Clin Exp Immunol 1996; **103**:335–342

Cytokine-induced differential expression of serum amyloid A genes in fetal and neonatal rabbits

M. RYGG *Department of Paediatrics, Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway*

(Accepted for publication 31 October 1995)

SUMMARY

Serum amyloid A (SAA) is an acute-phase plasma protein which increases 100- to 1000-fold in response to inflammatory stimuli. In this study pregnant rabbits were subjected to laparotomy and their fetuses were injected with lipopolysaccharide (LPS) or various cytokines. Newborn rabbits were likewise stimulated. Hepatic SAA mRNA was studied using Northern blot analyses and scanning densitometry. *In vitro* derived RNA was used as standard for quantitative mRNA analyses. Cytokine concentrations in amniotic fluid and serum were analysed by biological assays. Fetal rabbits responded to cytokine stimulation by increased hepatic SAA mRNA expression, both during late gestation and in the early neonatal period. However, differences in dose–responses, kinetics and mRNA concentrations were seen dependent on gestational age. IL-1 and tumour necrosis factor (TNF) induced hepatic accumulation of both SAA1, SAA2 and SAA3, while only SAA1 and SAA2 mRNA accumulation was seen after IL-6 stimulation. High levels of IL-1 and TNF found in amniotic fluid from LPS-stimulated fetal rabbits corresponded with high levels in fetal, but not in maternal, serum. High levels of IL-1 and TNF, but no IL-6, were seen in newborn control sera and in adult serum 1 day after a normal delivery. The study details the complexity of the cytokine-induced *in vivo* response of SAA mRNA in fetal and neonatal rabbits.

Keywords acute-phase protein IL-1 IL-6 tumour necrosis factor amniotic fluid

INTRODUCTION

The host response to inflammation involves several alterations in homeostatic mechanisms, including changes in concentrations of a number of plasma proteins—the acute-phase proteins [1]. Serum amyloid A (SAA), is a sensitive marker of the acute-phase response [2]. The plasma concentration of SAA may increase by 100- to 1000-fold [1] as a result mainly of enhanced transcription, but also by altered post-translational processing [3,4]. SAA is encoded by a family of genes in many animals [5,6] including man [7] and rabbit [8]. Several studies of the biosynthesis of SAA have identified the hepatocyte as the primary source of this protein [9], but expression of SAA in other cells is also found [10].

Bacterial infection is still a major cause of perinatal morbidity and mortality, and the recognition of infection is difficult since the clinical and biochemical manifestations are subtle in the neonatal period [11]. New tools for diagnosing potentially life-threatening infections in these vulnerable patients are therefore needed [11].

Correspondence: M. Rugg, Department of Paediatrics, Institute of Clinical Medicine, University of Tromsø, N-9037 Tromsø, Norway.

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Cytokines are biologically active signal molecules and the secreted products of many cell types, especially activated monocytes and macrophages [12]. They are recognized as major modulators of pregnancy events, such as trophoblast growth and embryo development, and have also been implicated in the mechanisms responsible for the onset of parturition [13]. Cytokines play a key role in the induction and subsequent modulation of the synthesis of acute-phase proteins [14]. IL-1, IL-6 and tumour necrosis factor (TNF) have been described to induce SAA mRNA during inflammation [15]. These multifunctional signal molecules act by directly stimulating the hepatocytes, and indirectly, by inducing the synthesis of other cytokines or corticosteroids [14].

During development hepatic gene expression is undergoing considerable alteration [16]. Although studies in several species have focused on the regulation of SAA gene expression [15,17], few data have been available regarding the regulation of these acute-phase transcripts during fetal and neonatal development [18]. The present study was undertaken to study in more detail the degree of inducibility of three rabbit SAA genes encoding one of the major acute-phase plasma proteins during fetal and early neonatal life, and to identify the mediators potentially responsible for such an effect. This basic information is useful in the search for better methods of diagnosing neonatal infection.

Table 1. Experimental regimes and mortality after maternal laparotomy and fetal injection through the uterine wall

Treatment	Dose (ng/g body weight)	Volume (μl/g body weight)	*n* (fetuses)		*n* (litter)	Mortality	
			Stim.	Ctr.†		Stim.	Ctr.†
IL-1	0·5–5–10*	2·5–5–10	6	3	1	6	3
	0·25–0·5–2·5*	2·5	6	3	1	3	0
	2·5	2·5	7	4	3	0	0
IL-6	2·5–25–50–150*	2·5–5–15	5	2	1	0	0
	25	2·5	11	8	3	0	0
TNF	2·5–25–50*	2·5–5	6	4	1	1	0
	50	5	9	8	3	0	0
LPS	1000	2	19	12	6	0	0
	2000	4	10	6	1	3	0
	2000	4	8	5	1	5	0
NaCl	—	2·5	10	—	1	0	—

*Different doses given to individual fetuses in the same litter.
†Controls are fetuses from the same litter receiving either PBS (in the cytokine experiments) or NaCl (in the LPS experiments).
TNF, Tumour necrosis factor; LPS, lipopolysaccharide.

