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Serum amyloid A gene expression in rabbit, mink and mouse

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SUMMARY

The expression of serum amyloid A (SAA) protein, a major acute-phase reactant in most species, was examined by *in situ* hybridization in multiple organs of rabbit, mink and mouse. In livers of unstimulated mice and rabbits a heterogeneous pattern of SAA expression in hepatocytes was observed. In all three species, lipopolysaccharide (LPS) administration resulted in extensive uniform hybridization of SAA probes to hepatocytes and in the rabbit SAA transcripts were detected in cells in the white pulp of the spleen, the adrenal cortex and ovary as well as in the mucosa and lymphatic vessels of the small intestine. Examination of hybridizing SAA signals in the rabbit myocardium showed a speckled distribution in myocytes. The rabbit endocardium was strongly positive, and in the kidney rabbit SAA mRNA was mainly confined to epithelial cells of the proximal and distal convoluted tubules. In the unstimulated mouse, SAA mRNA was detected in the liver and epithelial cells of the small and large intestine. After stimulation of an acute-phase response with LPS a strong response was seen in these organs as well as in the convoluted tubules of the kidney. In extrahepatic organs of the mink, no SAA mRNA was detectable in unstimulated animals, while the convoluted tubules of the kidney and uterine endometrium were strongly positive after systemic LPS injection.

Keywords serum amyloid A protein expression *in situ* hybridization lipopolysaccharide

INTRODUCTION

Proteins of the serum amyloid A (SAA) family are found in serum associated with high-density lipoproteins (HDL) [1] and are major acute-phase reactants [2]. SAA proteins are polymorphic and are encoded by multiple genes in many animals. Under resting circumstances low concentrations of SAA are detectable in serum, while elevations of more than 1000-fold may be documented following inflammation or tissue injury [3]. Two distinct types of SAA have been described in several species, including man and mouse. A-SAA is the acute-phase type of SAA, while constitutive SAA (C-SAA) does not alter in concentration in serum following inflammation [4–6]. Although the precise function of SAA proteins remains unknown, many studies have been directed towards this family of proteins, since they are expressed in so many species and have been conserved throughout evolution [7]. These investigations have focused upon the structure of SAA proteins and the genes encoding them, as well as regulation of expression and interspecies variation of both structure and expression [8–16].

In mouse sera, the principal constitutive SAA is SAA5 (later renamed SAA4), accounting for 90% of total serum SAA concentration in the resting state [17]. Although hepatic expression of SAA5 is inducible by lipopolysaccharide (LPS), SAA1, SAA2 and SAA3 are the isotypes for which profound increases in expression occur during inflammation. Under these circumstances, induction of SAA1 and SAA2 biosynthesis occurs principally in the liver, while significant accumulation of SAA3 transcripts is noted in lung, spleen, heart and kidney in response to LPS [18]. Previous immunohistochemical studies of the pattern of SAA accumulation in liver revealed a characteristic pattern of SAA protein in hepatocytes [19]. However, reports of precise tissue localization of extrahepatic synthesis of SAA have been limited and restricted largely to murine systems. SAA transcripts have been detected in mouse hepatocytes, convoluted tubules (proximal and distal) of the kidney and intestinal mucosa by *in situ* hybridization following LPS stimulation. In the liver, murine SAA1 and SAA2 probes failed to detect SAA expression in unstimulated animals [20]. In addition, murine SAA3 is expressed in adipocytes [21].

In man, the A-SAA isotypes, SAA1 and SAA2, are each encoded by distinct loci and occur as α and β forms, while a γ form of SAA1 has been identified. SAA4 in humans is the C-SAA and is encoded by a separate locus from SAA1 and SAA2, with SAA3 being a pseudogene [16]. Extrahepatic expression of SAA has been described in cells associated with atherosclerotic lesions including human endothelial cells, smooth muscle cells, macrophages and adipocytes [22].

While expression of SAA has been studied in other mammals, little is known concerning the precise localization of SAA biosynthesis outside the liver in those species. In rabbits, studies of

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SAA1, SAA2 and SAA3 using specific oligonucleotide probes reveals a similar pattern of extrahepatic expression to that observed in mice. All three isotypes are expressed in the liver, but SAA3, which has less sequence homology with SAA1 and SAA2 and was originally isolated from synovial fibroblasts, is the principal isotype synthesized at extrahepatic sites including the ovary, spleen, kidney and lung [23–27]. Similarly, studies of tissue distribution of SAA gene expression in the mink using RNA blot analysis have shown hepatic expression of SAA1 and SAA2, with a third transcript occurring predominantly outside the liver (kidney, adrenal, testis and ovary) [28].

Although constitutive expression of SAA occurs in the liver [17], analyses of transcript accumulation for SAA isotypes in non-hepatic tissues has been documented to be significant, especially following inflammatory stimuli [18,29]. These findings raise the possibility that extrahepatic expression of SAA could significantly contribute to the total SAA pool in the body. Therefore, further investigation of tissue localization of SAA gene expression and the interspecies differences therein was performed in these studies of SAA gene expression using *in situ* hybridization techniques in mice, rabbits and mink.

MATERIALS AND METHODS

Materials

LPS (*Escherichia coli* 026:B6) was purchased from Difco (UK). Polymerase chain reactions (PCR) were performed using the GeneAmp PCR kit with AmpliTaq (Perkin Elmer, Norwalk, CT) or Taq DNA polymerase (Boehringer, Mannheim, Germany). For generating murine SAA cDNA clones the Quick-Clone Mouse Liver cDNA and mouse 15-day embryo cDNA (Clontech, CA) were used as templates. After subcloning using the TA Cloning kit (Invitrogen, CA), plasmids were prepared using commercially available reagents (Qiagen Inc., CA). Promega Biotec (WI) supplied the Riboprobe Gemini II kit, Wizard PCR preparation reagents, molecular weight markers and restriction endonucleases. ^{35}S-UTP was purchased from NENResearch (DE) and LM-1 Hypercoat emulsions from Amersham Life Science (Aylesbury, UK). Fisher Scientific provided phosphate-buffered formaldehyde and xylene, while other chemicals were obtained from Sigma Chemical Co. (St Louis, MO).

