

PRETRANSLATIONAL MODULATION OF ACUTE PHASE
HEPATIC PROTEIN SYNTHESIS BY MURINE RECOMBINANT
INTERLEUKIN 1 (IL-1) AND PURIFIED HUMAN IL-1

By G. RAMADORI,* J. D. SIPE,[‡] C. A. DINARELLO,[§] S. B. MIZEL,^{||} and
H. R. COLTEN*

*From the *Division of Cell Biology, Children's Hospital, the Ina Sue Perlmutter Cystic Fibrosis Research Center, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; the [‡]Arthritis Center, Boston University School of Medicine, Boston, Massachusetts 02118; the [§]Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111; and the ^{||}Microbiology Program, Pennsylvania State University, University Park, Pennsylvania 16802*

The acute phase response is a systemic reaction to inflammation or tissue injury. It is characterized by complex changes that include fever, leukocytosis, increased muscle proteolysis, altered carbohydrate and trace metal metabolism, and a pronounced change in hepatic protein synthesis (1). Within several hours of an acute phase stimulus, the plasma concentrations of C-reactive protein and serum amyloid A (SAA)¹ are increased more than 100-fold. The concentrations of numerous serum glycoproteins are increased up to threefold (e.g. fibrinogen, haptoglobin, α_1 -glycoprotein, α_1 -antitrypsin, and several complement proteins), and the concentrations of a small number of proteins are decreased (albumin, prealbumin, transferrin, α_2 -HS-glycoprotein) (2). The mechanism by which injury at a distant site can effect changes in hepatic protein synthesis has been studied during the last fifty years. These studies have suggested that a circulating mediator, interleukin 1 (IL-1), derived from mononuclear phagocytes, elicits changes in the serum protein pattern similar to those observed during an acute phase response (3-4). However, none of these studies established that IL-1 solely and directly effects these changes in hepatocyte protein synthesis. That is, the role of IL-1 was inferred from the effect of in vivo administration of preparations of IL-1 purified to varying degrees, and from studies of primary hepatocyte cultures in which baseline (unstimulated) synthesis of acute phase proteins was rather high (5) or the IL-1 preparation was incompletely characterized.

To directly address these issues and thus to permit investigation of the mechanisms modulating hepatic protein synthesis by IL-1, we took advantage of two recent advances: (a) The cloning of mouse IL-1 cDNA into an expression vector, thus making available large quantities of homogeneous preparations of

This work was supported by grants AI15614, AM20613, AI20032, HD17461, AI21157, and NSF PCM 84-10202 from the U. S. Public Health Service.

¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hank's balanced salt solution; IL-1, interleukin 1; MEM, minimum essential medium; SAA, serum amyloid A; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloroacetic acid.

IL-1 synthesized as the product of a single gene (6) and (b) the isolation of cDNA specific for acute phase proteins as probes for assessing acute phase gene expression (7-9).

Accordingly, the effect of IL-1 on synthesis of quantitatively major (SAA) and minor (factor B) positive acute phase reactants, and on albumin, a negative acute phase reactant, was examined *in vivo* and in primary mouse hepatocyte cultures. A dose- and time-dependent increase in SAA and factor B synthesis, and a decrease in albumin synthesis was induced by recombinant-generated murine and highly purified human IL-1 in primary mouse hepatocyte cultures. Regulation of these changes was pretranslational, since changes in mRNA specific for SAA, factor B, and albumin were affected by both IL-1 preparations. A similar pretranslational regulation of positive and negative acute phase protein synthesis was demonstrated in mice injected with recombinant-generated IL-1.

Materials and Methods

Mice. Female and male C3H/HeJ and C3HeB/FeJ mice aged 6-12 wk were obtained from the Jackson Laboratories, Bar Harbor, ME. No significant sex differences have been observed in the acute phase (SAA) response of C3H mice, although it is known that a higher baseline SAA concentration has been observed in untreated male mice (10).

Primary Hepatocyte Cultures. Mouse hepatocytes were isolated according to a previously described method (11-13). Briefly, the liver was perfused *in situ* with collagenase (Boehringer Mannheim Diagnostics, Inc., Houston, TX) through the portal vein. Hepatocytes were separated from nonparenchymal cells by differential centrifugation, and the resulting contamination with Kupffer cells was always <1% (14). The viability (as assessed by trypan blue exclusion) was 85-95%. Yields were $1.5-2.5 \times 10^7$ cells/liver. The cells were plated either on collagen (Sigma Chemical Co., St. Louis, MO)-coated 24-well Linbro plates (Flow Laboratories, Inc., McLean, VA) at 10^5 cells/well, in 0.5 ml culture medium, or on collagen-coated six-well Falcon plates (Becton Dickinson & Co., Oxnard, CA) at 10^6 cells/well in 1.5 ml Dulbecco's modified Eagle's medium (DMEM), containing 25 mM Hepes (M. A. Bioproducts, Walkersville, MD), 4.5 g/liter glucose, 1 μ g/ml insulin, 0.2% fatty acid-free bovine serum albumin (BSA) (Boehringer Mannheim Diagnostics, Inc.). After the first 2 h of culture, the medium, containing 10% heat-inactivated fetal calf serum, was changed, to remove nonadherent cells. The cells were kept in culture at 37°C under a 5% CO₂, 95% air atmosphere for up to 12 d, with change of medium every day.

Mouse IL-1. Recombinant mouse IL-1 (pILI, 1-156; kindly provided by Dr. W. Benjamin, Hoffman-La Roche, Nutley NJ) with 6×10^6 U/mg sp act (6) and a pI of ~5, was stored in 5 M guanidine hydrochloride at -20°C.

