protein A (AA) is the acute phase protein serum amyloid A (SAA). The concentration of SAA in plasma increases up to 1000-fold within 24 to 48 h after trauma, inflammation or infection. Individuals with chronically increased SAA levels may develop AA amyloidosis. SAA has been divided into two groups according to the encoding genes and the source of protein production. These two groups are acute phase SAA (A-SAA) and constitutive SAA (C-SAA). Although the liver is the primary site of the synthesis of A-SAA and C-SAA, extrahepatic production of both SAAs has been observed in animal models and cell culture experiments of several mammalian species and chicken. The functions of A-SAA are thought to involve lipid metabolism, lipid transport, chemotaxis and regulation of the inflammatory process. There is growing evidence that extrahepatic A-SAA formation may play a crucial role in amyloidogenesis and enhances amyloid formation at the site of SAA production.

Key words: Extrahepatic production, Isoforms, Serum amyloid A, Acute phase protein, AA amyloidosis

Introduction

Amyloidosis is a group of diseases characterized by the extracellular deposition of protein that contains non-branching, straight fibrils on electron microscopy (amyloid fibrils) that have a high content of ß-pleated sheet conformation (Eanes and Glenner, 1968). In humans, at least twenty-four biochemically distinct proteins can transform into amyloid fibrils (Westermark et al., 2002). In animals, deposition of amyloid protein A (AA) is the most frequent amyloid form found.

AA amyloidosis or reactive amyloidosis is frequently associated with chronic inflammatory disease and tumors (Thysell et al., 1986; Friedman et al., 1989; Wens et al., 1989; Fievet et al., 1990; Krishnan et al., 1993; Agha et al., 2002), or it occurs as an idiopathic disorder (Sohar et al., 1967; Hawkins et al., 1993; Ordi et al., 1993). The precursor of AA is the acute phase serum amyloid A protein (SAA) which is associated with high density lipoprotein (HDL) from plasma and mainly originates in the liver (Benditt and Eriksen, 1977; Coetzee et al., 1986; Shephard et al., 1987). It may increase up to 1000-fold within 24-48 h after trauma, infection or inflammation. The synthesis and secretion of SAA is mediated by cytokines, mainly interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Husby et al., 1994). The functions of SAA are though to be related to lipid metabolism and lipid transport, chemotaxis and regulation of the inflammatory process (Table 1).

SAA has been divided into two groups that correspond to the encoding genes and the concentration in plasma under normal conditions or after acute phase response: acute phase SAA (A-SAA) and constitutive SAA (C-SAA). The A-SAA, which increases in concentration in the plasma during the acute phase response, is mainly synthesized by the hepatocytes, whereas the C-SAA, which is found to be associated with HDL under normal conditions and which is not significantly increased during the acute phase response, is produced both in the liver and in other organs (Whitehead et al., 1992; Steel et al., 1993, 1996; de Beer et al., 1994). In birds, only one SAA type, corresponding to mammalian A-SAA, has been described (Guo et al., 1996; Ovelgönne et al., 2001; Kovacs et al., 2005). There is not only a response difference between A-SAA and C-SAA, but various publications also describe different SAA genes that code for A-SAA and its

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isotypes in many species including man. In the present paper the SAA gene families in human and animals will be named following the nomenclature for the SAA gene families as outlined by the SAA Subcommittee of the Amyloidosis Nomenclature Committee (Sipe et al., 1999).

In humans, there are three different SAA genes coding for SAA proteins, and one pseudogene (*SAA3P*). *SAA1* and *SAA2* encode A-SAA proteins, whereas SAA4 encodes C-SAA protein (Whitehead et al., 1992). Mice have five different SAA genes coding for SAA proteins and one pseudogene (*Saa-psl*). The mouse *Saa1*, *2* and *3* encode A-SAA proteins and *Saa4* encodes C-SAA protein (Lowell et al., 1986). There are a few studies on the SAA gene family in other mammals (Sletten et al., 1989; Ray and Ray, 1991; Syversen et al., 1993).

All of the SAA genes in animals described to date, have four exons, including an untranslated 5'exon, while for the single duck SAA gene three exons have been mentioned (Guo et al., 1996). Comparison of the SAA amino acid sequence between duck and chicken revealed 82% homology including the 5'signal peptides. Amino acid sequence prediction from duck SAA cDNA showed that two SAA protein types (alleles) can be produced, whereas in chicken, only one type of SAA protein has been observed (Ovelgönne et al., 2001).

In humans (Table 2), fifteen SAA isoforms were found and no preferential SAA isoform is converted to AA fibrils (Dwulet et al., 1988; Strachan et al., 1988, 1989; Betts et al., 1991; Raynes and McAdam, 1991; Baba et al., 1992; Whitehead et al., 1992; Steel et al., 1993; Westermark et al., 1996). In mice (Table 2), ten SAA isoforms, of which SAA1.1, SAA1.2, and SAA4 are preferably converted to AA fibrils, were found (Hoffman et al., 1984; de Beer et al., 1991, 1992, 1993a, 1994; Meek et al., 1992; Cathcart et al., 1996; Foyn Bruun et al., 1998). In other animals, SAA isoforms have been also reported (Table 3).