Animals

One female rabbit, *Chinchilla* (Chbb:ch) (Thomae, Biberach, Germany) weighing 2 kg was given a subcutaneous (s.c.) injection of LPS (2 mg/kg) in sterile water. An equal volume of sterile water was administered to a second female rabbit as a control. Following sacrifice 20 h after LPS or water administration, organs to be investigated were immediately harvested and washed in PBS, fixed in 4% buffered formaldehyde for 72 h at 4°C and subsequently dehydrated in graded ethanols (30%, 50% and 70%). Before further processing, samples were stored in 70% ethanol at 4°C.

Six female mink, *Mustela vision*, were each given LPS (3 mg/kg) by s.c. injection. One animal each was killed by pentobarbital injection at 3, 6, 9, 12, 15 and 24 h following LPS administration, and organs for study were processed as described above. LPS (3 mg/kg) was injected subcutaneously into one male mink and testes were harvested at 24 h. As a control, one female mink received saline by s.c. injection and organs were harvested at 24 h. Three female mice, *Swiss Webster*, were each given LPS (50 μg) intraperitoneally. Organs were harvested at 20 h following injection, fixed and dehydrated as above. Two *Swiss Webster* mice served as controls, one receiving saline and the other without injection to exclude the possibility of i.p. needle puncture causing inflammation itself. The organs were also harvested at 20 h. For studies of testicular expression of SAA, one male mouse of the same species was given the same amount of LPS.

For investigation of expression of acute-phase plasma proteins during mammalian development, slides of mouse embryos at day 11 and day 14 gestation prepared for *in situ* hybridization were purchased from Novagen (WI). All animal experiments were performed in accordance with appropriate standards from the regulatory agencies of the USA and Norway (NIH guidelines and Federation of European Laboratory Animal Science Association).

Preparation of probes for in situ *hybridization*

For use with rabbit organs, antisense and sense single-stranded RNA probes were generated from prabSAA1, a previously described rabbit SAA1 cDNA clone with 569 bp insert in pBS SK[-], following cleavage with HindIII and BamHI, respectively, using T3 or T7 RNA polymerase [24]. The antisense probe transcribed by T3 RNA polymerase has been shown to recognize rabbit SAA1, SAA2 and SAA3 transcripts in a previous study [27].

Probes for study of SAA expression in mink were similarly generated. The previously described plasmid pmi9/5 SAA2 (insert size 552 bp) [30] was cleaved with HincIII for production of antisense probes (T3 RNA polymerase) and SmaI for sense probe (T7 RNA polymerase) transcription. This probe recognizes transcripts from mink SAA1 and SAA2 genes as well as another uncharacterized mink SAA isotype [28]. For murine studies, clones containing sequences complementary with mouse SAA transcripts were generated by PCR amplification using the Quick-Clone Mouse Liver cDNA as a template with primers (residues 246–264 and 523–541) from the published sequence of murine SAA1 [31]. After subcloning using the TA cloning kit, the approximate size of the PCR product (296 bp) was confirmed by restriction endonuclease digestion and agarose gel electrophoresis. The identity of the resulting product was authenticated by complete sequence analysis of both strands using an Applied Biosystems Automated DNA Sequencer. For transcription of RNA probes for murine SAA studies, XhoI and BamHI cleaved plasmids were used with SP6 RNA polymerase and T7 RNA polymerase for production of corresponding antisense (XhoI) and sense (BamHI) single-stranded probes. For comparison with expression of other murine acute-phase reactants, probes were generated in a similar manner for murine C-reactive protein (CRP) and serum amyloid P (SAP) transcripts. For CRP, the same murine hepatic cDNA was used as a template for production of a 597 bp CRP product by PCR (primer sites 217–235 and 796–814 from the published sequence of mouse CRP cDNA [32]). XhoI cleavage and SP6 RNA polymerase were used for transcription of the antisense probe, while BamHI linearlization and T7 RNA polymerase were utilized for the sense probe. Coding sequence (557 bp) for murine SAP component from amino acid residue 3–187 was amplified by PCR (primer sites 162–182 and 699–719 [33]) from the murine hepatic cDNA and similarly cloned. Plasmid linearized with EcoRV and SP6 RNA polymerase were used for producing the antisense probe, while BamHI cleavage and T7 RNA polymerase were used for the corresponding sense probe. In each case the identity of the cloned PCR product was confirmed by sequence analysis as above. Mouse α-fetoprotein (AFP) served as positive control for the murine fetal

experiments. The AFP probe was prepared by PCR amplification (primer sites 837–855 and 1382–1400 in the published murine AFP cDNA sequence [34]) using the Mouse 15-day Embryo Quick-Clone cDNA as template. The resulting 563 bp product represents part of the coding sequence for mature AFP. The antisense probe for AFP was generated by cleavage with XhoI and transcription using SP6 RNA polymerase, while the sense probe was prepared using T7 RNA polymerase following linearization of the plasmid with BamHI. The Riboprobe Gemini II Core System was used with 250 μCi ^{35}S-UTP/reaction for all labellings, and after separation of unincorporated nucleotides probes were diluted in hybridization solution as detailed below.

