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Regulation of rabbit acute phase protein biosynthesis by monokines

Andrzej MACKIEWICZ,* Mahrukh K. GANAPATHI, Debra SCHULTZ, David SAMOLS, James REESE and Irving KUSHNER†

Case Western Reserve University, Department of Medicine at Cleveland Metropolitan General Hospital, Cleveland, OH 44109 and Department of Biochemistry, Cleveland, OH 44106, U.S.A.

We defined the acute phase behaviour of a number of rabbit plasma proteins in studies (*in vivo*) and studied the effects of monokine preparations on their synthesis by rabbit primary hepatocyte cultures. Following turpentine injection, increased serum levels of C-reactive protein, serum amyloid A protein, haptoglobin, ceruloplasmin, and decreased concentrations of albumin were observed. In contrast to what is observed in man, concentrations of α_2-macroglobulin and transferrin were increased. Co-culture of primary hepatocyte cultures with lipopolysaccharide-activated human peripheral blood monocytes or incubation with conditioned medium prepared from lipopolysaccharide-activated human or rabbit monocytes resulted in dose-dependent induction of serum amyloid A, haptoglobin, ceruloplasmin and transferrin and depression of albumin synthesis, while C-reactive protein synthesis and mRNA levels remained unchanged. A variety of interleukin-1 preparations induced dose-dependent increases in the synthesis and secretion of serum amyloid A, haptoglobin, ceruloplasmin and transferrin and decreased albumin synthesis. Human recombinant tumour necrosis factor (cachectin) induced a dose-dependent increase in synthesis of haptoglobin and ceruloplasmin. In general, human interleukin-1 was more potent than mouse interleukin-1 and tumour necrosis factor. None of the monokines we studied had an effect on C-reactive protein synthesis or mRNA levels. These data confirm that C-reactive protein, serum amyloid A, haptoglobin and ceruloplasmin display acute phase behaviour in the rabbit, and demonstrate that, in contrast to their behaviour in man, α_2M and transferrin are positive acute phase proteins in this species. While both interleukin-1 and tumour necrosis factor regulate biosynthesis of a number of these acute phase proteins in rabbit primary hepatocyte cultures, neither of these monokines induced C-reactive protein synthesis. Comparison of these findings with those in human hepatoma cell lines, in which interleukin-1 does not induce serum amyloid A synthesis, suggests that the effect of interleukin-1 on serum amyloid A synthesis may be indirect.

INTRODUCTION

Among the broad range of systemic and metabolic changes which occur following tissue injury or infection are increases in hepatic synthesis of a number of proteins, the acute phase proteins (APP), and decreases in others, the negative APP [1]. There are substantial differences between species as to which proteins manifest acute phase behaviour. Even very closely related species such as rat and mouse vary markedly [1,2]. It is believed that the pattern of APP in man generally resembles the pattern observed in the rabbit. In both species C-reactive protein (CRP) and serum amyloid A (SAA) are major acute phase proteins [1,2]. α_2-Macroglobulin (α_2M), a major APP in the rat [2], does not show significant changes in man [2] and one study in the rabbit reports no acute phase behaviour for this protein [3]. Transferrin (Tf) is considered to be a negative APP in man [1,2], but we are not aware of reports concerning changes in its concentration in the rabbit following tissue injury.

It has recently been shown that activated monocytes produce several monokines, including interleukin-1 (IL-1), tumour necrosis factor/cachectin (TNF) and hepatocyte stimulating factor, which are capable of inducing different APP in rat [4–6] and mouse [4,7,8] primary hepatocyte cultures (PHC), established human hepatoma cell lines [4,9–11], and murine fibroblast L cells transfected with DNA bearing genes that encode certain human APP [10,12]. No studies of APP induction have been reported in rabbit hepatocytes.