MATERIALS AND METHODS

Materials

Molecular biological enzymes were obtained from Pharmacia (Uppsala, Sweden) and Boehringer, Mannheim (Mannheim, Germany). The SP6/T7 transcription kit and the Sephadex G-25 and G-50 Quick Spin Columns were from Boehringer Mannheim. Radioactive isotopes were obtained from New England Nuclear (Boston, MA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) was from Sigma (St Louis, MO). Lipopolysaccharide (LPS; *Escherichia coli*, serotype 026:B6) was obtained from Difco Labs (Detroit, MI). Human recombinant IL-6 (hrIL-6, specific activity $>1{\cdot}0 \times 10^8$ U/mg), hrIL-α (specific activity $>5{\cdot}0 \times 10^7$ U/mg), hrTNF-α (specific activity $>1{\cdot}0 \times 10^8$ U/mg) and a MoAb against hIL-6 were obtained from Boehringer Mannheim. Polyclonal antibodies against hIL-1β and hTNF-α were from British BioTechnology (Oxon, UK) and Genzyme (Cambridge, MA), respectively.

Fetal studies

Timed pregnant rabbits, *Chinchilla* (Chbb:CH), weight 3–4 kg, all specific pathogen-free, were purchased from Thomae (Biberach, Germany). Three experimental groups of multiparous rabbits were used.

In the first experimental group, 25 day pregnant rabbits (total pregnancy length 30–32 days) were subjected to laparotomy, and LPS (in saline) or cytokines (in PBS with bovine serum albumin (BSA) 1 mg/ml) were injected to all fetuses in one uterine horn through the uterine wall. Fetuses in the other uterine horn served as controls, receiving equal injection volumes of either saline (control for LPS stimulation) or PBS with BSA (control for cytokine stimulation). Fetal weight was 15–30 g. LPS was administered in doses of 1–2 μg/g body weight. Each cytokine was initially given in different doses to individual fetuses in a litter (Table 1). After Northern analyses, a suitable dose for each cytokine was chosen, based on the fetal acute-phase response. Animals were killed after

Table 2. Experimental regimes and mortality after subcutaneous injections to 1-day-old neonatal rabbits

Treatment	Dose (ng/g body weight)	Volume (μl/g body weight)	*n* (neonates)	*n* (litter)	Mortality
IL-1	0·25	0·25	2	1	0
	0·5	0·5	1	1	0
	2·5	2·5	4	2	0
IL-6	25	2·5	6	4	0
TNF	25	2·5	5	3	0
	50	5	1	1	0
LPS	500	0·5	2	1	0
	1000	2	10	5	1
	1500	1·5	2	1	0
NaCl/PBS	—	2·5	7	5	0

TNF, Tumour necrosis factor; LPS, lipopolysaccharide.

8 or 24 h. Maternal and fetal blood were sampled by heart puncture, and amniotic fluid was sampled individually from each fetus in the litter, centrifuged and stored at −20°C for further cytokine analyses. Maternal and fetal livers were immediately frozen in liquid nitrogen and stored at −70°C for RNA analyses. In addition to internal controls in each litter, all fetuses of one 25 day pregnant rabbit were given injections with sterile saline. Serum, amniotic fluid and livers were sampled after 24 h.

In the second experimental group, 25 day pregnant rabbits were given subcutaneous injections of LPS in saline, 0·5 mg/kg body weight. An equivalent volume of sterile saline was given to one control animal. Rabbits were killed 24 h after the injections, and serum, amniotic fluid and livers were harvested and frozen.

In the third experimental group, two 19 day pregnant rabbits were likewise subjected to laparotomy, and LPS in doses of 1–2 μg/g body weight, or saline, was administered to their fetuses, weight 2–3 g. Amniotic fluid, maternal and fetal livers were frozen at 24 h.

Neonatal studies

One-day-old neonatal rabbits, weight 35–65 g, were injected subcutaneously with LPS or cytokines (Table 2). Control animals received sterile saline or PBS with BSA in equivalent volumes. All animal experiments were performed in accordance with ethical guidelines of the Federation for Laboratory Animal Science.

Northern blot analyses

Isolation of total RNA from rabbit livers was prepared by the guanidinium thiocyanate method from frozen tissues [19]. The concentration of each RNA preparation was measured by photospectrometry at 260 nm. RNA samples (20 μg) were fractionated by agarose-formaldehyde gel electrophoresis and transferred to nylon membranes by vacuum blotting. Equal RNA loading and adequacy of blotting were confirmed by comparison of rRNA intensities in ethidium bromide-stained gels. For hybridization, a cRNA probe complementary to rabbit SAA1 cDNA [8] cross-hybridizing to SAA1, SAA2 and SAA3 mRNA, was used. Three 18-mer oligonucleotide probes [20] specific for rabbit SAA1, SAA2 [8] and SAA3 [21] cDNA, respectively, were also used. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [22] was used as a control probe. The methods used for α-^{32}P-labelling of the probes and the different hybridization conditions were detailed previously [20]. The hybridization intensity of the bands was measured by scanning densitometry using a PhosphorImager Personal Densitometer and analysed by the ImageQuant software package from Molecular Dynamics (Sunnyvale, CA). For autoradiographic visualization, blots were exposed on Hyperfilm-MP (Amersham, London, UK) at −70°C.