The pathogenesis of AA amyloidosis has been studied in a number of animal models, especially the mouse model. There are a number of factors that are involved in amyloidogenesis, such as amyloid enhancing factor (AEF), the isoform or proteolytic cleavage site of SAA, glycosaminoglycans (GAGs) and, maybe, local formation of extrahepatic A-SAA products.

This review focuses on the extrahepatic production of A-SAA in various animal tissues during *in vivo* and *in vitro* studies, and the possible role of extrahepatic A-SAA production in amyloidogenesis.

The extrahepatic production of A-SAA protein

SAA expression and production during in vivo studies

Although A-SAA is mainly synthesized by hepatocytes, the number of publications showing extrahepatic production of A-SAA is increasing. Several species have been reported to show extrahepatic A-SAA expression and production.

In humans (Table 4), the expression and production of the extrahepatic A-SAA and C-SAA were found in

Table 1. Summary of the functions of SAA.

FUNCTIONS	REFERENCES
Lipid metabolism	
Cholesterol esterification in human plasma	Steinmetz et al. (1989)
Acute phase HDL (HDL-SAA complex) has reduced ability to bind to hepatocytes but has a higher ability to bind to macrophages	Kisilevsky and Subrahmanyan (1992)
Inhibit HDL binding and selective lipid uptake	Cai et al. (2005)
Enhance cholesterol uptake into hepatoma cell line	Liang and Sipe (1995); Liang et al. (1996)
Decrease lipid biosynthesis	
Inhibit the synthesis of phospholipids, triglycerides and cholesterol	Schreiber et al. (1999)
Immunomodulation	
Induce MMPs production	Brinckerhoff et al. (1989); Sack and Talbot (1989); Mitchell et al. (1991); Strissel et al. (1997); Migita et al. (1998)
Inhibit thrombin-induced platelet aggregation	Zimlichman et al. (1990); Syversen et al. (1994)
Chemoattractant	Badolato et al. (1994); Olsson et al. (1999); Kumon et al. (2002); Xu et al. (1995); Hershkoviz et al. (1997a); Hershkoviz et al. (1997b)
Induce upregulation of adhesion molecules	Badolato et al. (1994)
Enhance phagocytic activity	Badolato et al. (2000)
Induce cytokine synthesis	Patel et al. (1998); Preciado-Patt et al. (1998); Ribeiro et al. (2003); Hatanaka et al. (2004)
Induce intestinal cells to produce mucin	
Inhibit pathogenic bacteria to adhere to the intestinal cells	Larson et al. (2003a); Mack et al. (2003)

Table 2. The isoforms of SAA in human and mouse.

SPECIES	NUMBER OF ISOFORMS	GENE	TYPE	PROTEIN ISOFORM	ISOELECTRIC POINT (pI)	REFERENCES
Human[a]	15	*SAA1*	A-SAA	SAA1		
				SAA1.1 (SAA1α)	pI 6.4	Strachan et al. (1988, 1989);
				SAA1.1 des-arg (SAA1α des-arg)	pI 6.0	Raynes and McAdam (1991)
				SAA1.2 (SAA1ß)	pI 6.1	Beach et al. (1992)
				SAA1.2 des-arg (SAA1ß des-arg)	pI 5.6	
				SAA1.3 (SAA1γ)	pI ?	Baba et al. (1992)
				SAA1.4 (SAA1δ)	pI ?	Westermark et al. (1996)
				SAA1.5 (SAA1ß)	pI ?	Betts et al. (1991)
		SAA2	A-SAA	SAA2		
				SAA2.1 (SAA2α)	pI 7.5	Strachan et al. (1988, 1989);
				SAA2.1 des-arg (SAA2α des-arg)	pI 7.0	Raynes and McAdam (1991)
				SAA2.2 (SAA2ß)	pI 8.0	
				SAA2.2 des-arg (SAA2ß des-arg)	pI 7.4	
		SAA4	C-SAA	SAA4		
				SAA4 pI 8.1	pI 8.1	Whitehead et al. (1992); Steel et al. (1993)
				SAA4 pI 7.9	pI 7.9	
				SAA4 pI 7.3	pI 7.3	
Mouse[a]	10	*Saa1*	A-SAA	SAA1.1 (SAA2)	pI 6.3	Hoffman et al. (1984); de Beer et al. (1991, 1993a); Cathcart et al. (1996)
				SAA1.2 ($SAA_{SJL/J}$)	pI 5.9	Hoffman et al. (1984); de Beer et al. (1991, 1992, 1993a; Cathcart et al. (1996)
				SAA1.3 (mc1)	pI ?	Cathcart et al. (1996)
				SAA1.4 (mc2)	pI ?	Cathcart et al. (1996)
				SAA1.5 (mm1)	pI ?	Cathcart et al. (1996)
				SAA1.6 (mm2)	pI ?	Cathcart et al. (1996)
		Saa2	A-SAA	SAA2.1 (SAA1)	pI 6.45	Meek et al. (1992)
				SAA2.2 ($SAA_{CE/J}$)	pI 6.15	Hoffman et al. (1984); de Beer et al. (1991, 1993a); Cathcart et al. (1996)
		Saa3	A-SAA	SAA3 (SAA3)	pI 9.2	Lowell et al. (1986)
		Saa4	C-SAA	SAA4 (SAA5)	pI 8.1	de Beer et al. (1994)

[a]: Nomenclature for human and mouse *SAA* genes and proteins is after guidelines of the SAA Subcommittee of the Amyloidosis Nomenclature Committee, 1999 (Sipe et al., 1999), parenthesis represent the old nomenclature.