In the present study we (i) defined the pattern of several APP in the serum of rabbits following induced inflammation *in vivo*, (ii) characterized changes in the patterns of APP synthesis in rabbit PHC following co-culture with human peripheral blood monocytes or exposure to conditioned medium prepared from activated

Abbreviations used: PHC, primary hepatocyte cultures; APP, acute phase proteins; CRP, C-reactive protein; SAA, serum amyloid A protein; Hp, haptoglobin; Cer, ceruloplasmin; α_2M, α_2-macroglobulin; Tf, transferrin; TNF, tumour necrosis factor/cachectin; hrTNF, human recombinant tumour necrosis factor/cachectin; PGE_1, prostaglandin E_1; IL-1, interleukin-1; SDS-PAGE, polyacrylamide gel electrophoresis; LPS, lipopolysaccharide; CM, conditioned medium.

* Permanent address: Department of Immunology and Rheumatology, Winogrady 144, 60-826 Poznan, Poland.

† To whom correspondence and reprint requests should be addressed at Cleveland Metropolitan General Hospital.

monocytes and (iii) evaluated the ability of well defined purified or recombinant monokines to alter synthesis of various rabbit APP in PHC.

MATERIALS AND METHODS

Antibodies to rabbit albumin, Tf and α_2M, all raised in the goat, and purified rabbit albumin and Tf were purchased from Cappel (Malvern, PA, U.S.A.). Goat anti-[human haptoglobin (Hp)] and ceruloplasmin (Cer), both of which cross-react with the corresponding rabbit proteins [13] and human standard serum-calibrator 1 were obtained from Atlantic Antibodies (Scarborough, ME, U.S.A.); sheep anti-(rabbit SAA) antibodies were a generous gift from Dr. J. B. Natvig (The National Hospital, Oslo, Norway). Rabbit anti-(sheep IgG) conjugated to alkaline phosphatase was from Southern Biotechnology Assoc. (Birmingham, AL., U.S.A.). Purified rabbit Apo-SAA was a generous gift from Dr. P. S. Tobias (Scripps Clinic and Research Foundation, LaJolla, CA, U.S.A.); RPMI-1640 with L-glutamine was from Mediatech-Fisher Scientific (Springfield, NJ, U.S.A.). William's Medium E, RPMI-1640 methionine free (prepared from RPMI-1640 Select Amino Kit) and fetal bovine serum were from Gibco (Grand Island, NY, U.S.A.).

Cell line-derived purified IL-1 (specific activity 10^4 U/mg) and human recombinant IL-1α (hrIL-1α) (specific activity 10^8 U/mg) were obtained from Genzyme (Boston, MA, U.S.A.); hrIL-1β (specific activity 60000 U/mg) was a generous gift from Dr. C. Dinarello (Tufts University Medical School, Boston, MA, U.S.A.); mouse recombinant IL-1 (mrIL-1) (specific activity 2×10^6 U/ml) was kindly provided by Hoffmann-LaRoche (Nutley, NJ, U.S.A.); human recombinant TNF (hrTNF), 1 mg/ml was a generous gift from Dr. B. Beutler, (University of Texas, Dallas, TX, U.S.A.); and prostaglandin E_1 (PGE_1) was from Upjohn, Kalamazoo, MI, U.S.A.

L-[^{35}S]Methionine (specific activity 1100 Ci/mmol) was from Dupont (Boston, MA, U.S.A.); lipopolysaccharide from *Escherichia coli* 055:B5 (LPS), collagenase from *Clostridium histolyticum* type I, glycine, Na_2EDTA, dexamethasone, Triton X-100 and sodium deoxycholate were purchased from Sigma (St. Louis, MO, U.S.A.); acrylamide, *NN*^{1}-methylene-bis-acrylamide, SDS, TEMED, 5-bromo-4-chloro-3-indolyl phosphate and Nitroblue Tetrazolium were from Bio-Rad (Richmond, CA, U.S.A.); nitrocellulose transfer membranes, 0.2 μm pore size were from Schleicher and Schuell (Keene, NH, U.S.A.); Sea-Kem LE agarose was from FMC Bio Products (Rockland, ME, U.S.A.); phosphocholine–agarose was from Pierce (Rockford, IL, U.S.A.); X-ray film, X-omat AR was purchased from Kodak (Rochester, NY, U.S.A.); Scinti Verse I was from Fisher Scientific (Springfield, NJ, U.S.A.). Plastic culture dishes (60 mm × 15 mm) were obtained from Becton-Dickinson (Lincoln Park, NJ, U.S.A.).