Quantification of gene-specific mRNAs

Quantification of SAA mRNA was performed according to a method described by Carter *et al*. [23]. PrabSAA2 [8], known to cross-hybridize to SAA1, was digested at a Hind III restriction site at the 3′ end of the cDNA insert, and used as template in an *in vitro* transcription reaction. The resultant iv-SAA RNA of 599 nucleotides corresponded to the distance from the T3 RNA polymerase start site to the end of the linear template at the 3′ end of the cDNA sequence. Following *in vitro* transcription, digestion of the DNA template with RNAse-free DNAse and purification on a Sephadex G-50 column, the iv-SAA concentration was determined by measuring absorbance at 260 nm. The mol. wt and concentration was used to calculate its molarity. Two-fold dilutions of the iv-SAA were electrophoresed and subjected to Northern analyses together with total RNA samples as described above. The logarithm of the densitometric units (area) of the radioactive signals resulting from the specific hybridization to the iv-SAA, and the logarithm of the two-fold RNA dilutions (moles), were used in simple linear regression analyses. The resultant regression line was used as an internal standard curve, and the concentration of SAA mRNA in each liver sample was determined, based on the intensity of the corresponding hybridization signal. These quantitative analyses were performed on liver RNA samples that had been run on the same gel as the iv-SAA used for the standard curve.

IL-1 bioassay

IL-1 activity was determined by a two-stage bioassay. The first stage involved the mouse thymocyte EL-4 NOB-1 cell line which produces high amounts of IL-2 in response to rhIL-1 [24]. Serial dilutions of rhIL-1 were induced as a standard. NOB-1 cells were seeded in 96-well microplates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) with duplicate sets of serial five-fold dilutions, starting at a 1:10 dilution of either rabbit serum or amniotic fluid, and incubated for 24 h at 37°C. Then, 100-μl aliquots of the samples were transferred to a replicate 96-well microplate for the next stage involving the IL-2-dependent mouse T cell line, Th2 [25]. Aliquots (100 μl) of Th2 cell suspension ($1{\cdot}5 \times 10^8$ cells/well) were added to each well of the microplate and incubated for a further 24 h before cell proliferation was determined. IL-1 activity found in rabbit amniotic fluid was neutralized by a polyclonal antibody against rhIL-1. Results are presented in pg/ml as a mean of duplicate measurements.

IL-6 bioassay

IL-6 activity was determined in a cell proliferation assay, using the IL-6-dependent mouse hybridoma cell line B13.29 clone B9 [26]. Duplicate sets of five-fold dilutions of rabbit serum or amniotic fluid, starting at a 1:20 dilution, and rhIL-6 as a standard were analysed. IL-6 bioactivity found in rabbit amniotic fluid was neutralized by a MoAb against rhIL-6. Results are presented in pg/ml as a mean of duplicate measurements.

TNF bioassay

TNF activity was determined by its cytotoxic effect on the fibrosarcoma cell line WEHI 164 clone 13, as described previously [27]. Duplicate sets of five-fold dilutions of rabbit serum or amniotic fluid, starting at a 1:20 dilution, and rhTNF as a standard were analysed. The TNF specificity of the assay was verified using a polyclonal antibody against rhTNF, which neutralized the cytotoxic effect found in rabbit amniotic fluid. Results are presented in pg/ml as a mean of duplicate measurements.

Cytotoxicity assay

Viability of the cells in the assays for IL-1, IL-6 and TNF was tested by incubation with MTT, which is converted to insoluble purple formazan by living cells [28].

RESULTS

Dose–responses of SAA mRNA accumulation

Tables 1 and 2 list the various experimental regimes in relation to gestational age, dose, number of injected animals, and survival. When IL-1 was administered to fetal rabbits in doses from 0·5 to

10 ng/g body weight, all animals in the litter including the controls died *in utero* within 24 h (Table 1). Placental haemorrhage and fetal maceration were found at autopsy. When doses were reduced to 0·25–2·5 ng/g body weight, half of the IL-1-injected animals died, while there were no deaths in the PBS-injected littermates. There were no intra-uterine deaths in nine fetal rabbits from four different litters after IL-1 injection with 2·5 ng/g body weight. No deaths were observed after fetal IL-6 stimulation in doses varying from 2·5 to 150 ng/g body weight. One of the 15 TNF-injected animals died *in utero* (Table 1). None of the newborn rabbits died after cytokine stimulation (Table 2). In most experiments the volumes of injections were standardized to 2·5 μl/g body weight. Increased volume in some experiments did not seem to affect either mortality or SAA mRNA response. The dose–response for hepatic SAA mRNA after administration of three cytokines to fetal and newborn rabbits is documented in Fig. 1. Based on these preliminary results, the doses for studying SAA mRNA response in the remaining experiments were chosen to be 2·5, 25 and 50 ng/g body weight for IL-1, IL-6 and TNF, respectively (Fig. 1).