Human IL-1. Human plateletpheresis byproducts were used as a source of mononuclear cells, which were adjusted to a concentration of 10^7 cells/ml in Eagle's minimum essential medium (MEM) without methionine. Cells were incubated at 37°C for 1.5 h in medium containing 1% dialyzed human AB serum, and the nonadherent cells were removed by vigorous shaking. The adherent cell population was incubated in MEM, and stimulated with opsonized heat-killed *Staphylococcus albus*. After 36 h, the supernatant was removed, centrifuged at 3,000 g, filtered, and applied to an immunoadsorbent. The preparation of the immunoadsorbent, and the purification procedures have been described previously (15, 16). Gel filtration was over Sephadex G-50 (fine), 170 \times 5 cm, which had been autoclaved in pyrogen-free water. The chromatofocusing gel was purchased from Pharmacia Fine Chemicals (Piscataway, NJ), and washed extensively in endotoxin-free imidazole buffer. 200 rabbit pyrogen doses were bound to the immunoadsorbent at 37°C, and the IL-1 was eluted with two column volumes of 0.1 M citric acid buffer, pH 2.5, containing 100 μ g/ml BSA. The eluted material was collected in 10% lysine (final concentration 1%) and immediately neutralized with NaOH. IL-1 assay of both the starting and eluted material revealed a recovery of ~75%. The IL-1 was

concentrated to 1/20 volume in autoclaved dialysis tubing (cutoff, 3.5 kilodaltons [kD]) against polyethylene glycol (8–10 kD), and applied to Sephadex G-50 in 0.15 M NaCl containing 100 $\mu\text{g}/\text{ml}$ BSA. Each fraction was assayed for IL-1, and the peak of IL-1 activity, eluting at 15–20 kD, was pooled, concentrated, dialyzed against 0.1 M imidazole buffer, pH 7.8. The dialyzed IL-1 peak was then applied to a chromatofocusing gel, and washed with two column volumes of imidazole buffer followed by polybuffer 94 (Pharmacia Fine Chemicals), to generate a pH gradient between 7.6 and 4.5. Each fraction was assayed for IL-1 (17), and the neutral pI peak (Fig. 1) was isolated (sp act $\sim 6 \times 10^6$ U/mg protein) and used.

Before addition to the hepatocyte cultures, both human and mouse IL-1 preparations were adjusted to the appropriate concentration by dilution in culture medium containing 2 mg/ml BSA, followed by filtration through a 0.45 μm Millipore filter. Control solution for human IL-1 contained the appropriate dilution of 1% BSA in saline. Control solutions for mouse IL-1 contained guanidine hydrochloride or nontransformed bacterial extract in the same concentration as that of the highest IL-1 concentration.

Antisera. Rabbit antiserum to mouse albumin was purchased from Miles Laboratories, Inc. (Elkhart, IN). IgG containing anti-human factor B that crossreacts with mouse factor B was purchased from Atlantic Antibodies (Scarborough, ME). SAA was precipitated with rabbit antibodies raised against mouse AA (amyloid A) protein, according to the method of Linke et al. (18). For immunoprecipitation, the antiserum was adsorbed with normal mouse serum in which no SAA was detectable by radioimmunoassay. For radioimmunoassay, the anti-AA antibodies were concentrated and purified on an affinity column (18).

Immunoprecipitation of Radiolabeled Proteins. Radiolabeling, immunoprecipitation, and sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were performed as previously described (19). Briefly, hepatocyte monolayers (Linbro plates) were pulsed for a suitable interval with methionine-free DMEM containing 800 $\mu\text{Ci}/\text{ml}$ [^{35}S]methionine (sp act 1,000 $\mu\text{Ci}/\text{mmol}$) (New England Nuclear, Boston, MA). Total protein synthesis was measured by trichloroacetic acid (TCA) precipitation of 1 μl of cellular lysates, and of supernatants (20) obtained as follows. The cells were washed on ice with cold Hank's balanced salt solution (HBSS), and lysed by freeze-thawing with a solution of phosphate-buffered saline, pH 7.5, containing 100 mM KCl, 1% Triton X 100 (Sigma Chemical Co.), 0.5% sodium deoxycholate (Sigma Chemical Co.), 2 mM phenylmethylsulphonyl fluoride (Sigma Chemical Co.), 10 mM EDTA, and 100 $\mu\text{g}/\text{ml}$ Leupeptin (Protein Research Foundation, Osaka, Japan). The culture supernatants and lysates were centri-

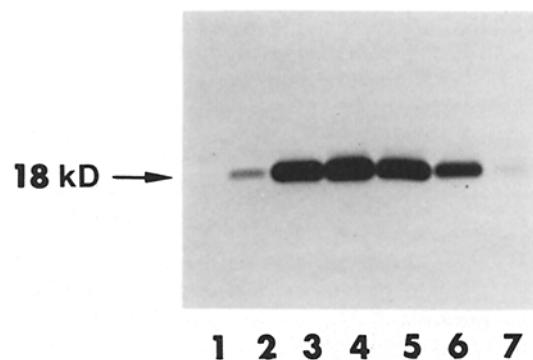


FIGURE 1. SDS-PAGE autoradiograph of purified human IL-1. For analytical purposes, an aliquot of the monokine was labelled with [^{35}S]methionine, and carried through the purification procedure. Fractions from the final chromatofocusing separation were analyzed by SDS-PAGE (17.5% acrylamide, 1 mM 2-mercaptoethanol, 10 mM Tris, pH 7.2) and fluorography. The biological activity of a 1:200 dilution of each fraction was measured by the thymocyte assay. Fractions 1–7 correspond to pH 7.5, 7.4, 7.19, 6.99, 6.65, 6.3, 6.03, and peak thymocyte stimulation was found in fraction 5. We used a pool of fractions 3–6.

fuged in a Eppendorf minifuge for 30 min at 4°C, diluted with the lysis buffer containing 1% SDS, and stored at -90°C. The balance of the procedure was carried out exactly as described by Auerbach et al. (19).