Table 3. The isoforms of SAA in different species.

SPECIES	NUMBER OF ISOFORMS	SAA TYPE	ISOELECTRIC POINT (pI)	REFERENCES
Hamster	5	SAA1	pI 9.15	Webb et al. (1989)
		SAA2	pI 9.3	Webb et al. (1989)
		SAA3	pI 9.44	Webb et al. (1989)
		SAA4	pI 9.45	Gervais and Suh (1990)
		SAA5	pI 5.85	de Beer et al. (1993b)
Horse	3	SAA pI 8.0 (minor)	pI 8.0	Hulten et al. (1997)
		SAA pI 9.0 (minor)	pI 9.0	Hulten et al. (1997)
		SAA pI 9.7 (major)	pI 9.7	Hulten et al. (1997)
Cow	6	Three major SAAs	pI 5.5	Alsemgeest et al. (1995)
			pI 6.0	
			pI 6.4	
		Three minor SAAs	pI 4.8[a]	Alsemgeest et al. (1995)
			pI 5.0	
			pI 7.3	
Mink	3	SAA pI 6.8 (SAA1-like)	pI 6.8	Foyn Bruun et al. (1994)
		SAA pI 6.5 (SAA1-like)	pI 6.5	
		SAA pI 6.0 (SAA1- like and SAA2-like)	pI 6.0	
Dog	2	Two major SAAs	pI 6.4	Sellar et al. (1991)
			pI 6.8	

[a]: One isoform was detected in sera obtained from a cow suffering from spontaneous AA-amyloidosis.

atherosclerotic lesions; SAA mRNAs were detected mainly in endothelial cells, macrophages, adipocytes and smooth muscle cells (Meek et al., 1994; Yamada et al., 1996). A-SAA was found to be expressed in the brain of patients suffering from Alzheimer's disease (Liang et al., 1997; Chung et al., 2000). In fact, A-SAA was produced in the epithelial cells of several normal tissues such as breast lobule, colon mucosa, prostate gland, kidney tubule, and lung (Urieli-Shoval et al., 1998). The extrahepatic A-SAA synthesis was detected in synovial fibroblasts of rheumatoid arthritis (RA) patients, but neither in osteoarthritis (OA) patients nor in normal human synovium, whereas C-SAA was found in both RA and OA patients (Kumon et al., 1999; O'Hara et al., 2000). In addition to synovium, chondrocytes of OA patients were found to produce A-SAA protein as well (Vallon et al., 2001).

In mice (Table 5), SAA mRNAs, mainly SAA3, were found to be expressed in heart, kidney, lung, intestine, spleen, and peritoneal macrophages (Ramadori et al., 1985). The liver and other tissues responded and expressed the main SAA types differently depending on the stimulators used. For example, casein induced the liver to express only SAA1 and SAA2, whereas lipopolysaccharide (LPS) strongly induced the liver to express SAA1, SAA2 and SAA3. Many extrahepatic tissues responded to LPS stimulation by expressing mainly SAA3, but not to casein (Meek and Benditt, 1986). SAA3 was detected in adipocytes in several tissues, Leydig cells of testis, mononuclear cells in the parafollicular zone of spleen, Kupffer cells and even peritoneal macrophages (Rokita et al., 1987; Benditt and Meek, 1989; Marhaug et al., 1997). Thus, in the mouse, extrahepatic organs express mainly SAA3 upon stimulation.

At least five genes encode SAA in Syrian hamsters: SAA1, SAA2, SAA3, SAA4 and SAA5 respectively. After injection with turpentine or LPS, A-SAA mRNA was detected in brain, heart, lung, ovary, testis, uterus, adrenal gland, kidney, urinary bladder, muscle (at the site of the abscess), diaphragm, esophagus, stomach, spleen, duodenum, jejunum and ileum. SAA3 was expressed in lung, muscle, spleen, and kidney (Table 6) (Webb et al., 1989; Hardardottir et al., 1997).

Similar to the mouse, rabbit SAA1 and SAA2 are exclusively formed in the liver, but SAA3 is found to be expressed extrahepatically (Table 7). The rabbit tissues differentially respond to stimulators. Turpentine and

Table 4. Extrahepatic sites of SAA production in human tissues.