Induction of APP *in vivo*

New Zealand White male rabbits weighing 2–3 kg (obtained from Howard Gutman, Madison, OH, U.S.A.) were injected in the thigh intramuscularly with 0.5 ml or 1 ml of turpentine. Blood was collected from the marginal ear vein before injection and on days 1, 2 and 3 following injection. Serum was stored at −20 °C until analysis.

Levels of albumin, Hp, Cer, Tf and α_2M in rabbit sera were measured by electroimmunoassay [14] using serial dilutions of 'acute rabbit serum' obtained from rabbits 48 h after turpentine injection as standards. APP levels were expressed as percentages of protein concentrations before the first turpentine injection. Serum CRP levels were measured by radial immunodiffusion using goat anti-(rabbit CRP) serum and CRP standard [15]. The sensitivity of this method is 1.5 mg/l.

Western blot analysis [16] was employed for detection of SAA. Serum or culture medium was subjected to electrophoresis in 15% SDS/polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. This was incubated overnight with sheep anti-(rabbit SAA) antibodies (dilution 1:2000) and then for 2 h with rabbit anti-(sheep Ig) antibodies conjugated with alkaline phosphatase (1:500) and the colour developed using 5-bromo-4-chloro-3-indolyl phosphate and Nitroblue Tetrazolium as substrates. The intensity of the staining reaction was graded on a scale of 1–4+.

Isolation of human and rabbit monocytes and production of conditioned medium

Monocytes were isolated from whole heparinized blood obtained from healthy human donors and from uninflamed rabbits. Mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation and 6×10^6 in 5 ml of RPMI-1640 containing 20% foetal bovine serum and tobramycin were attached to culture dishes for 1.5 h at 37 °C in a humidified atmosphere (5% CO_2/95% air). Adherent cells were then washed three times with Hank's Balanced Salt Solution and incubated in 2 ml of serum-free RPMI-1640 containing LPS (20 μg/ml) under the same conditions. After 24 h the conditioned medium was harvested and stored at −20 °C until use. In initial experiments adherent monocytes (after a 1.5 h incubation period) were detached by treatment with 0.2% Na_2EDTA for 20 min at 4 °C and co-cultured with hepatocytes.

Induction of APP in PHC

Hepatocytes were isolated from non-inflamed rabbits by a modification of the collagenase perfusion technique developed by Laishes & Williams [17]. Isolated hepatocytes were washed three times in William's medium E and suspended in the same medium supplemented with 10% fetal bovine serum, dexamethasone (1 μM), insulin (0.02 U/ml), and tobramycin (50 μg/ml). The concentration of viable cells was determined by Trypan Blue exclusion and 3×10^6 viable cells were placed in culture dishes in 5 ml of medium. After a 1.5 h period of attachment at 37 °C in a humidified atmosphere (19:1, CO_2/air), the culture medium was replaced with 5 ml of serum free RPMI-1640 supplemented with dexamethasone (1 μM), insulin (0.02 U/ml) and tobramycin (50 μg/ml).

Hepatocytes were maintained in serum-free RPMI-1640 for 3 days. At this point medium was replaced with a mixture of 80% methionine-depleted RPMI and 20% CM or diluted monokines in experimental plates. Cultures were then incubated for 24 h at 37 °C in 5% CO_2/95% humidified air and 200 μCi of L-[^{35}S]-methionine was added 7 h before harvesting. In independent studies we found that incubation with this concentration of unlabelled methionine did not diminish total protein synthesis. Various doses of human and

Table 1. Maximal changes of APP in rabbit sera following intramuscular turpentine injection

		Proteins whose maximal changes were usually 48 h after injection				Proteins whose maximal changes were usually 72 h after injection		
	n	CRP (mg/l)	SAA†	Hp (%)*	α_2-M(%)*	Cer(%)*	Tf(%)*	Albumin (%)*
Pre-injection	6	1.5	–	100	100	100	100	100
0.5 ml turpentine	3	74±20	+++	350±180	162±28	280±60	146±29	77±1
1 ml turpentine	3	112±93	++++	766±280	295±7	210±10	161±2	41±3

* Concentration expressed as percentage of pre-injection value (mean±S.D.).
† Concentration expressed as intensity of stained SAA band: (−) negative, (+, ++, +++, ++++) degrees of positivity.

rabbit conditioned medium, cell line-derived purified IL-1, hrIL-1α, hrIL-1β, mrIL-1 or hrTNF were employed in experimental plates.