Isolation of RNA. Hepatocyte monolayers in six-well Falcon plates were washed three times with HBSS, then lysed with 3 ml of 4 M guanidinium thiocyanate (Fluka A. G. Buchs, Basel, Switzerland), 25 mM sodium citrate, and 0.5% sodium-*N*-laurylsarcosinate (K and L Laboratories, Inc., Plainview, NY) (22). The cells were detached from the dish with a rubber policeman, and the DNA was sheared by repeated passage through a 23-gauge needle. The guanidinium lysate was then layered onto 2 ml of 5.7 M CsCl in a Beckman SW 50.1 rotor (Beckman Instruments, Inc., Fullerton, CA) for 17 h at 35,000 rpm at 20°C; the RNA pellet was washed twice in ethanol, then dissolved in water and reprecipitated in ethanol (15 min in dry ice/ethanol bath, or at -20°C overnight), and dissolved in water. RNA content was determined by measurement of absorption at 260 nm. Yields were in the range of 35-40 µg RNA/10⁶ cells.

RNA was extracted from whole liver, either by phenol/chloroform/isoamyl alcohol extraction (23) after homogenization of tissue, or from liver frozen in liquid nitrogen and then extracted with 4 M guanidinium thiocyanate (1:10 vol/vol), followed by ultracentrifugation as described above (22).

cDNA Clones and Northern Blot Analysis. The factor B cDNA clone pBmB2 employed in this study has been described elsewhere (8). It is 1.5 kilobases (kb) long, and spans 94% of the protein sequence for the murine Bb fragment. The SAA-specific cDNA (9) clone pAla, which contains the coding sequence for amino acid residues -10-55 of human apoSAAL- α was subcloned into λ phage M13 mp 11. The nucleotide sequence of the cDNA coding for amino residues 33-45 is identical to that coding for the corresponding constant region peptide of mouse SAA (9, 24). Mouse albumin cDNA (7) was a gift of Dr. S. Tilghman (Cancer Research Institute, Philadelphia, PA).

Chicken α -actin cDNA, which also hybridizes with β - and γ -actin (25), was kindly provided by R. J. Schwartz (Baylor College of Medicine, Houston, TX). Whereas factor B-, albumin-, and actin-specific cDNA were ³²P labelled by nick translation, radiolabeled SAA-specific single-stranded cDNA was prepared by primed synthesis with [³²P]dNTP (New England Nuclear), and by extension using the Klenow DNA polymerase large fragment (Boehringer Mannheim Diagnostics, Inc.) after hybridization of the M13 template with the universal primer (New England Biolabs, Boston, MA). The free nucleotides were removed by two spin dialyses. Detection of specific mRNA by Northern blot hybridization was accomplished using reagents and methods exactly as previously described (26).

SAA Measurements. SAA concentration was measured in serum obtained from the orbital plexus, an aliquot of which was heated at 60°C for 1 h. Several dilutions were analyzed in triplicate by the competitive binding radioimmunoassay, as described previously (27).

Results

Recombinant IL-1 Regulates SAA Gene Expression in Vivo. Recombinant-generated purified murine IL-1 was injected intravenously into C3H/HeJ mice at doses of 500 and 1,000 U/mouse. SAA gene expression was monitored by Northern blot analysis of liver mRNA and SAA protein concentration was estimated in sera obtained from individual mice at timed intervals. The concentration of hepatic SAA-specific mRNA increased within 2 h after IL-1 injection, peaked at 4 h postinjection, and remained elevated above baseline after 12 h (Fig. 2A). Similar kinetics were observed for both IL-1 doses, but the magnitude of the effect was dose dependent. A dose-dependent increase in serum concentration of the SAA protein after IL-1 injection was first detectable at 4 h (the peak response), after which concentrations declined to 15 µg/ml at 12 h (Fig. 2B).

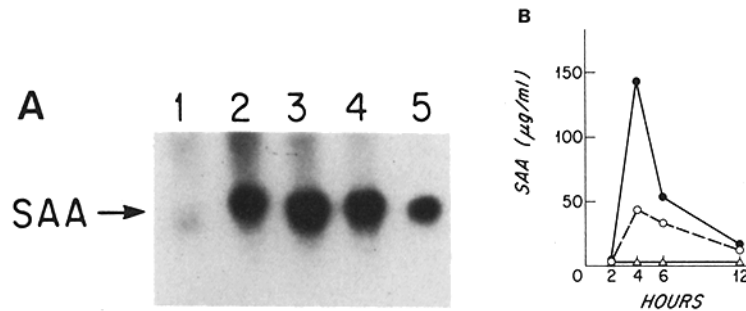


FIGURE 2. A, Northern blot analysis of SAA mRNA in C3H/HeJ mice after injection of 500 U recombinant IL-1. Each lane contains 15 μ g of RNA extracted with guanidinium isothiocyanate. 1, RNA extracted 4 h after intravenous injection of nontransformed bacterial extract. 2-5, RNA extracted 2, 4, 6, and 12 h after injection of mouse recombinant IL-1. Hybridization with 32 P-radiolabeled SAA cDNA was performed as described in Material and Methods. B, serum concentration of SAA after intravenous injection of recombinant mouse IL-1. SAA levels were measured in serum samples taken at different intervals after intravenous injection of 1,000 U IL-1 (●), U IL-1 (○), or nontransformed bacterial extract (△) into C3H/HeJ mice.

Controls included animals injected intravenously with an extract of nontransformed bacteria dissolved in 5 M guanidine hydrochloride (bacterial extract) or with the guanidine solution alone, each at an appropriate dilution in 250 μ l of pyrogen-free saline. No SAA protein was detected in sera of nine control mice, and only a small amount of specific RNA was detected in livers of two of five control animals (Fig. 2, A1).