TISSUES	CONDITION	SAA TYPE	mRNA SAA[a]	SAA PROTEIN[b]	REFERENCES
Cells in atherosclerotic lesion (endothelial cells, macrophages, adipocytes and smooth muscle cells)	Atherosclerotic lesion	ns	+	nd	Meek et al. (1994)
Atherosclerotic lesion	Atherosclerotic lesion	ns	nd	+	Yamada et al. (1996)
Brain	Alzheimer's disease	A-SAA	+	+	Liang et al. (1997); Chung et al. (2000)
Normal tissues	No lesion				Urieli-Shoval et al. (1998)
Brain		ns	+	+	
Pituitary gland		ns			
Breast (epithelial cell)		SAA1, SAA2, SAA4	+	+	
Kidney		SAA1, SAA2, SAA4	+	+	
Pancreas		ns	+	+	
Placenta		ns	+	+	
Prostate		ns	+	+	
Skin		ns	+	+	
Spleen		SAA1, SAA2, SAA4	+	+	
Thyroid		ns	+	+	
Tonsil		ns	+	+	
Esophagus		SAA1, SAA2, SAA4	+	nd	
Stomach		ns	+	nd	
Small intestine		ns	+	+	
Large intestine		SAA1, SAA2, SAA4	+	+	
Synovial tissue	Rheumatoid arthritis (RA)	SAA1, SAA2, SAA4	+	+	Kumon et al. (1999);
	Osteoarthritis (OA)	SAA4	+	nd	O'Hara et al. (2000)
Cartilage (chondrocytes)	Osteoarthritis (OA)	A-SAA	nd	+	Vallon et al. (2001)

ns: no specific type of SAA. nd: not done. [a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. [b]: SAA protein production, no distinct SAA isoforms mentioned.

Table 5. Extra hepatic sites of SAA mRNA expression in mouse.

TISSUES	CONDITION	SAA TYPE[a]	REFERENCES
Adipocytes	LPS	SAA3	Benditt and Meek (1989)
Adrenal	Casein LPS	SAA3 SAA1, SAA2, SAA3	Meek and Benditt (1986)
Brain	LPS	SAA3	Meek and Benditt (1986)
Pituitary	LPS	SAA3	Meek and Benditt (1986)
Heart	LPS	SAA3	Ramadori et al. (1985); Meek and Benditt (1986)
Stomach	LPS	SAA1, SAA3	Meek and Benditt (1986)
Small intestine	LPS	SAA2 (weak), SAA3	Ramadori et al. (1985); Meek and Benditt (1986)
Duodenum	LPS	SAA3	Meek and Benditt (1986)
Jejunum	LPS	SAA2 (weak), SAA3	Meek and Benditt (1986)
Ileum	LPS	SAA2, SAA3	Meek and Benditt (1986)
Large intestine	LPS	SAA1, SAA3	Meek and Benditt (1986)
Epithelial cells of the small and large intestine	LPS	A-SAA	Marhaug et al. (1997)
Epithelial lining the mucosa of the ileum and large intestine	LPS	SAA2	Meek et al. (1989)
Kidney (epithelial lining of the proximal and distal convoluted tubules)	LPS	SAA1, SAA2, SAA3	Ramadori et al. (1985); Meek and Benditt (1986); Meek et al. (1989); Marhaug et al. (1997)
Lung	LPS	SAA1, SAA2, SAA3	Ramadori et al. (1985); Meek and Benditt (1986)
Kupffer cells	LPS	SAA3	Benditt and Meek (1989)
Macrophages	LPS	SAA3	Ramadori et al. (1985); Meek and Benditt (1986);
	AEF+casein+ Freund's adjuvant		Rokita et al. (1987)
Pancreas	LPS	SAA3	Meek and Benditt (1986)
Skeletal muscle	LPS	SAA3	Meek and Benditt (1986)
Spleen and mononuclear cells in parafollicular zones of the spleen	LPS	SAA3	Ramadori et al. (1985); Meek and Benditt (1986); Benditt and Meek (1989)
Testes	LPS	SAA3	Meek and Benditt (1986)
Leydig cells of the testis	LPS	SAA3	Benditt and Meek (1989)

[a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. SAA protein production was not done.

Table 6. Extrahepatic sites of SAA mRNA expression in hamster.

TISSUES	CONDITION	SAA TYPE[a]	REFERENCES
Lung	Turpentine	SAA3	Webb et al. (1989)
Muscle	Turpentine, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAA3	Webb et al. (1989); Hardardottir et al. (1997)
Ovary	Turpentine	SAAs (weak)	Webb et al. (1989)
Spleen	Turpentine, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAA3	Webb et al. (1989); Hardardottir et al. (1997)
Testis	Turpentine	SAAs (weak)	Webb et al. (1989)
Uterus	Turpentine	SAAs	Webb et al. (1989)
Heart	Turpentine, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAAs	Webb et al. (1989); Hardardottir et al. (1997)
Intestine	Turpentine	SAAs	Webb et al. (1989)
Urinary bladder	Turpentine	SAAs (weak)	Webb et al. (1989)
Diaphragm	Turpentine	SAAs	Webb et al. (1989)
Esophagus	Turpentine	SAAs	Webb et al. (1989)
Brain	LPS	SAAs	Webb et al. (1989)
Stomach	LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAAs	Hardardottir et al. (1997)
Duodenum	Constitutively, LPS, IL-1ß TNF-α, or IL-1ß+TNF-α	SAAs	Hardardottir et al. (1997)
Jejunum	Constitutively, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAAs	Hardardottir et al. (1997)
Ileum	Constitutively, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAAs	Hardardottir et al. (1997)
Kidney	Turpentine, LPS, IL-1ß, TNF-α, or IL-1ß+TNF-α	SAA3	Webb et al. (1989)

[a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. Type of SAA was not analysed and protein production was not done.

casein induce A-SAA mRNA expression in liver and ovary to a limited extent, whereas LPS induces SAA mRNA expression in various extrahepatic tissues such as ovary, uterus, spleen, kidney, lung, brain, heart, small and large intestine (Rygg et al., 1993; Marhaug et al., 1997). In the rabbit model of Antigen-induced arthritis (AIA), when the rabbit was sensitized to methylated bovine serum albumin (mBSA) and it developed arthritis after intra-articular injection with the mBSA, SAA3 was detected in synovium and chondrocytes (Vallon et al., 2001).