In two experiments 2.5×10^6 hepatocytes were co-cultured with human monocytes, ranging from 1×10^3 to 4×10^6 cells/dish in RPMI-1640 containing insulin and tobramycin, with and without LPS. In some experiments hepatocytes were incubated with PGE_1.

The accumulation of Hp, Cer and α_2M in culture medium was estimated by electroimmunoassay [14] followed by autoradiography, and accumulation of SAA was estimated by Western blot analysis [16]. For quantifying rabbit Hp and Cer, which we measured using antibodies directed against the human proteins, human standard serum was used, permitting expression of results in absolute units with reference to the original immunizing antigens.

Synthesis and secretion of CRP, albumin and Tf were measured by immunoprecipitation followed by fractionation on SDS-PAGE. Briefly, an aliquot of culture medium was incubated for 24 h with specific antibody in the presence of the corresponding purified carrier protein (10 μg/sample) and detergents (1% Triton X-100 and 1% sodium deoxycholate). Purified rabbit albumin and Tf were obtained commercially; CRP was purified from 'acute rabbit serum' by affinity chromatography on phosphocholine–agarose [18]. Precipitates were washed, solubilized and subjected to SDS-PAGE. Gels were stained, fluorographed in 1 M-sodium salicylate, 0.1% glycerol, dried and exposed to X-ray film for 72 h at −70 °C. To determine the amount of radioactivity present in the protein, the stained protein band was cut, rehydrated in water, solubilized in H_2O_2, and counted in a scintillation counter.

RNA was isolated from PHC by the guanidine isothiocyanate procedure of Chirgwin *et al.* [19]. Rabbit CRP mRNA accumulation was measured with a quantitative S1 nuclease protection assay described previously [20] using a probe which detects the 5′ end of the rabbit CRP transcript.

RESULTS

Studies *in vivo*

Increases in the concentration of serum CRP, Hp, α_2-M, Cer and Tf and decreases in concentration of albumin were observed in all six rabbits, whether injected with 0.5 ml (three rabbits) or 1 ml (three rabbits) of turpentine. The magnitude of changes observed for CRP, SAA, Hp, α_2M and Cer was greater with the higher dose of turpentine (Table 1). The time course of changes in

