

Monocyte-conditioned Medium, Interleukin-1, and Tumor Necrosis Factor Stimulate the Acute Phase Response in Human Hepatoma Cells In Vitro

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Abstract. Human hepatoma cells mimic the acute phase response after treatment with monocyte-conditioned medium. Levels of secreted fibrinogen, α -1 acid glycoprotein, C-reactive protein, haptoglobin, and the third component of complement were elevated compared with control levels after 48 h of incubation with conditioned supernatant medium from an enriched fraction of normal peripheral monocytes. Albumin levels declined and α -1 antitrypsin remained unchanged. Levels of specific mRNA were measured

by hybridization to slot blots and Northern blots and changed in correspondence with protein alterations. Interleukin-1 and tumor necrosis factor stimulated the third component of complement, but did not elevate any other member of the acute phase group and were therefore only partially active in this system. The identification of an in vitro model of the human acute phase response will permit analysis of the molecular basis for coordinate regulation of this group of facultative genes.

IN response to inflammation and cellular damage, many of the proteins synthesized and secreted by the liver show quantitative changes in serum levels (15, 29). These alterations include two- to three-fold elevations of haptoglobin, α -1 antitrypsin (α 1AT),¹ α -1 acid glycoprotein (AGP), and fibrinogen. More dramatic increases are seen in C-reactive protein (CRP) and serum amyloid A, which elevate 100–1,000-fold in man. The role that each of these proteins plays in the acute phase response has not been fully defined for all products, but is well reviewed elsewhere (15, 22, 29). Albumin, in contrast to the other proteins described, decreases in abundance during the response. Several animal models have been used for the analysis of the acute phase response, including rats (2), mice (3, 34), and rabbits (26), each of which has a slightly different subset of proteins that responds to the appropriate stimulation in vivo (e.g., injection of bacteria or turpentine).

Whereas animal models have provided much information regarding the kinds of proteins secreted by the liver during the acute phase response and the time course in which they are expressed, in vivo studies are complicated by the multiplicity of cell types in the body and pose some questions about whether the stimulus acts directly or indirectly upon the hepatocyte.

Attempting to address these issues, other investigators have used primary hepatocyte cultures (3, 31, 17). These in

vitro systems have permitted the conclusion that stimulating agents such as turpentine and bacteria do not operate directly on hepatocytes. Rupp and Fuller (32) have shown that one acute phase protein, fibrinogen, was elevated when supernatants derived from isolated peripheral monocytes were added to primary rat hepatocytes in vitro. This demonstration clarified the role of cellular intermediates in the acute phase response although the primary hepatocyte culture system is not a totally pure population of parenchymal cells.

Some characterization of cell-derived mediators that act humorally to exert their effect on the hepatocyte has been made. Sipe et al. (33) have reported the induction of serum amyloid A after the injection of mice with purified fractions of mouse interleukin-1 (Il-1) and rabbit endogenous pyrogens. Woloski and Fuller (38) have shown that a separate factor, which they have called hepatocyte-stimulating factor, elevates fibrinogen in primary rat hepatocyte cultures.

To study the acute phase response in man at the molecular level, we sought an established human hepatoma cell line in which to analyze the coordinate expression of the acute phase response genes. Four human hepatoma lines, Hep SK (13), PLC/PRF-5 (27), HepG2, and Hep 3B2 (1) were screened for the expression of acute phase proteins, and we report here the identification of HEP 3B2 as a useful in vitro model system for the investigation of the human inflammatory response. This cell line reproducibly responds to conditioned supernatant medium from cultivated monocytes by elevating several proteins involved in the acute phase response and decreasing albumin production. The elevations and declines in protein production are correlated with similar changes in

1. *Abbreviations used in this paper:* α 1AT, α -1 antitrypsin; AGP, α -1 acid glycoprotein; C3, third component of complement; CRP, C-reactive protein; hIl-1, human interleukin-1; Il-1, interleukin-1; mIl-1, mouse Il-1; TNF, tumor necrosis factor.

specific mRNA levels. We further show that Il-1 and tumor necrosis factor (TNF) independently stimulate production of the third component of complement (C3) but do not elevate any other acute phase gene examined.

Materials and Methods

Isolation of Monocytes

A population of peripheral monocytes and lymphocytes was obtained from whole human blood by dextran sedimentation of red cells followed by enrichment of the monocyte and lymphocyte fraction over Ficoll-Hypaque (Pharmacia Fine Chemicals, Pharmacia Inc., Piscataway, NJ). In brief, 100 ml of peripheral blood was mixed with 10 ml of 6% dextran, (400,000 mol wt in PBS) and the red cells allowed to sediment at unit gravity for 45 min at room temperature. The plasma containing leukocytes was removed from the red cell fraction and spun at 400 g for 10 min. The cell pellet, enriched for leukocytes, was resuspended in PBS and layered over 5 ml of Ficoll-Hypaque in three centrifuge tubes. Sedimentation of polymorphonuclear leukocytes and granulocytes was accomplished by centrifugation at 675 g for 30 min. Cells located at the interface of the supernatant and the Ficoll-Hypaque were isolated by suction, washed twice with PBS, and plated in RPMI (Gibco, Grand Island, NY) plus 10% fetal bovine serum.