In mink, SAA mRNA is not detectable in unstimulated animals, while the convoluted tubules of the kidney and uterine endometrium are strongly positive after systemic LPS injection (Table 7) (Marhaug et al., 1997).

In chickens suffering from amyloid arthropathy, SAA mRNA was detected in synoviocytes and in the vessel walls of the synovium (Table 7) (Ovelgönne et al., 2001).

In cow, horse, sheep and pig a SAA3, the so-called mammary-associated serum amyloid A3 (M-SAA3), was

Table 7. Extrahepatic sites of SAA production in other species.

SPECIES	TISSUES	CONDITION	SAA TYPE	mRNA SAA[a]	SAA PROTEIN[b]	REFERENCES
Rabbit	Brain	LPS	SAA3	+	nd	Rygg et al. (1993);
	Heart		SAA3	+	nd	Marhaug et al. (1997)
	Lung		SAA3	+	nd	
	Spleen		SAA3	+	nd	
	Kidney		SAA3	+	nd	
	Small and large intestine		SAA3	+	nd	
	Ovary		SAA3	+	nd	
	Synovium	Antigen-induced arthritis	SAA3	+	+	Vallon et al. (2001)
	Cartilage (chondrocytes)	Antigen-induced arthritis	SAA3	++	+	
Mink	Convoluted tubules of the Kidney		SAA1, SAA2	+	nd	Marhaug et al. (1997)
	Endometrium		SAA1, SAA2	+	nd	
Chicken	Amyloidotic synovium	Amyloid arthropathy	SAA	+	nd	Ovelgönne et al. (2001)
Duck	Lung	Avian leucosis virus-C infection	SAA	+	nd	Stepanets et al. (2005)
	Bursa of Fabricius	Avian leucosis virus-C infection	SAA	+	nd	
Cow	Colostrum	Post-parturition	M-SAA3	nd	+	McDonald et al. (2001)
Sheep			M-SAA3	nd	+	
Horse			M-SAA3	nd	+	
Pig			M-SAA3	nd	+	

nd: not done. [a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. [b]: SAA protein production, no distinct SAA isoforms mentioned

Table 8. Extrahepatic SAA expression and/or SAA protein production in primary cell culture.

SPECIES	CELL TYPE	INDUCTION	SAA TYPE	SAA mRNA[a]	SAA PROTEIN[b]	REFERENCES
Human	Adult aortic smooth muscle cells	Dex+IL-1 or Dex+IL-6	SAA1 SAA2 SAA4	+	+	Meek et al. (1994) Kumon et al. (2001)
	Synovial fibroblasts from RA synovium	Dex+IL-1ß	SAA1, SAA2, SAA4	+	nd	Kumon et al. (1999)
	Synovial fibroblasts from RA synovium	Quiescent	A-SAA	+	+	O'Hara et al. (2000)
	Chondrocytes	IL-1ß	A-SAA	+	+	Vallon et al. (2001)
Mouse	Granulosa cells	TNF-α	SAA3	+	nd	Son et al. (2004)
Rabbit	Neonatal aortic smooth muscle cells	IL-1ß	SAA3	+	+	Kumon et al. (1997)
	Synovial fibroblasts	PMA or IL-1	SAA3	+	+	Mitchell et al. (1991)
	Chondrocytes	IL-1ß, IL-1ß+TNF-α	SAA3	+	+	Vallon et al. (2001)
Cow	Mammary epithelial cells	Quiescent	M-SAA3	+	nd	McDonald et al. (2001)
Chicken	Synovial fibroblasts	LPS	SAA	+	+	Upragarin et al. (2002)

nd: not done. [a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. [b]: SAA protein production, no distinct SAA isoforms mentioned.

found to be highly elevated in colostrum. It decreased within 4 days post-parturition (Table 7). The amino acid sequence of M-SAA3 revealed a conserved four amino acid motif, which is Thy-Phe-Leu-Lys (TFLK), within the first eight residuals (McDonald et al., 2001).