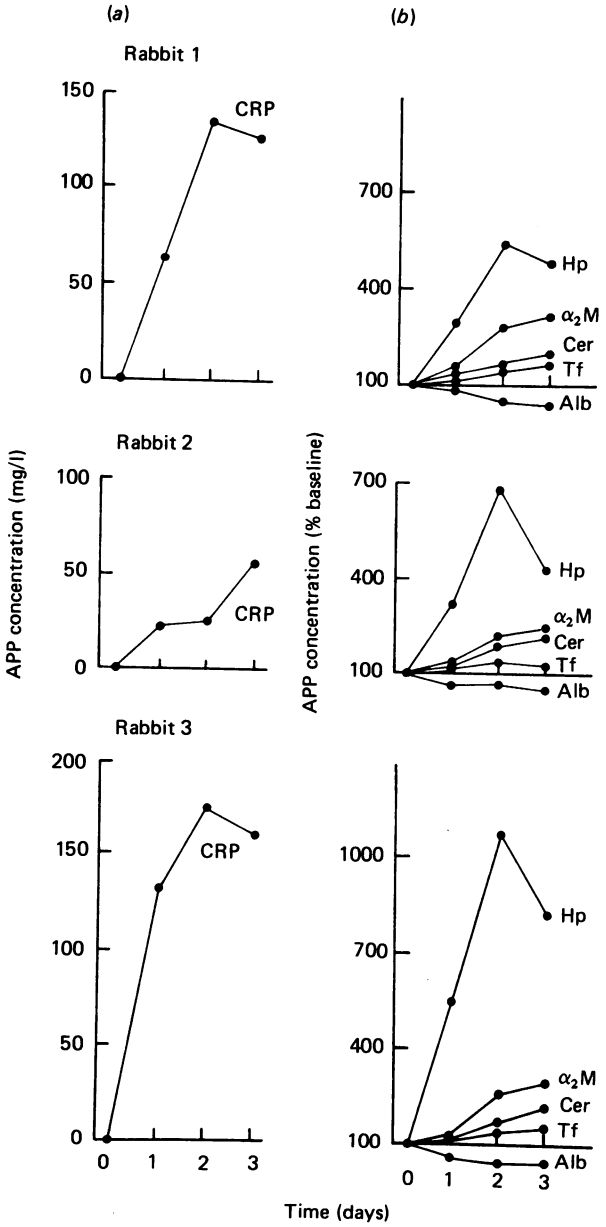

Fig. 1. Time course of changes in concentration of APP in serum of three rabbits following intramuscular injection of 1 ml turpentine

Abbreviations as described in the text. (*a*) CRP concentrations expressed in absolute units. (*b*) Concentrations of Hp, α_2M, Cer, Tf and albumin expressed as a percentage of pre-injection value.

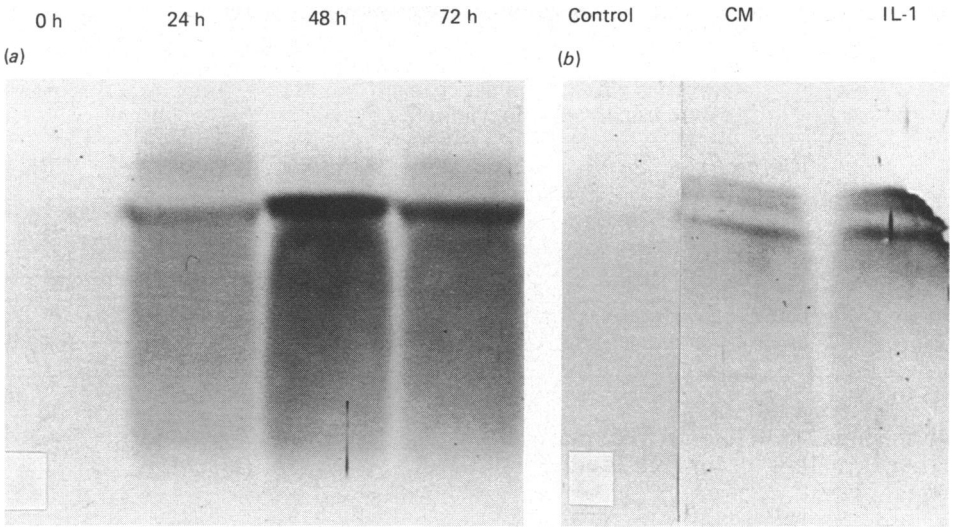

Fig. 2. Western blot analysis of SAA

(*a*) Rabbit serum (1 μl) collected before (0 h), and 24 h, 48 h and 72 h after intramuscular injection with 1 ml of turpentine. (*b*) 100 μl of culture media from PHC incubated in the absence (control) and presence of 20% conditioned medium or 4 U/ml of hrIL-1β.

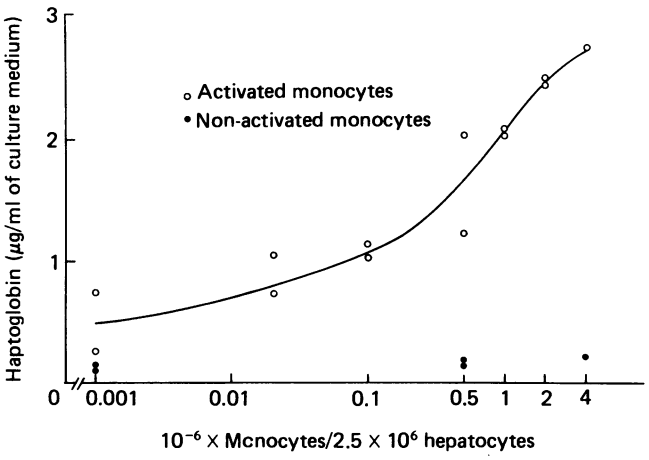

Fig. 3. Dose–response curve of haptoglobin accumulation in medium of PHC co-cultured with human peripheral blood monocytes