Time course and quantitative calculations of SAA mRNA levels
Autoradiographs of the *in vitro* derived RNAs and liver RNA samples used to make direct quantitative calculations of the SAA mRNA response are shown in Fig. 2. Eight hours after injections with cytokines, the SAA mRNA signals had a higher mol. wt than after 24 h in both the fetal (Fig. 2) and neonatal rabbits (Fig. 2b).

Densitometric scanning analyses of the Northern blots demonstrated that in fetal rabbits (Fig. 3, open circles), the SAA mRNA levels in two controls were below $0{\cdot}1 \times 10^{-17}$ mol/μg total liver RNA. No differences were seen between non-stimulated and saline-injected controls. Eight hours after stimulation of two fetal rabbits from each experimental group, the SAA mRNA levels were increased to 3–4 in the IL-1 group, 11–14 in the IL-6 group, and 3–7×10^{-17} mol in the TNF group (Fig. 3a–c). No further increase was seen after 24 h. In contrast, the increase in SAA mRNA levels after LPS stimulation were more pronounced and continued to rise to twice the level seen at 8 h (Fig. 3d).

In neonatal rabbits (Fig. 3, closed squares), relatively high levels of SAA mRNA ($10–19 \times 10^{-17}$ mol) were seen in the two controls, and nearly no changes were seen after IL-1 stimulation (Fig. 3a). As in fetal rabbits, SAA mRNA levels in neonatal rabbits peaked at 8 h in response to IL-6 stimulation (Fig. 3b). However, after TNF administration the rise in SAA mRNA was higher and seemed to peak later, reaching about 50% of the level seen 24 h after LPS stimulation (Fig. 3c,d). No differences were observed in GAPDH mRNA levels in the dose–response or kinetic experiments (data not shown).

Differential expression of SAA genes
Using SAA gene-specific oligonucleotide probes, hepatic SAA1 and SAA2 mRNAs were seen after stimulation with all three cytokines, as well as LPS (Fig. 4a,b). In contrast, SAA3 mRNA was only seen after IL-1, TNF or LPS administration in fetal, and only after TNF or LPS administration in neonatal rabbits (Fig. 4c). Despite being a major inducer of SAA1 and SAA2 mRNA, IL-6 did not elicit any SAA3 mRNA response (Fig. 4).

Cytokine concentration in amniotic fluid and serum
High levels of amniotic fluid TNF and IL-1 were detected after fetal LPS stimulation both at gestational day 19 and day 25

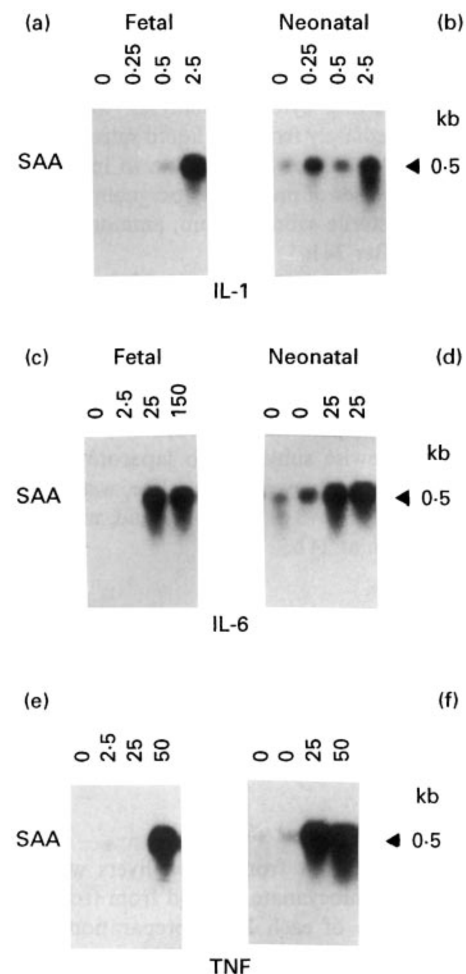


Fig. 1. Serum amyloid A (SAA) mRNA induction following administration of cytokines to fetal and neonatal rabbits. Northern blots with total liver RNA, 20 μg per lane, from fetal (a,c,e) or neonatal (b,d,f) rabbits were hybridized with a SAA RNA probe. Rabbits had been given increasing doses of IL-1 (a,b), IL-6 (c,d) and tumour necrosis factor (TNF) (e,f). Doses in ng/g body weight are indicated above each lane. Exposure: 8 h (a), 3 h (b), 2 h (c,d), 4 h (e) and 1 h (f).