SAA expression and production during in vitro studies

Studies of SAA expression and production in cell culture have predominantly been focused on human cells. Most *in vitro* studies were established to investigate the role of local SAA production at the inflammatory site, or in lipid transportation and metabolism (Table 8). Cultured human adult aortic smooth muscle cells (HASMC) from non-diseased persons express three SAA mRNAs (SAA1, SAA2 and SAA4) after incubation with dexamethasone (Dex), proinflammatory cytokines, or corticoid hormones (Meek et al., 1994; Kumon et al., 2001). Unlike HASMC, rabbit cultured aortic smooth muscle cells constitutively express SAA3 at a low level, and increasingly express SAA3 after induction with proinflammatory cytokines (Kumon et al., 1997). Several non-hepatic human cell lines were found to express C-SAA constitutively and expressed A-SAA after induction with Dex or proinflammatory cytokines (Steel et al., 1996) (Table 9). The human SAA3 mRNA, encoded by the *SAA3P* gene which has been previously considered as a pseudogene, could not be detected in several tissues originating from acute phase response patients treated with IL-1 and IL-6 (Kluve-Beckerman et al., 1991). However, in a later study, SAA3 mRNA was

Table 9. Extrahepatic SAA expression and/or SAA protein production in non-hepatic cell lines.

SPECIES	CELL NAME	INDUCTION	SAA TYPE	SAA mRNA[a]	SAA PROTEIN[b]	REFERENCES
Human	THP-1 (monocyte/macrophage cell)	LPS Dex Dex+PMA Dex+D3 Dex+IL-1 Dex+IL-6	SAA1 SAA2 SAA4	+	+	Urieli-Shoval et al. (1994); Ray and Ray (1997b)
	KB (oral epidermal carcinoma)	Quiescent IL-1 IL-6 Dex	SAA4 SAA2	+		Steel et al. (1996); Vreugdenhil et al. (1999); O'Hara et al. (2000)
	INT-407 (negroid cervix carcinoma)	IL-1ß+IL-6+TNF	ns	nd	+	Vreugdenhil et al. (1999)
	Caco-2 (colonic adenocarcinoma)	IL-1ß+IL-6+TNF CMc+Dex	SAA2 SAA4	+	+	Steel et al. (1996); Vreugdenhil et al. (1999)
	MRC5 (fetal lung fibroblast)	CM+Dex	SAA2 SAA4	+	nd	Steel et al. (1996)
	RT4/31 (bladder papilloma)	CM+Dex	SAA2 SAA4	+	nd	Steel et al. (1996)
	Hela (ohio cervical carcinoma)	CM+Dex	SAA2 SAA4	+	nd	Steel et al. (1996)
	HCT81 (ileocecal carcinoma cell line)	CM+Dex	SAA2 SAA4	+	nd	Steel et al. (1996)
	SW13 (adrenal cortex carcinoma)	CM+Dex	SAA2 SAA4	+	nd	Steel et al. (1996)
	ECV304 (umbilical cord endothelial)	CM+Dex IL-1ß IL-1ß TNF-ß	SAA2 SAA4	+	nd	Steel et al. (1996)
	MCF-7 (mammary gland epithelial cell)	LPS Prolactin	SAA3	+	nd	Larson et al. (2003b)
	T47-D (mammary gland epithelial cell)	LPS Prolactin	SAA3	+	nd	Larson et al. (2003b)
Mouse	J-774.1 (mouse macrophage cell)	LPS	SAA3	+	+	Meek et al. (1992)
Hamster	Hamster embryo fibroblasts transformed by herpes simplex virus type 2	CM	SAA3	+	nd	Gervais and Suh (1990)
Chicken	HD11 (chicken macrophage cell)	LPS	SAA	+	nd	Upragarin, N.[d]

ns: no specific type of SAA. nd: not done. [a]: mRNA expression was detected by either *in situ* hybridization or northern blot analysis or by RT-PCR. [b]: SAA protein production, no distinct SAA isoforms mentioned. [c]: CM, conditioned medium from peripheral blood mononuclear cells cultured with LPS. [d]: unpublished data

found to be expressed by human mammary gland epithelial cell lines (MCF-7 and T47-D) after incubation with prolactin or LPS (Larson et al., 2003a).

Mouse granulosa cells express SAA3 mRNA after incubation with TNF-α (Son et al., 2004). Similarly, a mouse macrophage cell line (J-774.1) produces SAA3 after LPS stimulation (Meek et al., 1992). A human monocyte/macrophage cell line (THP-1) expresses SAA1, SAA2 and SAA4 mRNA after incubation with Dex or combinations with other substances such as phorbol 12-myristate 13-acetate (PMA) or 1ß-25-dihydroxycholecalciferol (D_3). SAA4 was not detected in quiescent THP-1 cells, but was detected after stimulation (Urieli-Shoval et al., 1994). There is inconsistency in the expression of A-SAA by the THP-1 cell line after incubation with LPS (Ray and Ray, 1997a). In the case of human primary hepatocytes and a human hepatoma cell line (Hep3B), the mRNA of A-SAA is upregulated after stimulation with LPS or combinations of inflammatory cytokines (Castell et al., 1988; Ganapathi et al., 1991). Moreover, SAA1 and SAA2 were found to be upregulated in the synovium of RA patients and cultured synovial fibroblasts originating from RA synovium after incubation with a Dex and IL-1 combination (Kumon et al., 1999). A-SAA was constitutively produced by the quiescent synovial fibroblasts originating from RA patients (O'Hara et al., 2000). Cultured human chondrocytes expressed A-SAA after incubation with IL-1ß (Vallon et al., 2001). Thus in man, the expression of various SAA isoforms is not different between hepatic cells and non-hepatic cells.