Protein Biosynthesis in Hepatocytes as a Function of Time in Culture. Hepatocyte monolayers in replicate dishes were examined at timed intervals, from 1.5 h to 10 d in culture, for specific mRNA content and protein synthesis. Total protein synthesis (TCA-precipitable radioactivity) decreased from 250,000 cpm in 5 μ l on day 1, to 70% of the initial value by day 5, then gradually increased again up to 220,000 cpm in 5 μ l by day 8, and declined again thereafter. Normal liver, and unstimulated hepatocytes at 1.5 h in culture generally did not contain detectable SAA mRNA, but a spontaneous increase in SAA mRNA and SAA synthesis was noted in cells at day 1 and 2, regardless of whether liver from a lipopolysaccharide responder (C3HeB/FeJ) or nonresponder (C3H/HeJ) strain was used (Fig. 3A). After day 2 in culture, baseline synthesis of SAA protein and SAA mRNA were low or undetectable. Factor B mRNA was also relatively abundant on days 1 and 2, decreased, then increased to a second peak on day 8 in culture. At all time points tested between day 3 and 8, mouse recombinant IL-1 or human IL-1 induced significant increases in SAA-specific mRNA (Fig. 3B) and protein synthesis. A similar but less pronounced effect of IL-1 on the induction of factor B-specific mRNA and protein synthesis was noted. In the cultures fed with medium alone, albumin synthesis was maintained for at least 10 d in culture. Because of the changes in baseline gene expression with time in culture, the maximal IL-1-mediated effect on SAA factor B and albumin gene expression was observed after day 3, therefore all subsequent studies were performed using cells from day 4-9 in culture.

Kinetics of IL-1 Action on Hepatocytes in Tissue Culture. The amount of time required for IL-1 to effect changes in SAA, factor B, and albumin gene expres-

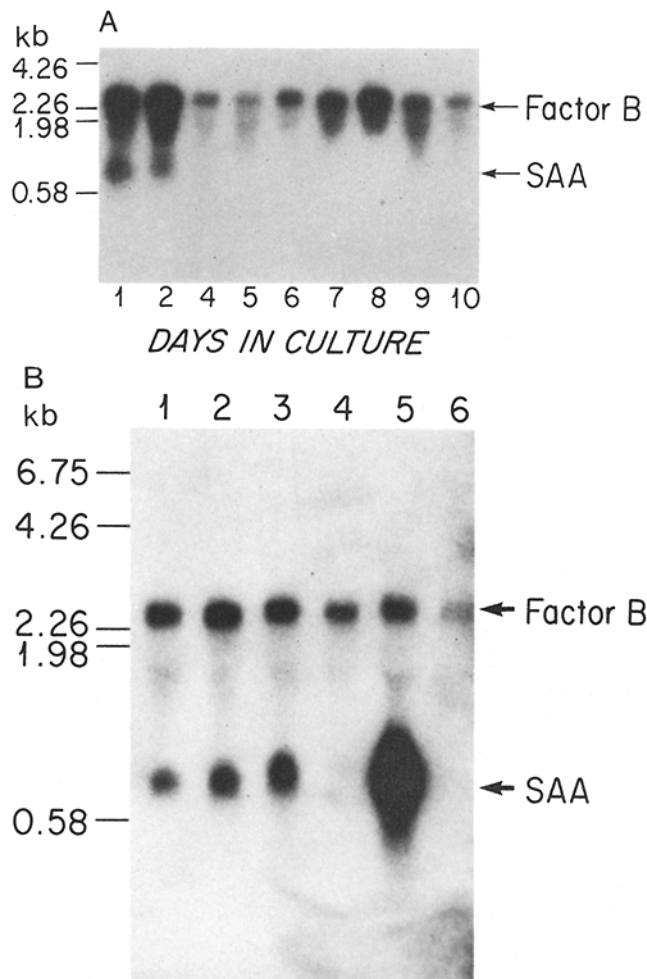


FIGURE 3. *A*, Northern blot analysis of RNA samples extracted from C3HeB/FeJ mouse hepatocytes cultured for a 10-d period. Aliquots of liver RNA from each day were electrophoresed, transferred, and probed with ^{32}P -labeled SAA and factor B cDNA. *B*, Northern blot analysis of RNA extracted from hepatocytes isolated from a C3H/HeJ mouse and kept in culture for varying times, up to 8 d. 1, hepatocytes cultured for 2 d, then incubated for 20 h with medium containing bacterial extract. 2, 2-d cultures incubated for 20 h with medium containing 200 U/ml of mouse recombinant IL-1. 3, 8-d cultures incubated for 20 h with 200 U/ml of mouse IL-1. 4, 8-d culture incubated for 20 h with medium containing nontransformed bacterial extract. 5, liver RNA from a C3HeB/FeJ mouse 16 h after intraperitoneal injection of 10 μg endotoxin (*Salmonella typhosa* W, purchased from Difco Laboratories, Detroit, MI), and used as positive control. 6, liver RNA from C3HeB/FeJ (control) injected with saline. ^{32}P -labeled factor B and SAA cDNA were used as probes.

sion was monitored by Northern blot analysis and immunoprecipitation of newly synthesized protein. Hepatocytes were incubated with IL-1 at intervals up to 24 h, and were then either pulsed for 2 h with [^{35}S]methionine, or were lysed for RNA extraction. IL-1-induced inhibition of albumin synthesis was detectable (Fig. 4,A) within 2 h, and progressively decreased up to 24 h, the end of the observation period. A corresponding decrease in the albumin mRNA level was