In situ *hybridization*

Prior to hybridization, 5-μm tissue sections were prepared and deparaffinized in xylene at 60°C for 1·5 h followed by washing in xylene at room temperature. After rapid hydration in sequential ethanols (100%, 95%, 80%, 70%, 50% and 30%), the slides were washed in 1× PBS and treated with proteinase K (0·001%) for 30 min followed by acetylation with acetic anhydride. Following washing in 2× SSC and rapid dehydration ending with 100% ethanol, the slides were completely dried under vacuum with desiccant before hybridization [35]. All prehybridization treatments were performed using ribonuclease-free conditions. Hybridization (4×10^7 ct/min per ml hybridization solution) was performed in formamide 50%, dextran sulphate 10%, Denhardt's solution 1×, tRNA 0·5 mg/ml, dithiothreitol 0·01 M, sodium chloride 0·3 M, and EDTA 0·001 M in a Tris buffer (0·01 M at pH 8·0). Hybridization proceeded for 18 h and temperatures varied between 57°C and 61°C depending upon the probe. After removal of the coverslips in 4× SSC, slides were treated with RNAse A 20 μg/ml for 30 min at 37°C, washed in decreasing salt concentrations ending in 0·1× SSC for 30 min at 65°C. After washing, the slides were rapidly dehydrated and dried. Preliminary evaluation of hybridization was performed by exposing the slides to autoradiographic film for 18 h at room temperature. For microscopic analysis, Kodak NTB-2 or LM-1 Hypercoat emulsions were used. Exposure time was 3–12 days depending on signal intensity. Slides were developed in Kodak D-19 developer at 14°C, fixed in Kodak rapid fixer and stained with haematoxylin and eosin. Signal detection over labelled cells compared with background was analysed by conventional light microscopy and darkfield illumination.

RESULTS

SAA gene expression in rabbit tissues

In the unstimulated rabbit liver, areas of positive hepatocytes were randomly spread over the whole liver without obvious relationship to a particular anatomic unit of organization. In the intervening hepatocytic areas, no signal was observed (Fig. 1). However, following LPS administration, positive hybridization signals for SAA transcripts were detected uniformly in all hepatocytes. Signal was not evident over vessels, including their endothelial cells, and bile ducts (Fig. 2A). In the kidney, positive signals were confined to proximal and distal convoluted tubules in the cortices and were not evident at all in glomeruli (Fig. 2B). In the renal medulla, the loops of Henle and collecting tubules showed weak hybridization for the SAA probe. SAA mRNA was detectable by *in situ* hybridization in the white pulp of the spleen, whereas the red pulp revealed only background signal activity (Fig. 2C). Epithelial cells of small intestinal villi were strongly positive, while minor amounts of signal were evident in the crypts. The endothelial cells of the intestinal submucosal vessels had even more intensely positive signals (Fig. 2D). In the rabbit myocardium, a speckled pattern was noted with some strongly positive myocytic areas (Fig. 2E). The signal in the endocardium was very positive (Fig. 3). Positively hybridizing signals were confined to the cortical cells of the adrenal glands, while the adrenal medulla was completely without hybridizing signal (Fig. 4A). Following LPS administration, no testicular SAA transcripts were detected by this method, but in the female rabbit positive hybridization for SAA was noted in the connective tissue of the ovary between follicles (Fig. 4B). Strongly positive signals were found in cells of the atretic follicles of the unstimulated rabbit ovary (Fig. 5A), as well as epithelial cells of the Fallopian tubes (Fig. 5B).

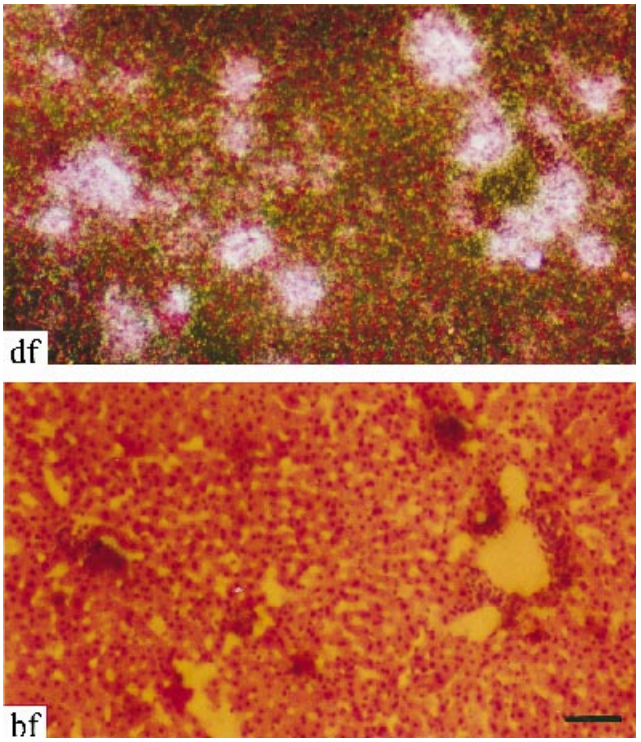
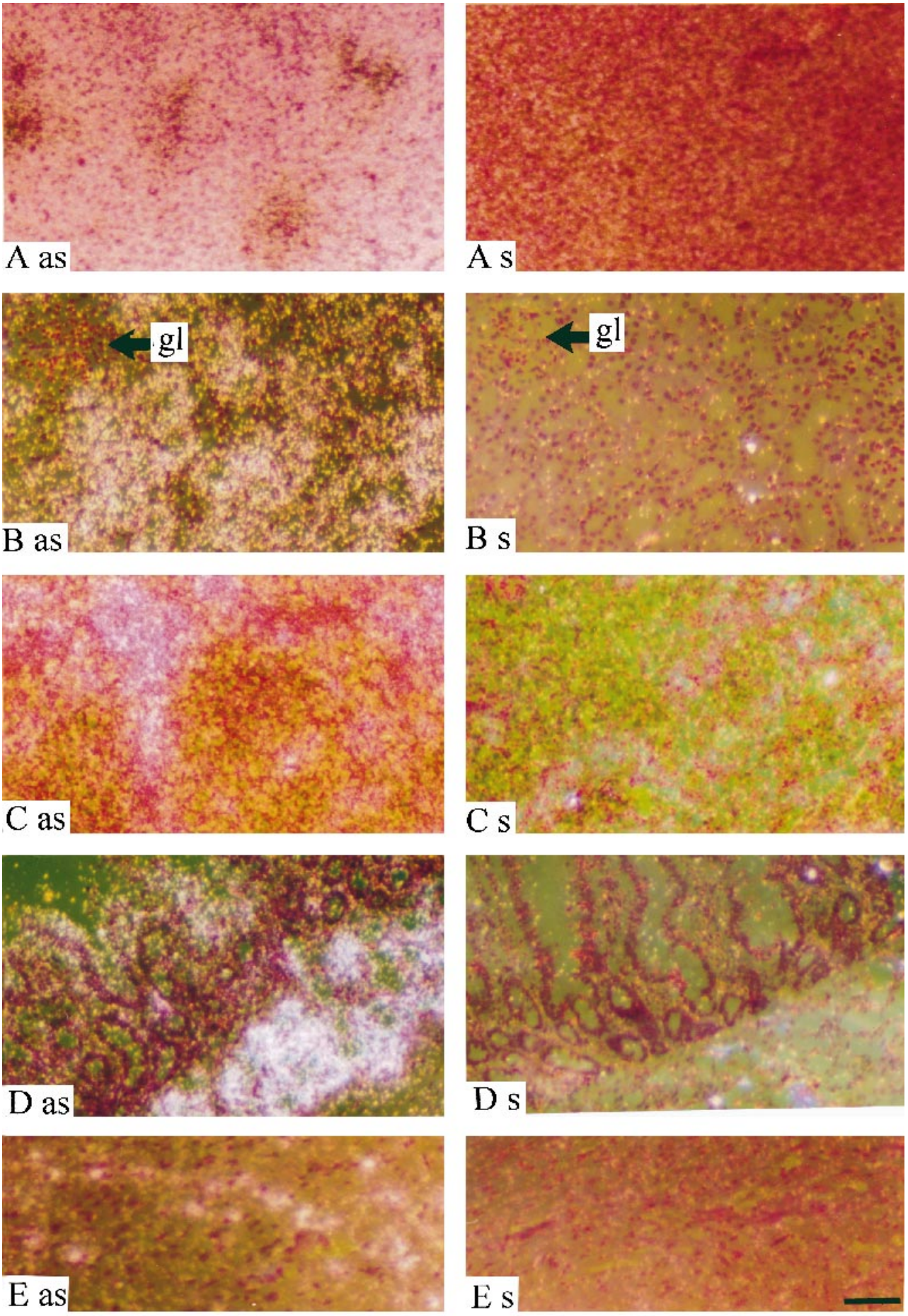