Hamster embryo fibroblasts transformed by herpes simplex virus type 2 express SAA3 after stimulation (Gervais and Suh, 1990). Rabbit chondrocytes produce SAA3 after incubation with cytokines (Vallon et al., 2001). Furthermore, rabbit synovial fibroblasts produce SAA3 constitutively, which was dramatically increased after stimulation (Mitchell et al., 1991). Similar to rabbit, primary cultured chicken synovial fibroblasts express SAA mRNA constitutively and increase SAA mRNA production abundantly after LPS incubation and SAA protein was found after LPS incubation but not shown in unstimulated cells (Upragarin et al., 2002). Thus, the main extrahepatic production of A-SAA isoform in animals is SAA3.

Extrahepatic production of A-SAA and amyloidogenesis

It is well known that AA amyloidosis mainly occur after a prolonged period of ongoing inflammation. The AA protein also may be deposited without overt inflammatory lesions, but it does not occur in the absence of an acute phase response and in the absence of elevated SAA levels in plasma.

In the process of amyloidogenesis, there are two phases during amyloid fibril formation. This process starts with a slow preamyloid phase that is believed to comprise accumulation and nucleation of the amyloid precursor protein, which is shortened by the presence of AEF, and is followed by a more rapid deposition phase (Teilum, 1964; Sipe et al., 1978; Axelrad et al., 1982; Shirahama et al., 1990; Alizadeh-Khiavi et al., 1992). Besides AEF, there are a number of local environmental factors that stimulate or enhance amyloidogenesis during amyloid fibril deposition such as GAGs, amyloid P component (AP), and ubiquitin (UB) (Ali-Khan et al., 1996).

AEF can be isolated from amyloidotic tissues. It was found to consist of amyloid A fibrils, having a homogeneous ß-pleated sheet structure (Lundmark et al., 2002). The AEF is thought to represent a nucleation site for nascent amyloid fibrils to be formed without any intracellular uptake (Magy et al., 2003). In addition to a homogeneous ß-pleated sheet structure, a heterogeneous ß-pleated sheet structure, such as the ß-pleated sheet protein originating from silk, is able to act as AEF in the AA amyloidosis mouse model as well (Kisilevsky et al., 1999). Moreover, heparan sulfate proteoglycans, a type of GAGs which was found on the cell surface and in the extracellular matrix (ECM) and was co-deposited with amyloid fibrils, is able to increase folding to the ß-pleated sheet structure of SAA in murine AA amyloidosis (de Beer et al., 1993a). AP, which is identical to the serum amyloid P component (SAP), was found to be a constituent of all amyloid types. Various roles were assigned to AP, such as binding to amyloid fibrils, protection of amyloid fibrils against proteolysis, and to represent a structural component of the amyloid fibrils (Pepys et al., 1997; Inoue and Kisilevsky, 1999). However, studies in SAP-deficient mice revealed that SAP was not essential for amyloid deposition, but it enhanced the AA amyloid formation (Togashi et al., 1997; Usui et al., 2001). Finally, ubiquitin (UB), one of the heat shock proteins, was found to be a constituent of AEF and appeared to have AEF-activity. Furthermore, UB-bound AA amyloid fibrils were present in endosome-lysosome-like vesicles of activated monocytoid cells (Alizadeh-Khiavi et al., 1992, 1994; Chronopoulos et al., 1992).

A definite role for extrahepatically formed SAA is still unclear. However, there is some evidence linking the extrahepatic SAA production to amyloid deposition, such as in a rat synapsin I-Saa1 (SYNI) transgenic mouse model (Guo et al., 2002) and in the brown chicken joint after intra-articular injection with various agents (Landman et al., 1998). In the SYNI-*Saa1* transgenic mouse, SAA is produced locally by brain tissue cells and this mouse-brain develops AA amyloid deposits after systemic inflammation induction. This phenomenon did not occur in normal mice. In the brown chicken model, local SAA production could be demonstrated in the synovial lining cells (Ovelgönne et al., 2001; Upragarin et al., 2002) and deposition of amyloid was only found in the injected joint and in central organs, but not in the other joints (Landman et al., 1998).

Extrahepatically formed SAA at the site of

production may thus act either as a source of the AA-fibril precursor, or as a nidus or amyloid fibril core to enhance amyloid formation from systemic SAA (Fig. 1).

Conclusion

SAA has been divided into two groups: A-SAA, mainly produced by the liver and other tissues after inflammation, and C-SAA, produced by liver and other tissues under normal conditions. In birds, only one SAA type has been described which is produced in liver and other tissues. Although A-SAA is predominantly expressed and produced by liver cells, there are a growing number of reports describing its extrahepatic production by many tissues. In these reports, several species and the expression and production during *in vivo* and *in vitro* studies have been regarded. The extrahepatic production of human A-SAA has been described; however, there is no difference in the isoforms produced by the liver. In mouse, hamster, and rabbit, the main extrahepatic A-SAA isoform is SAA3. The role of C-SAA, expressed in various tissues, is not completely understood. There is growing evidence that local SAA production might be crucial in amyloidogenesis. A first example of this evidence concerns transgenic expression of SAA in mouse brain tissue, which renders them susceptible to the development of brain AA amyloidosis after induction of a systemic acute phase response. In chickens, SAA expression and production have been demonstrated in cultured synovial cells and local amyloid deposition can be induced by intra-articular injection with inflammatory agents. SAA mRNA expression was shown *in vivo* in amyloidotic synovial membrane of chickens with amyloid arthropathy and SAA protein was produced by cultured synoviocytes after stimulation. It can be concluded that extrahepatic