levels of all APP studied, except SAA, in a representative rabbit is illustrated in Fig. 1. SAA was undetectable in serum before injection, was detectable at 24 h, reached a peak at 48 h and had decreased by 72 h (Fig. 2). Serum levels of CRP, SAA and Hp increased rapidly, whereas a slower change in the level of the other APP was observed.

Studies *in vitro*

Co-culture of PHC with LPS-activated human monocytes induced a dose-dependent increase in accumulation of SAA, Hp and Cer and in synthesis of Tf and a decrease in synthesis of albumin. Fig. 3 shows increasing accumulation of Hp in culture medium during 24 h of co-culture with increasing numbers of activated human monocytes. In contrast, neither synthesis of CRP by PHC nor accumulation of CRP-specific mRNA was affected by co-culture with activated monocytes. α_2M was not detected in the medium of either control or experimental cultures. The addition of LPS alone to PHC had no effect on synthesis or accumulation of APP.

Exposure of PHC to human or rabbit CM led to the same changes in protein synthesis and secretion as did co-culture with activated monocytes. The magnitudes of changes were related to the amount of CM added to the medium (Table 2). Again, no significant increases in CRP synthesis were observed and no increase in mRNA levels of CRP was found.

Cell line-derived purified human IL-1 and human rIL-1α and β were tested at concentrations of 0.04, 0.4, 1, 2, and 4 U/ml (Table 3). All IL-1 preparations induced a dose-dependent increase in accumulation of SAA, Hp and Cer and synthesis of Tf and a dose-dependent reduction in albumin synthesis. As little as 0.04 U of IL-1/ml was able to induce significant synthesis of SAA, Hp and Cer, while a higher dose (2 U/ml) was required to induce Tf. Mouse recombinant IL-1 was studied in four different concentrations: 1, 10, 50 and 100 U/ml. Only the highest concentration caused induction of SAA, Hp and Cer and reduction of Alb, but synthesis of Tf was not affected. Human rTNF was tested at concentrations of 1, 2 and 4 μg/ml. Only the highest dose increased synthesis of Hp and Cer; this dose did not influence synthesis of SAA, Tf and Alb. Accumulation of newly synthetized CRP and its mRNA was not increased by any IL-1 preparation or by TNF. In addition, similar results were obtained when rabbit PHC were incubated with medium from COLO-16, known to contain hepatocyte-stimulating factor activity (kindly given by Dr. H. Baumann, Buffalo, NY, U.S.A.): increase in synthesis of SAA, Hp, Cp and Tf, decrease in albumin, and no change in CRP. α_2M was not detected in any experiment. PGE_1 did not induce any of the APP studied and did not decrease the synthesis of albumin at doses of 0.01, 0.1 and 1 μg/ml.

Table 2. Induction *in vitro* of APP in rabbit PHC by co-culture with human monocytes or by incubation with human or rabbit conditioned media

			APP accumulation over 24 h vs. control cultures			APP synthesis over 7 h vs. control cultures		
		n	SAA*	Hp†	Cer†	Tf†	CRP†	Albumin†
Control		10	−	1	1	1	1	1
Monocyte co-culture (0.5×10^6 cells)		2	+++	15±5	6±2	2±0	0.9±0.1	0.70±0.04
Human conditioned medium	5%	2	+	5±2	3±1	1.1±0.2	1.1±0.1	0.85±0.02
	10%	2	++	13±2	5±1	1.8±0.3	1.2±0.3	0.80±0.03
	20%	10	+++	20±6	8±4	2.1±0.5	1.3±0.3	0.71±0.06
Rabbit conditioned medium	5%	2	+	6±1	3±1	1.2±0.2	1.0±0.1	0.74±0.06
	10%	10	++	14±4	6±3	1.9±0.6	0.9±0.2	0.62±0.07
	20%	10	+++	21±7	10±2	2.9±0.3	0.9±0.1	0.36±0.06

* Accumulation expressed as intensity of stained SAA band: (−) negative; (+, ++, +++) degrees of positivity.
† Ratio of accumulation or synthesis in experimental cultures to that in control cultures (mean±S.D.).