Fig. 1. *In situ* hybridization of normal rabbit liver with a ^{35}S-labelled cRNA antisense rabbit serum amyloid A (SAA) probe examined under darkfield (df) and brightfield (bf) illumination. Bar, 75 μm; ×130.

SAA gene expression in mink tissues

In the unstimulated mink no SAA expression was detectable by *in situ* hybridization in liver, heart, lung, spleen, adrenal, uterus, ovary, small intestine or testes. Following administration of LPS, the pattern of LPS expression in the livers and kidneys of the mink was the same as described for the rabbit. However, no SAA transcripts would be detected in any other organ of the mink,

Fig. 2. (See next page.) *In situ* hybridization of lipopolysaccharide (LPS)-stimulated rabbit organs with ^{35}S-labelled cRNA antisense (as) and sense (s) rabbit serum amyloid A (SAA) probes examined under dark field illumination. (A) Liver. (B) Kidney; gl, glomerulus. (C) Spleen. (D) Small intestine. (E) Myocardium. Bar, 75 μm; ×130.



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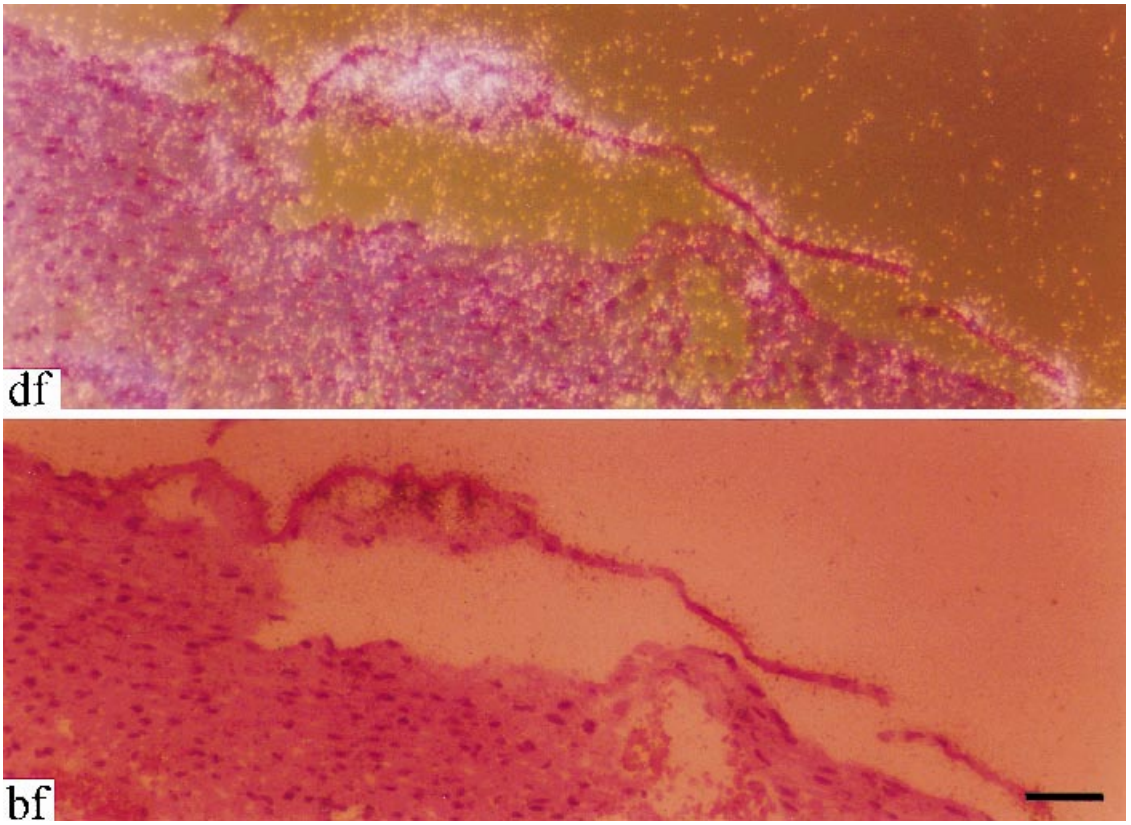

Fig. 3. *In situ* hybridization of lipopolysaccharide (LPS)-stimulated rabbit endocardium with a ^{35}S-labelled cRNA antisense rabbit serum amyloid A (SAA) probe examined under darkfield (df) and brightfield (bf) illumination. Bar, 75 μm; ×130.