(Fig. 5a,b). Amniotic fluid IL-6 levels were moderately elevated after fetal LPS stimulation at day 19, while normal IL-6 levels were found 24 h after LPS stimulation to 25-day-old fetal rabbits (Fig. 5c). None of the amniotic fluid samples from 10 fetal rabbits of LPS-treated mothers had detectable levels of either cytokines at 24 h, nor had any of the amniotic fluid samples from the nine saline-injected controls (Fig. 5). Amniotic fluid TNF and IL-1 levels corresponded with fetal serum levels, but not with maternal levels (Fig. 5).

Eight and 24 h after TNF stimulation of fetal rabbits, amniotic fluid and corresponding fetal serum TNF levels were as high as those measured after LPS stimulation, but little or no IL-1 and IL-6 was detected (Fig. 6a). Likewise, after IL-1 stimulation, moderately elevated amniotic fluid and corresponding fetal serum IL-1 were detected with near normal levels of TNF and IL-6 (Fig. 6b). In contrast, after IL-6 stimulation to fetal rabbits, elevated levels of all three cytokines were measured both in amniotic fluid and in corresponding fetal serum (Fig. 6c).

In neonatal serum, however, high levels of TNF and IL-1,

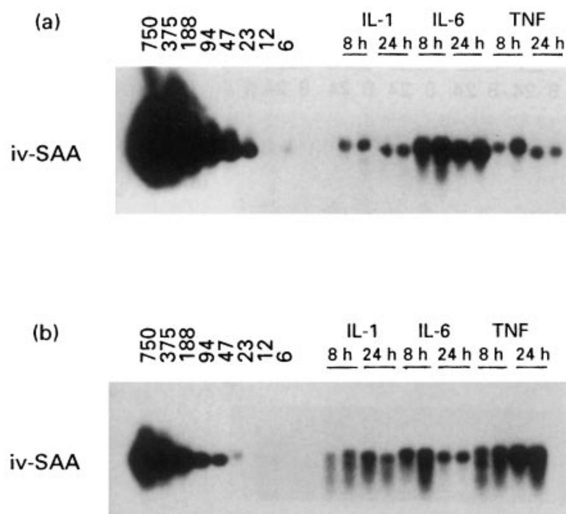


Fig. 2. Quantitative analyses of hepatic serum amyloid A (SAA) mRNA from fetal (a) or neonatal (b) rabbits 8 h and 24 h after stimulation with IL-1, IL-6 or tumour necrosis factor (TNF). Samples of liver RNA from two rabbits in each treatment group were run together with two-fold dilution series of iv-SAA RNA starting at 750×10^{-16} mol. After hybridization with a SAA RNA probe, the blots were exposed for 2 h (a) and 1 h (b).

but no IL-6, were detected 8 h and 24 h after administration of LPS, IL-1, IL-6 or TNF, but also in saline-injected controls (Fig. 7). The cytokine levels found in neonatal serum corresponded well with the levels measured in one adult control rabbit 1 day after a normal delivery (Fig. 7).

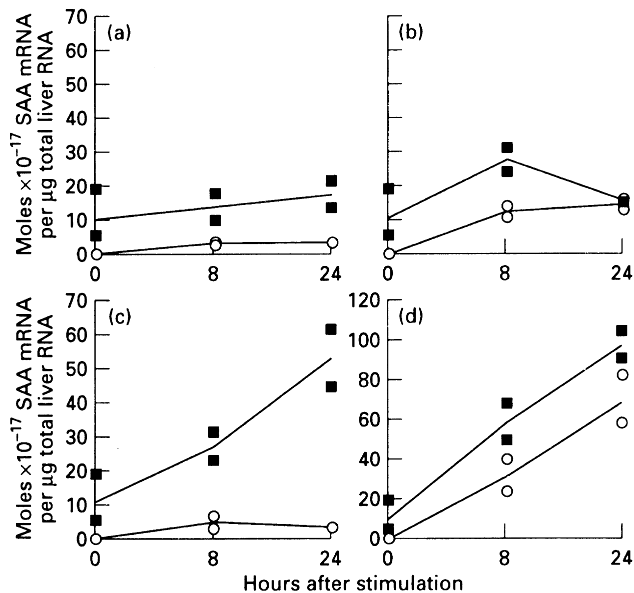


Fig. 3. Hepatic serum amyloid A (SAA) mRNA measurements after acute-phase stimulation to fetal, gestational day 25 (○) and 1-day-old neonatal rabbits (■). Values are given as the mean and range of scanning data from two rabbits in each experimental group after administration of IL-1 (a), IL-6 (b), tumour necrosis factor (TNF) (c) or lipopolysaccharide (LPS) (d).