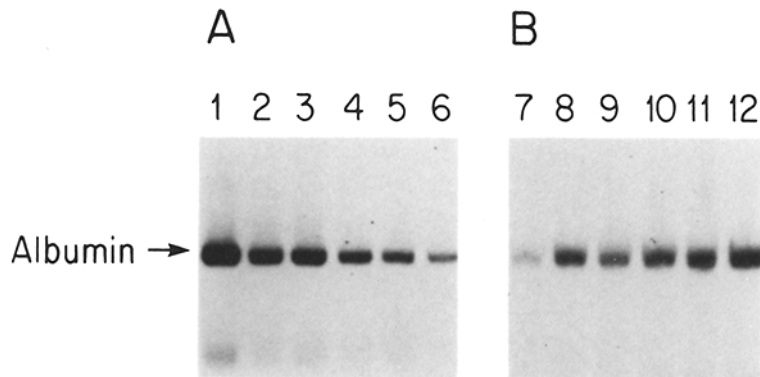


FIGURE 4. Kinetics of mouse recombinant IL-1 inhibition of (A) albumin synthesis and kinetics of release, from (B) the IL-1 effect. SDS-PAGE (7.5%) of secreted [³⁵S]methionine-labeled albumin immunoprecipitated from media of hepatocyte cultures after preincubation with mouse IL-1 (100 U/ml). A 1, control (medium alone); 2, recombinant IL-1 during the 2-h pulse-labelling period; 3, after 3 h; 4, after 7 h; 5, after 12 h; and 6, after 24 h with recombinant IL-1. B, 7, hepatocytes were pulse-labeled for 2 h, 4 h after washing out recombinant IL-1; 8, control (no IL-1 preincubation); 9, 10 h after release from recombinant IL-1; 10, 10-h control; 11, 22 h after release from IL-1; 12, 22-h control.

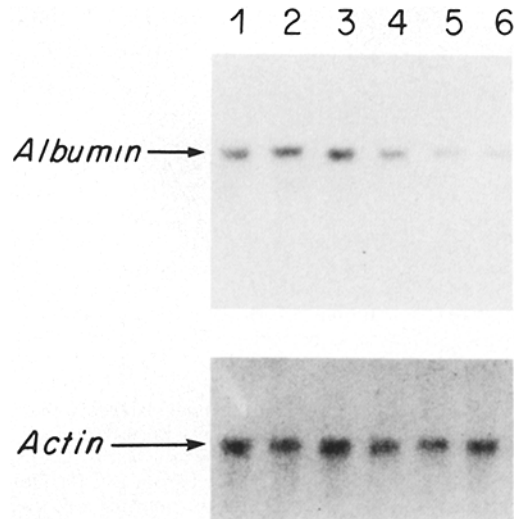


FIGURE 5. Kinetics of mouse IL-1 effect on hepatocyte albumin and actin mRNA content. Northern blot analysis of albumin and actin mRNA. RNA was extracted from 8-d cultures of C3H/HeJ hepatocytes. The controls were incubated for 24 h with medium alone (1), or with medium containing nontransformed bacterial extract (2). 100 U/ml mouse recombinant IL-1 for 3 h (3); 7 h (4); 12 h (5), or 24 h (6).

also detectable, but not until after 3 h, and continued to decrease in the presence of IL-1 up to 24 h (Fig. 5). Increases in SAA and factor B mRNA were detectable by 3 h, and continued to rise up to 24 h (data not shown). Total protein synthesis remained constant throughout the observation period.

The kinetics of release from the effect of IL-1 was studied in the following way. Cells were incubated with IL-1 for 20 h, washed thoroughly, and incubated for varying lengths of time with medium lacking IL-1, after which the cultures