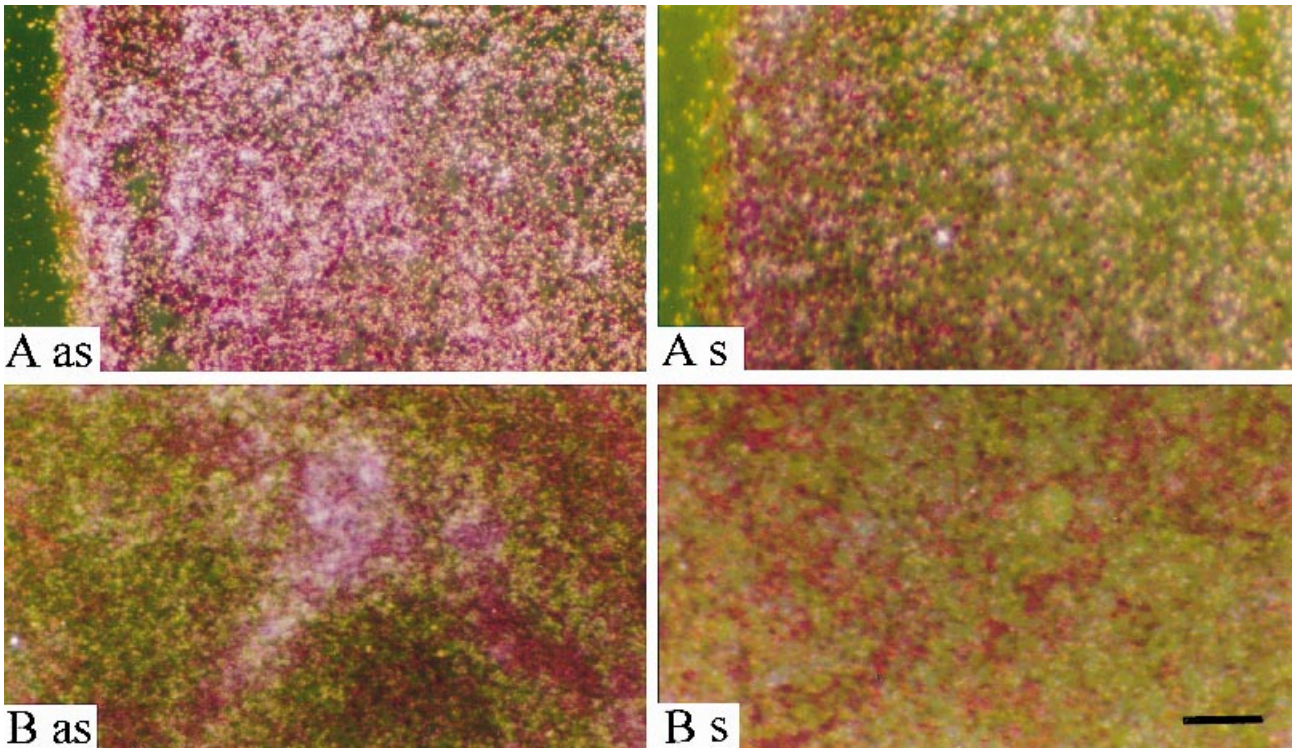

Fig. 4. *In situ* hybridization of lipopolysaccharide (LPS)-stimulated rabbit adrenal cortex (A) and ovary (B) with ^{35}S-labelled cRNA antisense (as) and sense (s) rabbit serum amyloid A (SAA) probe examined under darkfield illumination. Bar, 75 μm; ×130.