DISCUSSION

Previous studies have demonstrated that administration of either IL-6, IL-1 or TNF will increase the levels of hepatic SAA mRNA in a dose-dependent manner in adult hamsters [17]. In fetal hamsters, however, no accumulation of SAA mRNA was observed after stimulation with either of these cytokines [18]. The current data, however, demonstrate an increase in liver SAA mRNA in fetal rabbits after injection with either of the three cytokines. These results in rabbits represent the first observation, that SAA genes may respond to cytokines during fetal development. It has previously been pointed out that in order to define the potency of a cytokine as an acute-phase inducer, it is important to consider the kinetics dose and route of administration [29]. In the study of fetal hamsters, the cytokines were administered transplacentally with the possibility of losing some of the agent to the maternal circulation, and the doses were lower than in our study [18]. This might explain the lack of SAA mRNA response in fetal hamsters.

Near maximal elevation of SAA mRNA levels occurred 8 h after injection with each of the three cytokines in fetal rabbits. Similar results, at least for IL-6 and TNF, have previously been shown in adult hamsters [17]. IL-6 seems to be the major inducer of SAA mRNA in fetal rabbits. In neonatal rabbits, however, the kinetic experiments show a strong and delayed response to TNF. This might reflect an additive or synergistic effect of endogenously produced IL-1 and/or TNF, which seemed to be elevated also in non-stimulated neonates after birth. This elevated cytokine level found in neonatal control sera might indicate that some of the physiologic events characteristic of birth might induce IL-1 and TNF in these animals. This would explain the high level of SAA mRNA found in neonatal control rabbits.

Cytokine-inducible C/EBP- and NF-κB-like transcription factors have been shown to interact with two major promoter regions upstream of the transcription start site of rabbit SAA gene [30]. An active NF-κB-like transcription factor is detectable in LPS-induced, but not in turpentine-induced liver extract, suggesting that there are at least two alternative pathways for SAA gene expression [31]. A recently published *in vitro* study of murine SAA3 has indicated that the synergy between NF-κB- and C/EBP-like transcription factors is essential for SAA3 gene expression during inflammation [32]. The study further demonstrated that IL-1, but not IL-6 alone, induced murine SAA3 gene expression [32]. We have previously shown that LPS induces expression of rabbit SAA1, SAA2 and SAA3 genes, while only SAA1 and SAA2 mRNA induction is seen after turpentine or casein stimulation [20]. Now we confirm the murine SAA3 *in vitro* study [32] by demonstrating that, similar to LPS, both IL-1 and TNF can stimulate the *in vivo* induction of all three SAA genes, while IL-6, like turpentine, does not induce SAA3 mRNA. The differential regulation of the three rabbit SAA genes might be explained by a cytokine-dependent induction of different transcription factors.

The biological assays used to determine cytokine concentrations in rabbit amniotic fluid and serum were standardized with human recombinant cytokines, a possible source of inaccuracy in the measurements. Biological activities induced by rabbit and human IL-1 are, however, dose-dependent and of comparable magnitude [33]. The specificity of each assay was demonstrated by neutralizing antibodies against human IL-1, IL-6

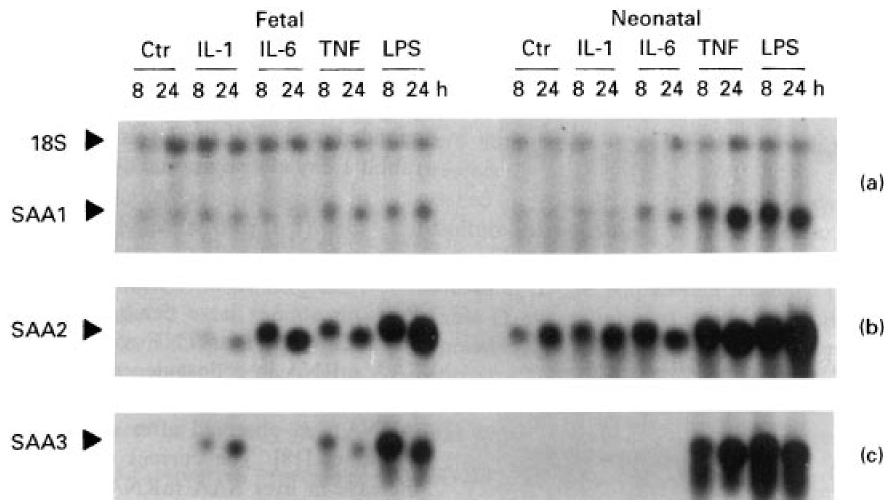
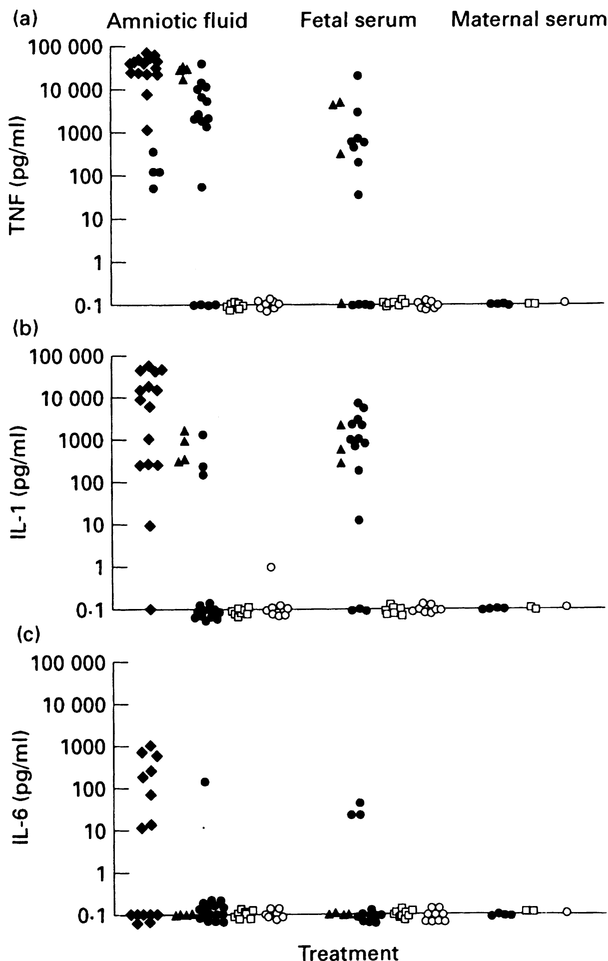