Table 3. Induction of APP in rabbit PHC by IL-1 and TNF

	Concentration (U/ml)		APP accumulation over 24 h vs. control cultures			APP synthesis over 7 h vs. control cultures		
			SAA*	Hp†	Cer†	Tf†	CRP†	Albumin†
Control		14	−	1	1	1	1	1
hIL-1	4	2	+++	20±3	8±2	2.5±0.2	1.1±0.2	0.68±0.30
	2	4	+++	19±2	8±1	2.0±0.0	1.2±0.3	0.74±0.04
	1	2	++	11±4	6±2	1.1±0.1	0.9±0.0	0.82±0.02
	0.4	2	+	9±3	4±1	0.9±0.1	1.0±0.1	0.91±0.01
	0.04	2	+	5±3	3±1	1.0±0.2	1.1±0.2	0.98±0.05
hrIL-1α	2	4	+++	22±4	9±2	2.3±0.2	1.0±0.3	0.84±0.05
	0.04	2	+	4±2	3±0.5	1.1±0.2	0.9±0.2	1.02±0.06
hrIL-1β	2	14	+++	18±5	10±4	2.2±0.5	1.1±0.3	0.75±0.04
	0.04	4	+	3±0.5	2±0.4	0.9±0.1	1.0±0.2	0.99±0.03
mrIL-1	100	4	++	5±1	3±0.2	1.1±0.1	1.1±0.1	0.75±0.03
	50	4	−	1±0.1	1.1±0.1	1.0±6.1	1.1±0.1	1.03±0.04
hrTNF	4	4	±	4±1	2±0.5	0.9±0.2	1.1±0.2	0.99±0.02

* As in Table 2.
† As in Table 2.

DISCUSSION

Our major findings further illuminate several levels of heterogeneity of the acute phase response: the variability between species in the acute phase behaviour of different plasma proteins, the recognition that multiple cytokines are capable of altering expression of APP genes, and perhaps most intriguing, the recognition that different model systems may respond differently to the same cytokines.

While it has been recognized that CRP, SAA, Hp and Cer are positive APP and albumin is a negative APP in the rabbit, the behaviour of α_2M and Tf during the acute phase response has not been clearly established in this species. Our data *in vivo* demonstrate that, in contrast to man, both α_2M and Tf are positive APP in the rabbit. Transferrin is generally considered to be a negative APP in other species [2], although conflicting data about the acute phase behaviour of rat Tf mRNA have recently appeared [21,22]. Transferrin levels rose only moderately, while the magnitude of increase in α_2M in the serum was greater than the increase observed for Cer, but less than that for Hp. Serum levels of α_2M and Tf did not start to increase until 24 h after turpentine injection and kept increasing until 72 h, while CRP, SAA and Hp levels started to increase more rapidly and reached a maximum by 48 hours. Our studies *in vitro* confirmed the positive acute phase behaviour of Tf.

These studies *in vitro* also indicated that changes in synthesis of several rabbit APP can be mediated by at least two monokines. Co-culture with monocytes and incubation with either human or rabbit CM or with IL-1 preparations all led to increased accumulation of SAA,

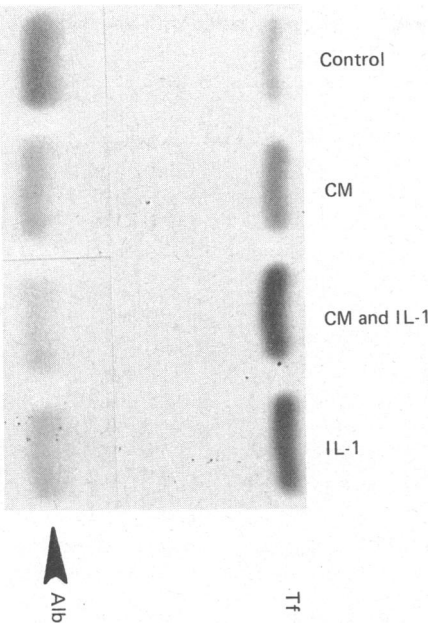

Fig. 4. SDS-polyacrylamide gel electrophoresis autoradiograms of transferrin (Tf) and albumin (Alb) synthesized by PHC