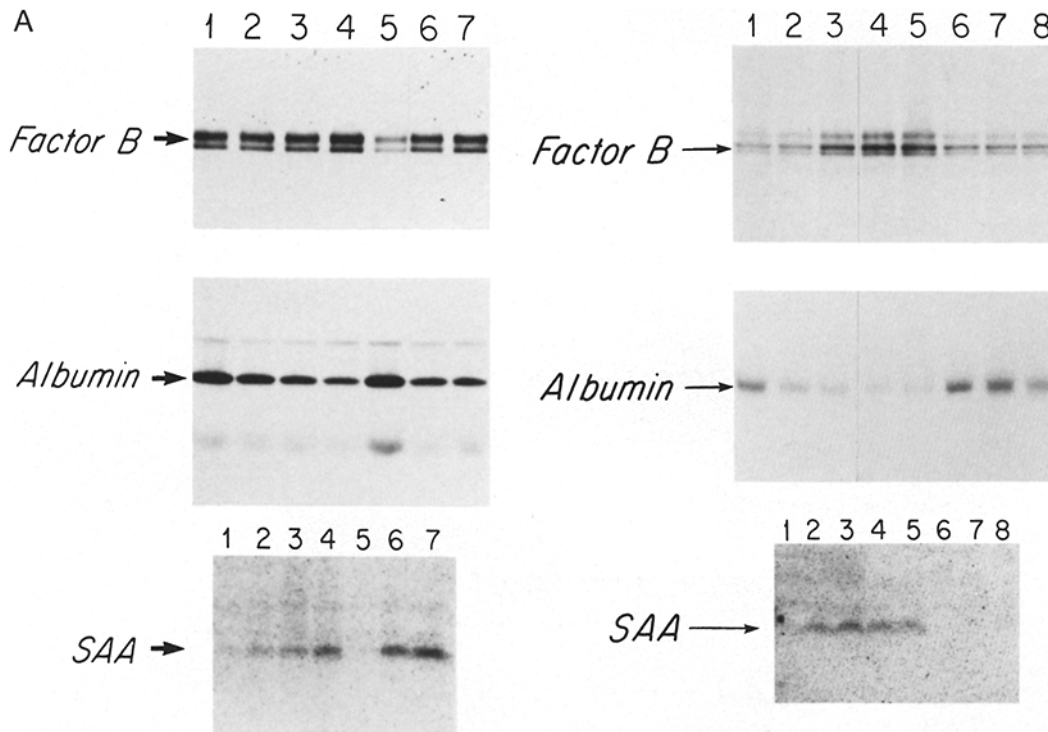


FIGURE 6. A, dose-response regulation of factor B, albumin and SAA synthesis by hepatocytes in culture. SDS-PAGE (7.5% for albumin and factor B, 15% for SAA) of extracellular factor B, albumin, and SAA immunoprecipitated from hepatocyte culture media, after a 2-h pulse with [35 S]methionine. Lanes 1-4, 0.5, 2.5, 5.0, and 10 U/ml human IL-1, lane 5, negative control (medium lacking human IL-1), 6 and 7, 10, and 40 U/ml of mouse recombinant IL-1, respectively. B, factor B, albumin, and SAA biosynthesis by hepatocytes in culture; dose-response to mouse recombinant IL-1. SDS-PAGE (7.5% for albumin and for factor B, 15% for SAA) of intracellular factor B, albumin, and SAA immunoprecipitated from cellular lysates after 22-h pulse with [35 S]methionine. Lane 1, hepatocytes incubated with medium alone; 2-4, hepatocytes incubated with medium containing 5, 20, 100 U/ml mouse recombinant IL-1; 5, 30 U/ml human IL-1; 6, nontransformed bacterial extract; 7, medium containing dilution of guanidine hydrochloride; 8, medium containing 10 μ g LPS/ml. Film exposed 2 d for factor B, 24 h for albumin, and 21 d for SAA. The triplet of intracellular factor B represents different stages of glycosylation of the molecule.

were pulse-labeled for 2 h with [35 S]methionine. Reversal of the inhibition of albumin synthesis was detectable 12 h after removal of IL-1, reaching baseline levels by 24 h (Fig. 4B), whereas increased SAA synthesis above baseline was still detectable 24 h after removing IL-1 (data not shown).

Dose-response Effect of Mouse and Human IL-1. Changes in SAA, factor B, and albumin synthesis were dependent on the concentration of either recombinant mouse IL-1 or purified human monocyte IL-1 present in the culture medium. The effect on these proteins (assessed after 20 h) was detectable at 0.5-10 U/ml in the case of both the human and mouse monokines (Fig. 6, A and B), both at the protein level and at the mRNA level (Fig. 7). The changes in factor B mRNA was significant, but the magnitude of this change was less than the relative increase in SAA mRNA. No significant effect was seen when the cells were

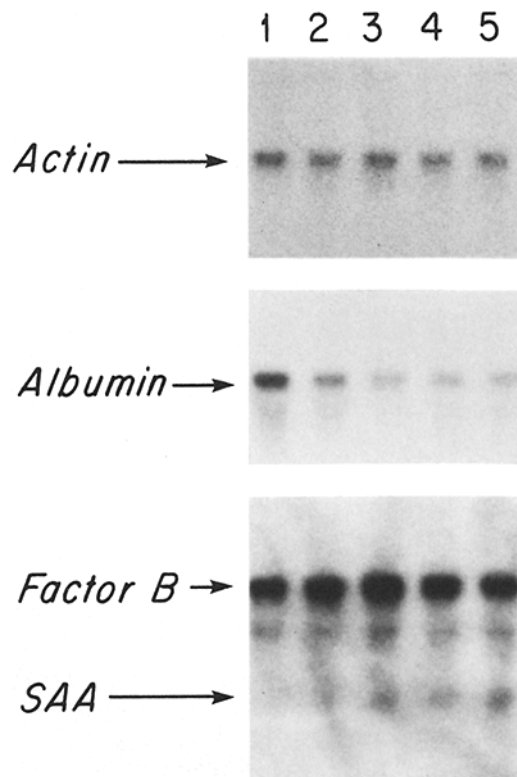


FIGURE 7. Northern blot analysis of actin, albumin, factor B, and SAA mRNA in hepatocyte cultures; dose-response to mouse recombinant IL-1. RNA extracted from C3H/HeJ hepatocytes isolated and cultured for 8 d, then incubated for 20 h with varying doses of mouse recombinant IL-1. 1, control (nontransformed bacterial extract in guanidine hydrochloride); 2, 20 U/ml; 3, 100 U/ml; 4, 200 U/ml; 5, 400 U/ml. 32 P-labeled probes specific for albumin, factor B, SAA, and for actin were used for hybridization. Soft-laser densitometry of the factor B signal showed an increase in specific message of approximately twofold with 100 U IL-1.

incubated with the nontransformed bacterial extract or with 10 μ g/ml LPS (Fig. 6B). There was no effect of IL-1 on expression of the actin gene at any dose examined (Fig. 7).

The specificity of the observed effects of IL-1 was confirmed by blocking experiments with goat anti-mouse IL-1 (28) (data not shown) in which inhibition of albumin synthesis induced by 50 U/ml of recombinant mouse IL-1 was reversed by the presence of the antibody.

Discussion

Previous studies (29–34) of the response to tissue injury or acute inflammation strongly suggested that the monokine IL-1 was an important mediator of the characteristic changes in plasma protein concentration. We provide direct evidence that recombinant-generated IL-1 increases expression of the genes for SAA and factor B, two positive acute phase reactants, and the same mediator decreases expression of the albumin gene, the product of which is a negative acute phase protein. The fact that IL-1 has opposite effects on expression of

albumin and the other two genes indicates that either transduction of the signal from IL-1 receptor to the hepatocyte nucleus is transmitted by multiple mechanisms, or that the regulatory sequences for the positive and negative acute phase genes respond differentially to the same signal. Recombinant-generated IL-1 induced these changes when injected intravenously into mice of the endotoxin-resistant strain, C3H/HeJ, and in primary murine hepatocyte cultures. This finding, and the lack of response to a control bacterial extract or to endotoxin in tissue culture ruled out the possibility that endotoxin contamination of the recombinant-generated IL-1 preparation was responsible for the effector function.

Parallel changes in protein synthesis and specific mRNA levels establish that both the positive (SAA and factor B) and negative (albumin) regulatory effects of IL-1 on hepatic gene expression are pretranslational. Though it is likely that changes in transcription rates account for these changes in mRNA levels, there is, as yet, not direct evidence for this conclusion.