Fig. 4. Different serum amyloid A (SAA) mRNAs 8 h or 24 h after administration of lipopolysaccharide (LPS), IL-1, IL-6 or tumour necrosis factor (TNF) to fetal rabbits, gestational day 25, or 1-day-old neonatal rabbits. Control were given saline injections. Northern blots with liver RNA (20 μg/ml) from one representative rabbit in each treatment group were hybridized with three oligonucleotide probes specific for SAA1 (a), SAA2 (b) and SAA3 (c), respectively. (a) was exposed for 15 days, (b) for 15 h and (c) for 4 days.

and TNF. Determination of cross-reactivity to corresponding cytokines from rabbit has not previously been demonstrated for these antibodies. However, species crossreactivity of a recombinant human IL-1 receptor antagonist (IL-1Ra) has been demonstrated [33]. It must be emphasized that the IL-1 bioassay is not totally specific, as it provides a measure of the balance between IL-1α, IL-1β, IL-1Ra and soluble IL-1 receptors.



Several studies have shown that amniotic fluid concentrations of IL-1, IL-6 and TNF are elevated in the presence of infection [13,34–36]. In addition to uteroplacental macrophages, several other cell types, such as decidual cells and endometrial stromal cells, have been implicated as the source of such local cytokine production [13]. The data presented in this study demonstrate that fetal rabbits are protected against maternal LPS stimulation, but react to direct LPS stimulation with elevated serum levels of IL-1 and TNF. These signals are somehow conferred both to fetal hepatocytes, inducing high levels of SAA mRNA and to decidual or amniotic cells, inducing high levels of amniotic fluid IL-1 and TNF. IL-1, TNF and IL-6 are each capable of inducing their own production [12], which might explain the high levels found in both fetal serum and amniotic fluid 24 h after stimulation with each agent. We have, however, no explanation of why all three cytokines are induced after IL-6 stimulation, as IL-6 is not known to induce IL-1 or TNF [12]. Other studies have demonstrated that IL-6 is a sensitive parameter for diagnosing neonatal bacterial infection [37]. We could not confirm this in our study, as we have not done a full time course. The lack of IL-6 response might be due to the time of sampling.

Fig. 5. Biologically active cytokine levels, tumour necrosis factor (TNF) (a), IL-1 (b) and IL-6 (c), in rabbit amniotic fluid, fetal and maternal serum in response to lipopolysaccharide (LPS) or saline. Each point represents individual samples from fetal rabbits gestational day 19, 24 h after LPS stimulation (◆), fetal rabbits gestational day 25, 8 h (▲) and 24 h (●) after LPS stimulation, fetal rabbits gestational day 25, 24 h after maternal LPS stimulation (□), and saline-injected fetal rabbits gestational day 25 (○). Corresponding maternal cytokine serum levels are represented by similar points from 25 day pregnant rabbits subjected to laparotomy and fetal LPS injections (●), 25 day pregnant LPS-stimulated rabbits (□), and a 25 day pregnant saline-injected rabbit (○), all 24 h post-injection.