Control, PHC incubated with culture medium alone; CM, PHC incubated with 20% conditioned medium; CM and IL-1, PHC incubated with 20% CM and 4 U of human purified IL-1/ml; IL-1, PHC incubated with human purified IL-1 (4 U/ml).

Hp, Cer and Tf and decreased synthesis of albumin. Human IL-1, purified or recombinant, was much more potent than mouse rIL-1. A distinct monokine, hrTNF, was also able to induce production of Hp and Cer in PHC, confirming recent reports of the APP-inducing behaviour of this monokine in human hepatoma cell lines [9,10] and murine fibroblasts transfected with cosmid DNA bearing APP genes [10]. The doses of TNF required to induce APP seemed to be much higher than doses of IL-1, based on other biological activities. Significant changes in synthesis of albumin and Tf were not observed with TNF at the doses employed.

Differences in the responsiveness of CRP and SAA to monokines were found when we compared two model systems, the rabbit PHC here reported and the human Hep 3B cell line evaluated in our laboratory in parallel studies [23]. SAA was found to be induced by IL-1 preparations in rabbit PHC, which is consistent with reported studies of SAA-induction in mouse PHC and in mouse fibroblasts transfected with the human SAA gene [7]. However, in our studies in human Hep 3B cells [23], IL-1 did not have this effect, while CM did cause SAA induction. Perlmutter *et al.* have similarly reported failure of IL-1 to alter biosynthesis of SAA in human hepatoma cell lines [24]. These differences may be related to differences between species or between cell lines and primary cultures. However, an attractive explanation is that IL-1 may not itself be the SAA-inducing cytokine, but rather that it stimulates the contaminating non-parenchymal cells which are commonly present in PHC, or the fibroblasts in transfection studies, to produce an authentic SAA-inducing cytokine.

In the present studies we failed to induce CRP in rabbit PHC by CM or any purified or recombinant monokine, as judged by secretion of newly synthesized protein and by mRNA levels. In contrast, Darlington *et al.* [9] and Goldman & Liu [11] have recently reported induction of CRP in human hepatoma cell lines by monocyte CM, but not by IL-1 or TNF, and we have confirmed this finding [23]. There are several possible explanations for this discrepancy between the responsiveness of CRP to induction in human hepatoma cell lines and in rabbit primary hepatocyte cultures. It is possible that CRP induction in the rabbit is not mediated by a product of human or rabbit peripheral blood monocytes, or that activation of monocytes under different conditions might produce monokines capable of inducing CRP in rabbit PHC. The putative CRP-inducing factor in the rabbit may be a product of tissue macrophages, such as Kupffer cells or other cell types, rather than of peripheral blood monocytes. Alternatively, factors may arise in the hepatic non-parenchymal cells contaminating PHC which are capable of inhibiting CRP induction. Other possible explanations are that additional co-factors might be required for rabbit CRP induction or that rabbit hepatocytes cultured *in vitro* do not express receptors for the CRP-inducing cytokines, while they do express receptors for inducers of other APP.

Finally, our data have bearing on the role of prostaglandins in APP induction. Reports showing increased levels of Hp in rabbits [13,25] and CRP in man [26] following intravenous injection of PGE_1 and PGE_2 have raised the question of whether these PGEs act directly or indirectly upon hepatocytes. We have previously presented evidence that administration of prostaglandins does not influence CRP synthesis by rabbit hepatocytes [27]. In the current studies, prostaglandin E_1 had no effect on production of other APP by hepatocytes *in vitro*, further supporting the view that PGE_1 does not induce the synthesis of APP directly.

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