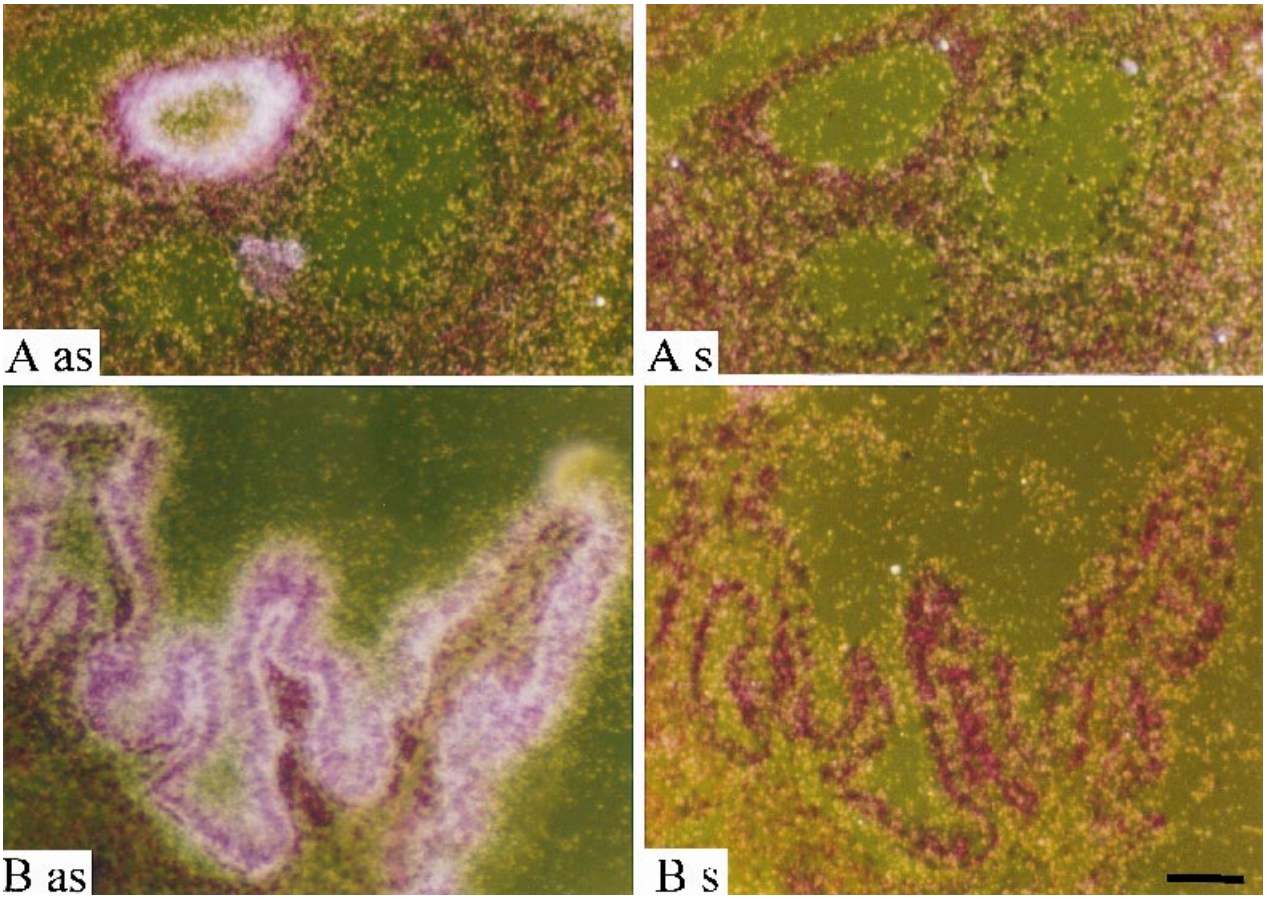

Fig. 5. *In situ* hybridization of normal rabbit ovary (A) and fallopian tube (B) with ^{35}S-labelled cRNA antisense (as) and sense (s) rabbit serum amyloid A (SAA) probes examined under darkfield illumination. Bar, 75 μm; ×130.

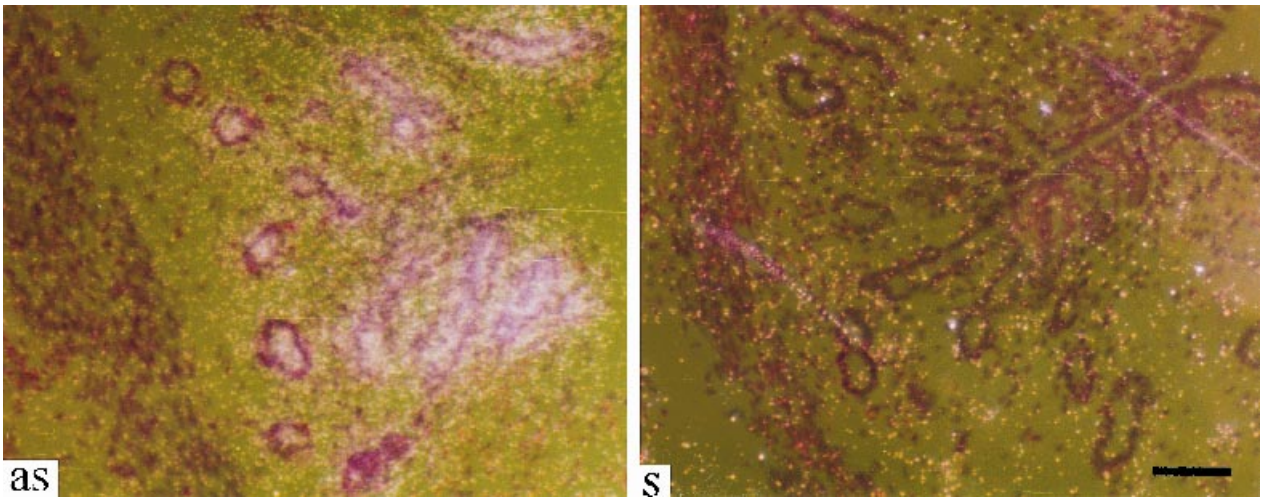

Fig. 6. *In situ* hybridization of lipopolysaccharide (LPS)-stimulated mink uterine endometrium with ^{35}S-labelled cRNA antisense (as) and sense (s) mink serum amyloid A (SAA) probes examined under darkfield illumination. Bar, 75 μm; ×130.

except the mucosal epithelial cells of the endometrium where strongly positive signals were noted (Fig. 6). In the time-course study, hepatocytes, convoluted tubules of the kidney and the endometrial signals were evident at 3 h after injection of LPS. Some individual variation in the expression of SAA in these tissues was noted from animal to animal at the later time points, but at no later time did other organs yield a positive signal for SAA mRNA.