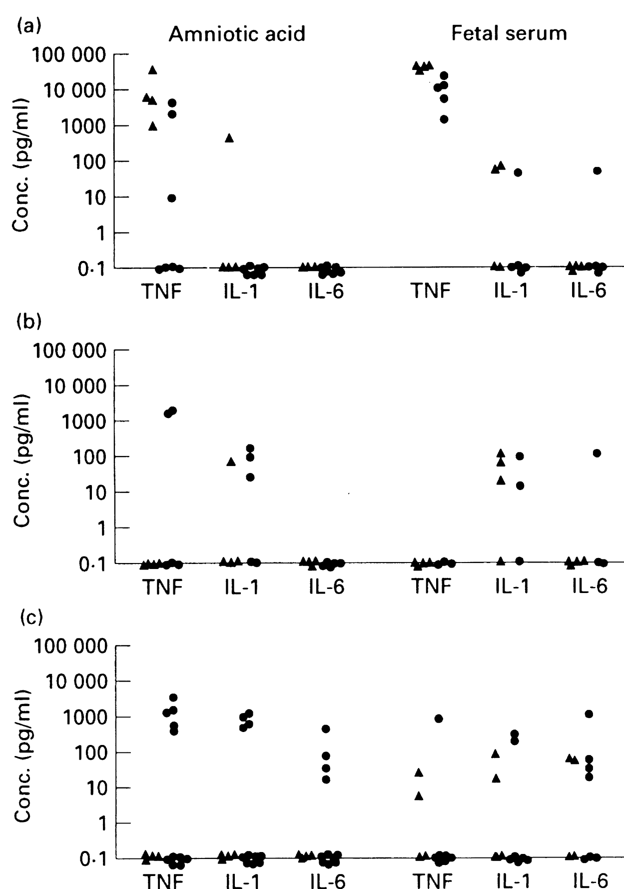


Fig. 6. Biologically active cytokine levels in rabbit amniotic fluid and corresponding fetal serum after direct fetal administration of tumour necrosis factor (TNF) (a), IL-1 (b) and IL-6 (c). Each point represents individual samples from fetal rabbits, gestational day 25, at 8 h (▲) or 24 h (●) post-injection.

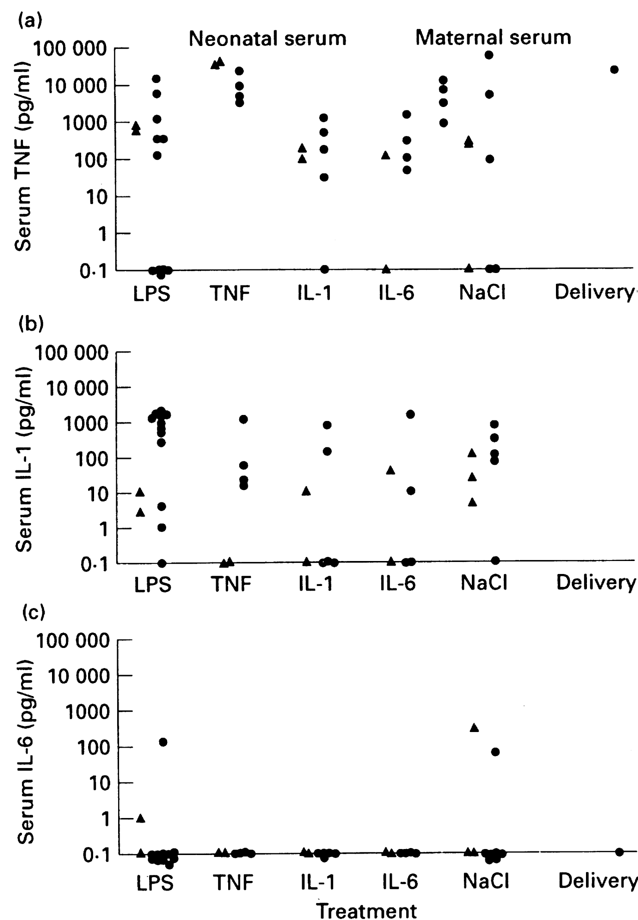


Fig. 7. Biologically active cytokine levels of serum tumour necrosis factor (TNF) (a), IL-1 (b) and IL-6 (c) from 1-day-old neonatal rabbits, after administration of lipopolysaccharide (LPS), TNF, IL-1, IL-6 or equal volume of sterile saline. Each point represents individual samples, at 8 h (▲) or 24 h (●) post-injection. Corresponding serum samples from one adult rabbit, 24 h after a normal delivery, are also included.

The sensitivity of measurements of the acute-phase protein C-reactive protein (CRP) has been disappointingly low in neonatal infections [11]. Any laboratory investigation which increased the sensitivity and specificity of this diagnosis would be welcome. Serum samples of SAA alone or in combination with CRP and/or cytokine analyses from amniotic fluid or cord blood could be of interest in the effort to improve diagnostic accuracy in neonatal infections.

This study has confirmed that the expression of SAA genes is induced by cytokines both in fetal and neonatal rabbits. It is further suggested that the expression of SAA genes may be developmentally regulated, and that some of the observations made might be explained by variations in the complex pattern of cytokine interactions observed during fetal development and parturition. It has also been demonstrated that IL-6 alone is unable to induce SAA3 gene expression in fetal and neonatal rabbits.

ACKNOWLEDGMENTS

This work was supported by the Research Council of Norway and the Inger Haldorsen's Grant. I am indepted to Gudmund Marhaug for valuable advice, to Gunnar Husby for his support, and to Ellinor Hareide for excellent technical assistance. I also wish to thank Baldur Sveinbjørnsson, Department of Experimental Pathology, Institute of Medical Biology, University of Tromsø, for the analyses of cytokines performed in his department.

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