Selection of the optimum culture conditions for liver cells permitted a detailed study of the kinetics and dose-response to IL-1 in preparations containing 99% hepatocytes. That is, low baseline levels of constitutive SAA gene expression permit substantial regulation by IL-1 (i.e., maximal signal-to-background). The IL-1 response is rapid in onset (<7 h), and relatively long lasting (>24 h), but is reversible for each of the genes studied when IL-1 is removed. Finally, highly purified human IL-1 had similar effects on SAA, factor B, and albumin gene expression. This is of interest because previous reports (6, 35) showed primary structural differences between the murine and human IL-1 polypeptides sequenced thus far.

Each of the genes studied here, and other IL-1-responsive genes, such as IL-2 (36), have been cloned. Detailed structural information is therefore available for each. A comparison of potential regulatory sequences among these genes will be useful for further defining the mechanisms regulating the response to inflammation. For some genes, stable transfected cells in which the cloned gene is expressed have been generated (36–38), allowing further investigation of the structural requirements for IL-1 responsiveness.

Others (39) have suggested that a factor distinct from IL-1, namely the hepatocyte-stimulating factor (HSF), effects changes in synthesis of fibrinogen. HSF is less well-defined than either of the two IL-1 preparations used in this study. However, the possibility that other mediators in addition to IL-1 may regulate expression of SAA, factor B, and albumin genes in hepatocytes is not excluded.

Summary

During the acute phase response to tissue injury or inflammation, the concentration of several plasma proteins change. Previous work (29–34) suggested a role for interleukin 1 (IL-1) in the acute phase response. The availability of recombinant-generated mouse IL-1 prompted a study designed to directly test the function of IL-1 and its mechanism of action on hepatic synthesis of two positive acute phase proteins (serum amyloid A [SAA] and complement factor B), and a negative acute phase reactant (albumin). Intravenous injection of

purified recombinant-generated murine-IL-1 into C3H/HeJ endotoxin-resistant mice induced a dose-dependent increase in SAA-specific hepatic messenger RNA (mRNA), and an increase in SAA plasma protein concentration. In primary murine hepatocyte cultures, both the recombinant IL-1 and highly purified human IL-1 induced a dose- and time-dependent, reversible increase in expression of the SAA and factor B genes, and a decrease in albumin gene expression. This regulation is pretranslational, since the kinetics and direction of change in specific mRNA for SAA, factor B, and albumin correspond to the changes in synthesis of the respective proteins. Moreover, the effect of IL-1 was specific, since actin gene expression was unaffected, and the IL-1 response was inhibited by antibody specific for IL-1. These data provide direct evidence that a single mediator, IL-1, can effect the positive and negative changes in specific hepatic gene expression characteristic of the acute phase response.

We thank Drs. K. H. Meyer zum Bueschenfelde, and A. S. Cohen for encouragement and support of this work, Dr. W. Benjamin for helpful discussions, and Ms. H. Hourihan for assistance in the preparation of this manuscript.

Received for publication 6 May 1985 and in revised form 11 June 1985.

References

1. Koj, A. 1974. Acute-phase reactants. *In* Structure and Function of Plasma Proteins. A. C. Allison, editor. Plenum Press, New York. 73-132.
2. Kushner, I., J. E. Volonakis, and H. Gewurz. 1982. C-reactive protein and the plasma protein response to tissue injury. *Ann. N. Y. Acad. Sci.* 389:1.
3. Dinarello, C. 1984. Interleukin-1 and the pathogenesis of the acute phase response. *N. Engl. J. Med.* 311:1413.
4. Dinarello, C. A. 1984. Interleukin-1. *Rev. Infect. Dis.* 6:51.
5. Tatsuta, E., J. D. Sipe, T. Shirahama, M. Skinner, and A. S. Cohen. 1983. Different regulatory mechanism for serum amyloid A and serum amyloid P synthesis by cultured mouse hepatocytes. *J. Biol. Chem.* 258:5414.
6. LoMedico, P. T., U. Gubler, C. P. Hellmann, M. Dukovich, J. G. Giri, Y. C. E. Pan, K. Collier, R. Semionow, A. O. Chua, and S. B. Mizel. 1984. Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. *Nature (Lond.)*. 312:458.
7. Kioussis, D., F. Eiferman, P. van de Rijn, M. B. Gorin, R. S. Ingram, and S. M. Tilghman. 1981. The evolution of alpha-fetoprotein and albumin II. The structures of the alpha-fetoprotein and albumin genes in the mouse. *J. Biol. Chem.* 256:1960.
8. Sackstein, R., H. R. Colten, and D. W. Woods. 1983. Phylogenetic conservation of a class III major histocompatibility complex antigen, factor B isolation and nucleotide sequencing of mouse factor B cDNA clones. *J. Biol. Chem.* 258:14693.
9. Sipe, J. D., H. R. Colten, G. Goldberger, M. D. Edge, B. F. Tack, A. S. Cohen, and A. S. Whitehead. 1985. Human serum amyloid A (SAA): Biosynthesis and postsynthetic processing of preSAA and structural variants defined by complementary DNA. *Biochemistry*. 24:2931.
10. McAdam, K. P. W. J., and J. D. Sipe. 1976. Murine model for human secondary amyloidosis. Genetic variability of the acute-phase serum protein SAA response to endotoxin and casein. *J. Exp. Med.* 144:1121.
11. Seglen, P. O. 1973. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exp. Cell. Res.* 82:391.
12. Ramadori, G., H. P. Dienes, K. H. Meyer zum Bueschenfelde, and D. Falke. 1984.