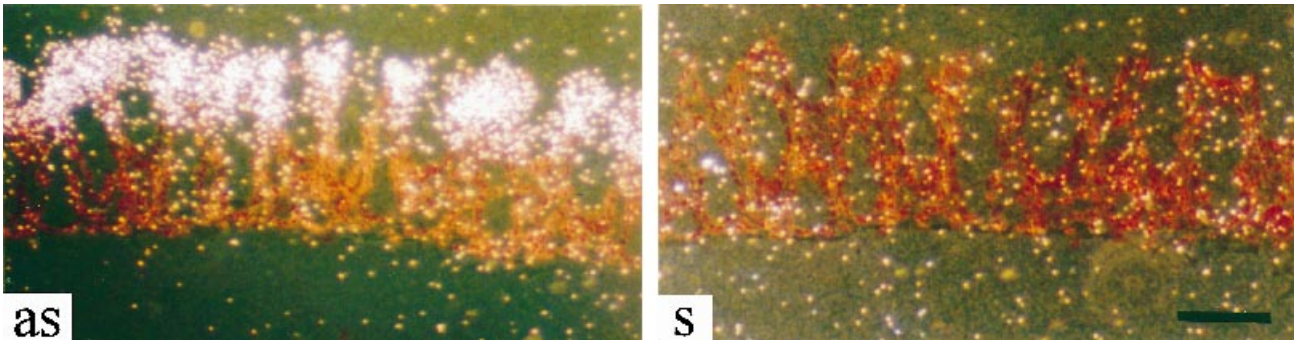

Fig. 7. *In situ* hybridization of normal mouse small intestine with ^{35}S-labelled cRNA antisense (as) and sense (s) mouse serum amyloid A (SAA) probes examined under darkfield illumination. Bar, 75 μm; ×130.

SAA gene expression in murine tissues

The pattern of expression in murine hepatocytes was similar to that observed for rabbit and mink following exposure to LPS. In mice SAA mRNA was also detected in mucosal epithelial cells of the small and large intestine, as well as in epithelial cells of the convoluted tubules of the renal cortex. The pattern was similar to that observed for rabbit and mink in these organs. No signal was detected in any other organ, including the endometrium.

In the absence of LPS stimulation, scattered hepatocytes were noted with intense hybridization signals for SAA, as seen previously in the livers of untreated rabbits (Fig. 1). In the mice without exposure to LPS, signals were also noted in small and large intestine, but signal was absent in kidney (Fig. 7). The results from the rabbit, murine and mink studies of SAA are summarized in Table 1.

Expression of CRP and SAP in murine tissues

When the same murine organs were hybridized with the murine CRP and SAP antisense probes, positive hybridization was noted only in the liver. In the unstimulated control samples, homogeneous patterns of hybridization with CRP and SAP probes were noted in all hepatocytes. Although not able to be quantified by the *in situ* hybridization method, there appeared to be an increase in hybridizing signal for these probes in samples from animals treated with LPS.

Table 1. Serum amyloid A (SAA) mRNA-positive cells in rabbit, mink and mouse organs in normal animals (control) and after lipopolysaccharide (LPS) stimulation

	Mink		Rabbit		Mouse	
	LPS	Control	LPS	Control	LPS	Control
Liver	Most hepatocytes	0	Most hepatocytes	Heterogeneous pattern. Areas of strongly positive hepatocytes, but most hepatocytes negative.	Most hepatocytes	Heterogeneous pattern. Areas of strongly positive hepatocytes, but most hepatocytes negative.
Spleen	0	0	Lymphocytes in the white pulp	0	0	0
Adrenal	0	0	Cortical cells	0	0	0
Small intestine	0	0	Epithelial cells of villi Endothelial cells in submucosa.	0	Epithelial cells of villi	Epithelial cells of villi
Ovary	0	0	Unidentified cells in connective tissue	Atretic follicles	0	0
Endometrium	Epithelial cells	0	ND	ND	0	0
Lung	0	0	0	0	0	0
Mycardium	0	0	Heterogenous pattern of positive cells	0	0	0
Endocardium	0	0	Endothelial cells	0	0	0
Testes	0	0	ND	ND	0	0
Colon	0	0	ND	ND	Epithelium	0
Kidney	Convoluted tubules	0	Convoluted tubules	0	Convoluted tubules	0
Fallopian tube	ND	ND	ND	Epithelial cells	ND	ND

ND, Not done; 0, no detectable SAA mRNA.

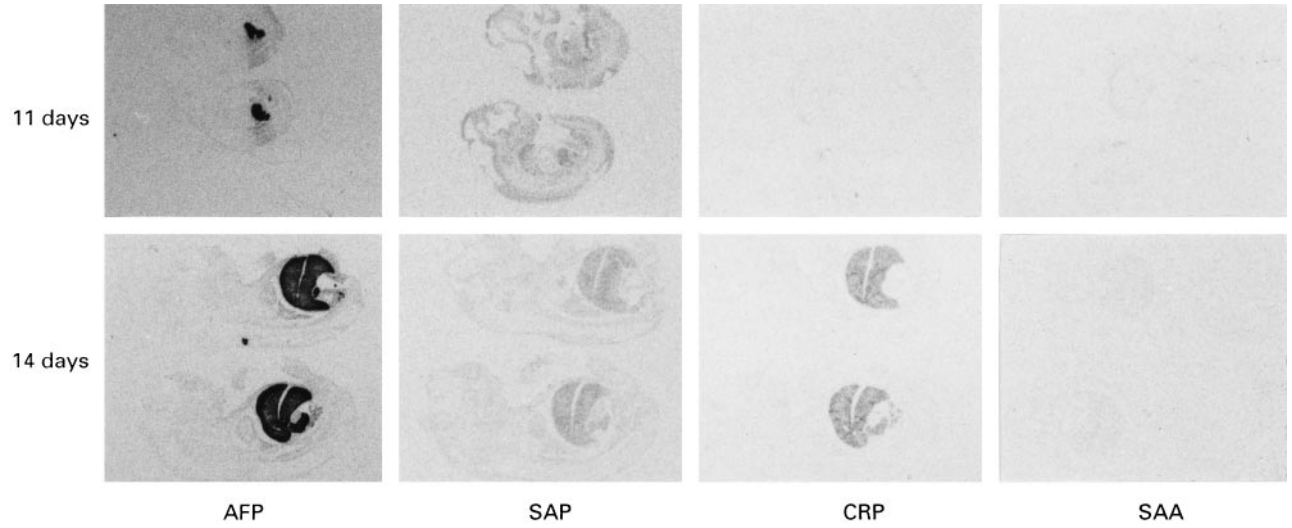

Fig. 8. *In situ* hybridization of fetal mouse embryos at 11 and 14 days of gestation using ^{35}S-labelled cRNA antisense probes for mouse α-fetoprotein (AFP), serum amyloid P component (SAP), C-reactive protein (CRP), and serum amyloid A protein (SAA), all exposed on the same x-ray film for 18 h.