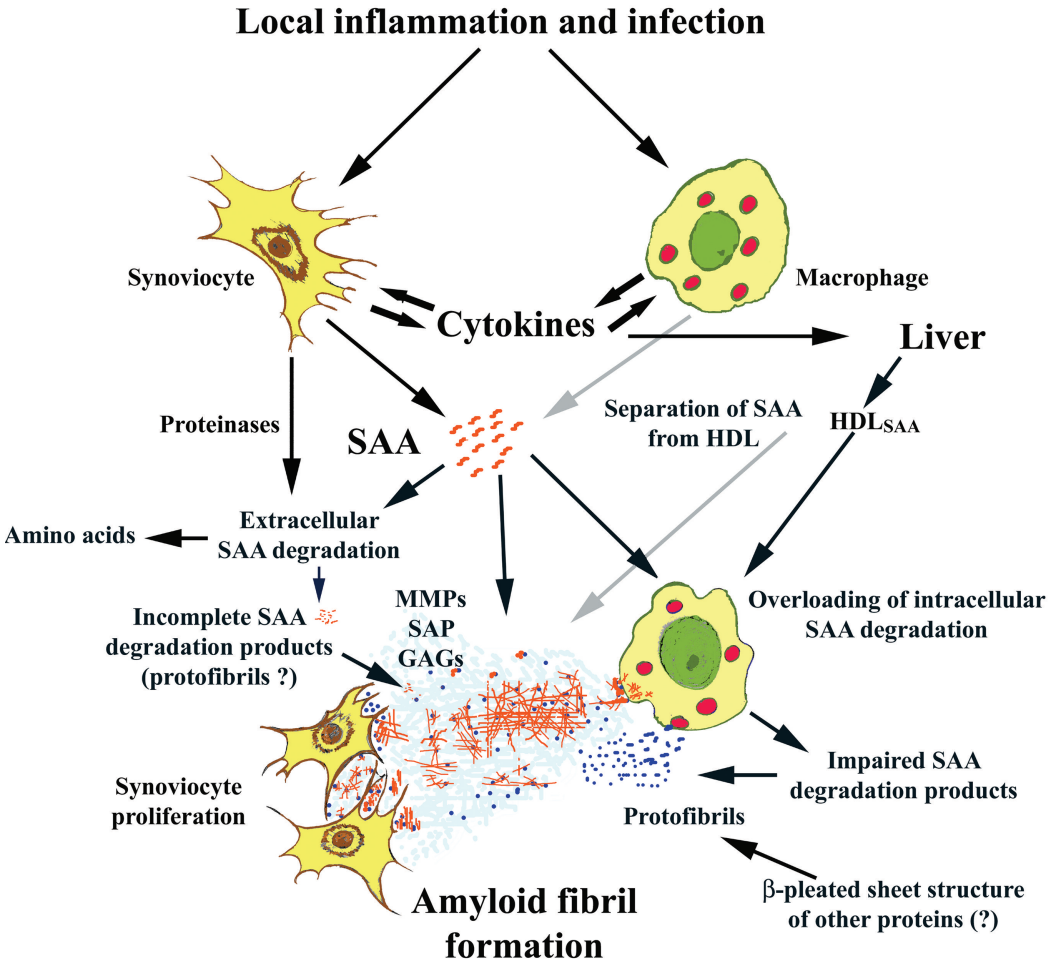

Fig. 1. The role of local production of SAA involved in AA amyloidogenesis in chicken joint (amyloid arthropathy). Synoviocytes (type B cells) and macrophages (type A cells) locally respond to inflammatory agents by producing proinflammatory cytokines (IL-1, IL-6, and TNF-α), SAA, proteolytic enzymes (proteinases), and/or extracellular matrix (ECM). The released proinflammatory cytokines will lead to a systemic acute phase response which is characterized by increasing amounts of plasma acute phase proteins. SAA is one of the plasma acute phase proteins that is mainly synthesized by hepatocytes and is associated with HDL, acute phase HDL (HDL_{SAA}). At the inflammatory sites SAA is degraded by extracellular and intracellular pathways. Extracellularly, SAA is degraded by proteinases such as MMPs. The intracellular SAA degradation pathway, which occurs in particular within macrophages, is processed by a lysosomal pathway whereby SAA is degraded by enzymes under acidic conditions. Macrophage SAA catabolism may be overloaded due to a high concentration of SAA at the inflammatory site and consequently they release impaired SAA degradation products that act as protofibrils or cores for amyloid deposition in the extracellular environment. The protofibrils bind to extracellular matrix (ECM), mainly glycosaminoglycans (GAGs). Finally, SAA and extracellular incomplete SAA degradation products, which may also act as protofibrils, bind to protofibril-ECM complex to form nascent fibrils and develop a mass deposition of amyloid fibrils at the site of hyperplasia of synoviocytes.

SAA may enhance amyloid formation at the site of production.

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