- Infection of murine hepatocyte cultures by herpes simplex virus (HSV) 1 and 2. *Liver*. 4:318.
13. Dienes, H. P., G. Ramadori, G., D. Falke, and W. Thoenes. 1984. Electron microscopic observations on herpes simplex virus type I and II infected primary hepatocyte culture cells. *Virchows Arch. B*. 46:321.
 14. Ramadori, G., G. Rasokat, H. Burger, K. H. Meyer, Bueschenfelde, and D. Bitter Suermann. 1984. Quantitative determination of complement components produced by purified hepatocytes. *Clin. Exp. Immunol.* 55:189.
 15. Dinarello, C. A., L. Renfer, and S. M. Wolff. 1977. Production of an antibody against human leukocytic pyrogen. *J. Clin. Invest.* 60:465.
 16. Dinarello, C. A., L. Renfer, and S. M. Wolfe. 1977. Human leukocytic pyrogen: Purification and development of a radioimmunoassay. *Proc. Natl. Acad. Sci. USA*. 74:4624.
 17. Kaye, J. S. Gillis, S. B. Mizel, E. M. Shevach, T. R. Malek, C. A. Dinarello, L. B. Lachman, and C. A. Janeway. 1982. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: Interleukin-1 is required for the expression of receptors for IL-2. *J. Immunol.* 133:1333.
 18. Linke, R. P., J. D. Sipe, P. S. Pollock, T. F. Ignaczak, and G. G. Glenner. 1975. Isolation of a low molecular weight serum component antigenically related to an amyloid fibril protein of unknown origin. *Proc. Natl. Acad. Sci. USA*. 72:1473.
 19. Auerbach, H. A., R. D. Baker, W. J. Matthews, and H. R. Colten. 1984. Molecular mechanism for feedback regulation of C4 biosynthesis in guinea pig peritoneal macrophage. *J. Exp. Med.* 159:1750.
 20. Roberts, B. E., and B. M. Patterson. 1973. Efficient translation of tobacco mosaic virus RNA and rabbit globin Gs RNA in a cell free system from commercial wheat germ. *Proc. Natl. Acad. Sci. USA*. 70:2330.
 21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680.
 22. Chirgwin, J. M., A. E. Przybyla, R. J. McDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.
 23. Woods, D. E., A. F. Markham, A. T. Ricker, G. Goldberger, and H. R. Colten. 1982. Isolation of cDNA clones for the human complement protein factor B, a class III major histocompatibility complex gene product. *Proc. Natl. Acad. Sci. USA*. 79:5661.
 24. Stearman, R. S., C. A. Lowell, W. R. Pearson, and J. F. Morrow. 1982. Regulation of synthesis of amyloid A-related protein. *Ann. N. Y. Acad. Sci.* 389:106.
 25. Schwartz, R. J., J. A. Haron, K. N. Rothblumm, and A. Dugaiczuk. 1980. Regulation of muscle differentiation: Cloning of sequences from alpha-actin messenger ribonucleic acid. *Biochemistry*. 19:5883.
 26. Sackstein, R., and H. R. Colten. 1984. Molecular regulation of MHC class III (C4 and factor B) gene expression in mouse peritoneal macrophage. *J. Immunol.* 133:1618.
 27. Sipe, J. D., T. F. Ignaczak, P. S. Pollock, and G. G. Glenner. 1976. Amyloid fibril protein AA: Purification and properties of the antigenically related serum component as determined by solid phase radioimmunoassay. *J. Immunol.* 116:1151.
 28. Mizel, S. B., M. Dukovic, and J. Rothstein. 1983. Preparation of goat antibodies against interleukin-1: Use of an immunoadsorbent to purify interleukin-1. *J. Immunol.* 131:1834.
 29. McAdam, K. P. W. J., and C. A. Dinarello. 1980. Induction of serum amyloid A synthesis by human leukocyte pyrogen. In *Bacterial Endotoxin and Host Response*. M. K. Agarwal, editor. Elsevier North-Holland, Amsterdam, the Netherlands. 167-178.

30. Sztejn, M. B., S. N. Vogel, J. D. Sipe, P. A. Murphy, S. B. Mizel, J. J. Oppenheim, and D. L. Rosenstreich. 1981. The role of macrophages in the acute-phase response: SAA inducer is closely related to lymphocyte activating factor and endogenous pyrogen. *Cell. Immunol.* 63:164.
31. Sipe, J. D., S. N. Vogel, J. L. Ryan, K. P. W. J. McAdam, and D. L. Rosenstreich. 1979. Detection of a mediator derived from endotoxin-stimulated macrophages that induces the acute phase serum amyloid A response in mice. *J. Exp. Med.* 150:597.
32. Baumann, H., G. P. Jahreis, D. N. Sauder, and A. Koj. 1984. Human keratinocytes and monocytes release factors which regulate the synthesis of major acute phase plasma proteins in hepatic cells from man, rat and mouse. *J. Biol. Chem.* 252:7331.
33. Mortensen, R. F., K. Sarlo, and P. T. Le. 1984. Monokine-induced hepatocyte synthesis of acute-phase reactants in mice. *Lymphokine Res.* 3:17.
34. Selinger, M. J., K. P. W. J. McAdam, M. M. Kaplan, J. D. Sipe, S. N. Vogel, and D. L. Rosenstreich. 1980. Monokine-induced synthesis of serum amyloid A protein by hepatocytes. *Nature (Lond.)*. 285:498.
35. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Nat. Acad. Sci. USA.* 81:7907.
36. Clark, S. C., S. K. Arya, F. Wong-Staal et al. 1984. Human T-cell growth factor: Partial amino acid sequence, cDNA cloning, and organization and expression in normal and leukemic cells. *Proc. Natl. Acad. Sci. USA.* 84:2543.
37. Chaplin, D. D., R. Sackstein, D. Perlmutter, J. H. Weis, J. Coligan, H. R. Colten, and J. G. Seidman. 1984. Expression of hemolytically active murine fourth component of complement in transfected L cells. *Cell.* 37:569.
38. Perlmutter, D. H., H. R. Colten, D. Grossberger, J. Strominger, J. G. Seidman, and D. D. Chaplin. 1985. Expression of complement proteins C2 and factor B in transfected L cells. *J. Clin. Invest.* In press.
39. Wolosky, B. N. R. N. J., G. M. Fuller. 1985. Identification and partial characterization of hepatocyte-stimulating factor from leukemia cell lines: Comparison with interleukin 1. *Proc. Nat. Acad. Sci. USA.* 82:1443.