Developmental expression of SAA, CRP and SAP in mice

The developmental expression of these acute-phase proteins was investigated by hybridizing each corresponding probe with murine whole mount preparations at 11 and 14 days gestation. AFP was utilized as a control and yielded strongly positive hybridization signals at both gestational ages. At day 11, SAP expression could be detected in the liver and became more prominent by day 14. However, CRP expression was not noted in 11 day samples, while strong hybridization was evident 3 days later. In contrast, no significant SAA signal was detected in either 11 day or 14 day gestation samples (Fig. 8).

DISCUSSION

The results of these studies using *in situ* hybridization to detect SAA transcripts in a variety of organs confirm previously reported studies using RNA isolated from whole organs in rabbits, mink and mice [18,27–29]. The current experiments have permitted a more detailed analysis of the cellular origin of SAA transcripts in many tissues and have shown that transcripts from this gene family are present in a wide variety of cell types including ectodermal, mesodermal and endodermal lineages. In addition to what has been reported previously, SAA transcripts were detected in the white pulp of the rabbit spleen, cortical cells of the rabbit adrenals, atretic follicles of the rabbit ovary, in rabbit myocardium and endothelial cells of rabbit endocardium and in epithelial cells of rabbit fallopian tubes, in epithelial cells of mink endometrium and in epithelial cells of mouse colon. Although discordant with a recent study in which no SAA mRNA signal was noted in an *in situ* hybridization study of unstimulated mouse livers [20], the focal SAA expression found in both the control murine samples as well as the livers of rabbits is in agreement with previously reported patterns of SAA protein expression studied using immunohistochemical techniques [19]. In the present study, there was no obvious connection of the distribution of SAA transcript-positive hepatocytes to defined anatomic structures within the liver. Local production of cytokines by Kupffer cells followed by stimulation of and recruitment of neighbouring hepatocytes to SAA production may be an explanation for the rather heterogeneous involvement of normal hepatocytes. Expression of SAA genes has been described in monocyte/macrophage cell lines [9], and the methods used in this study can not exclude the possibility that the SAA mRNA detected is derived from macrophages in the unstimulated liver. The reasons underlying the interspecies differences in basal and stimulated responses to LPS are not entirely clear. No SAA mRNA could be detected in unstimulated mink organs, whereas heterogeneous hepatocyte and ovarian follicular expression was noted in unstimulated rabbits as well as in hepatocytes and intestinal mucosa of mice. These differences probably indicate real differences in the species, whereas differences noted following LPS administration might be explained by dosage effects as well as interspecies variation in sensitivity to LPS.

In the longitudinal study of SAA expression in organs of the mink at varying times following LPS injection, no augmentation of SAA levels in positively hybridizing tissues (hepatocytes, endometrial epithelium, epithelium of the proximal and distal convoluted tubules) occurred following the initial dramatic increase noted at 3 h. This finding is in accord with data from transcription rates for SAA genes during the initial stages of a response following LPS administration showing that peak transcription is reached after 3 h [36]. Since the principal function of SAA remains to be elucidated, interpretation of the detection of organs and cell lineages in which SAA is expressed is difficult. SAA expression in the mucosa of the intestine, also shown by Meek *et al.* [20], as well as in the endometrium may reflect a role in surface epithelial protection for SAA. Since other apolipoproteins (apoE) may have a local function [20], perhaps the same is true for apoSAA. Additional evidence that SAA is expressed in endothelial cells [22] lends support to the postulate that SAA may be a factor in barrier functions of epithelial and endothelial cells. However, data from the current study show that endothelial expression does not occur in endothelial cells of all organs, since hepatic and renal vessels did not hybridize in their endothelial layers with SAA probes. Other functions of SAA proteins may be specific to local activities in the microenvironments of the organs involved in SAA production. In the unstimulated rabbit ovary intense SAA transcript levels were

seen in the walls of atretic follicles. Since interstitial collagenases are important in follicular rupture [37], local production of SAA in the ovary is not surprising since SAA1 and SAA3 may act in an autocrine or paracine fashion to stimulate collagenase synthesis in fibroblasts [26].

The probes used in the current study did not permit discrimination of signals arising from the various isotypes of SAA. However, based upon previous RNA blot data it is most likely that most of the extrahepatic transcripts detected in this study are SAA3, since that is the principal extrahepatic SAA isotype in rabbit and mice, and probably in mink also [27,28].

The comparative studies of CRP and SAP expression in murine tissues underscore the tissue specificity of expression of different acute-phase plasma proteins as well as the complexity of differential regulation of acute-phase plasma protein biosynthesis. CRP and SAP were found to be homogeneously expressed throughout the hepatocyte population of the liver in mice, and they were also noted to be under distinct patterns of regulation of expression during fetal development. The finding that SAP and CRP are expressed during normal fetal life, while SAA is not, perhaps suggests homeostatic roles for the pentraxins during fetal development for which SAA is not necessary. The regulation of these acute-phase plasma proteins has been extensively studied, and although many of the same cytokines and signal transduction mechanisms are involved, there are distinct differences in response. Indeed, within the SAA gene family differential regulation has been demonstrated in human hepatocytic and macrophage cultures [8,9]. Studies of transcriptional induction of SAA genes have revealed several regulatory elements in the 5′ regions of SAA genes, and NK-kB response elements are present in rat [10], rabbit [11] and human [12] SAA genes. Data from the current study confirm that SAA genes are expressed in many tissues in response to inflammation. Cells of various embryological lineages are involved, although at least in mice SAA does not appear to be expressed in fetal life. Additional studies will be necessary to clarify further the role of SAA in the sites identified in this study.

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