Stimulation of Monocytes

Monocyte cultures were stimulated to increase the production of hepatocyte-stimulating factors (31) by the addition of 10 µg/ml of lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) for 48 h.

Hepatoma Cell Culture

HEP 3B2 cells are routinely grown in MM (three parts per volume Eagle's minimal essential medium and one part MAB 87/3) plus 10% fetal bovine serum (Gibco) and passaged by trypsinization once a week. For expression of the acute phase response, HEP 3B2 cells were plated at high density and allowed to reach plateau. Equal volumes of conditioned medium from stimulated monocytes and fresh MM plus fetal bovine serum were added and allowed to incubate for 48 h. After 48 h the supernatant medium was collected and analyzed by radioimmunoassay for the levels of secreted proteins. Control cultures contained fresh medium alone or medium with 5 µg/ml lipopolysaccharide. The cellular monolayer was solubilized in 8 M guanidine hydrochloride, 20 mM sodium acetate (pH 7.5) for determination of total protein and for isolation of RNA. To measure the kinetics of induction, replicate flasks were harvested at various time points throughout a 48-h period.

Radioimmunoassay of Secreted Proteins

Supernatant media obtained from hepatoma cell cultures treated as described above were collected and frozen before analysis. Radioimmunoassay was performed using antibody coupled to polystyrene beads as a solid phase immunoabsorbant (Kelly, J. H., and G. J. Darlington, manuscript submitted for publication). Protein values were calculated as micrograms protein secreted in 24 h/mg cell protein in monolayer (Table I) or as micrograms protein secreted in 24 h/ml conditioned medium (Tables II and III).

Isolation of RNA

Cells were solubilized in guanidine HCl (8) and RNA was isolated by the protocol of Glisin et al. (16).

cDNA Probes

We are grateful to the following investigators for the cDNA probes used in our studies. Human α -1 AT (ϕ 631) was obtained from K. Matteson and A. Beaudet (Baylor College of Medicine, Houston, TX) (4), and human Alb (F-47) from R. Lawn (Genentech, Inc., South San Francisco, CA; 24). Two anonymous probes (clone 15 and clone 3) isolated from a human cDNA library generated from RNA obtained from an acute phase liver were given to us by S. Woo (Baylor College of Medicine, Houston, TX). Clone 15 has been shown by sequence identity of 150 base pair (bp) to be C3 (II). Clone 3 has a 750-bp insert and has been identified as AGP by three criteria. The first of these is its strong cross-hybridization to the pAGP-1 clone of Dente

et al. (12). It has the same size internal restriction fragments as pAGP-1 in a Taq I digest and in a Taq I-Hind III double digest. Clone 3 also hybridizes to an RNA of the appropriate size, and will subsequently be referred to as AGP. CRP cDNA (pCRP-5) was obtained from S. A. Whitehead (Harvard School of Medicine, Boston, MA; 37); α -Fib cDNA (pl15.1) from S. Lord (University of North Carolina, Chapel Hill, NC; 19), β -actin from D. Cleveland (Johns Hopkins University School of Medicine, Baltimore, MD) (10), and hypoxanthine phosphoribosyl transferase from C. T. Caskey (Baylor College of Medicine, Houston, TX; 6).

Analysis of mRNA

For quantitative slot blot analysis, total cellular RNA was diluted serially and blotted onto nitrocellulose using the slot blot apparatus of Schleicher & Schuell, Inc. (Keene, NH). The diluting solutions contain buffer and formaldehyde as described in the manufacturer's manual. Prehybridization of the filters was carried out for 4–18 h at 42°C in 50% formamide, 5× SSC (0.15 M sodium chloride, 0.015 sodium citrate), 0.1% polyvinylpyrrolidone, 0.1% Ficoll, 0.1 mg/ml salmon sperm DNA, 20 mM sodium phosphate (pH 6.8), and 0.1% SDS. Hybridization was done at 42°C for 18–24 h. The hybridization solution contained 10 mg/ml poly A, 9.5 µg/ml poly C, and the appropriate nick-translated probe in addition to the compounds present in the prehybridization mixture.

For Northern blot hybridization of α 1AT transcripts, a 475-bp internal Hinc II fragment was isolated from ϕ 631. The insert was recovered after electrophoresis through a 1% low melting point agarose gel (18) and labeled by nick translation. Nick-translated, whole plasmids were used for the analysis of albumin, CRP, and AGP. Washing conditions for both slot blots and Northern blots were essentially those of Maniatis et al. (28). The final two washes were carried out at 65°C in 0.2× SSC, 0.2% SDS.

Analysis of Il-1 Activity

Mouse Il-1 (mIl-1) is a recombinant DNA product isolated from bacteria and was kindly provided by Dr. Peter Lomedico (Hoffman-La Roche, Nutley, NJ; 25). MIl-1 was diluted and the guanidine solvent exchanged over a G25 column that had been equilibrated with growth medium. Several concentrations were tested by incubating the HEP 3B2 cells with mIl-1 for 48 h at which time the medium was collected and assayed for secreted serum proteins. Human Il-1 (hIl-1) (Collaborative Research, Inc., Waltham, MA) was hydrated and added to the medium of HEP 3B2 cells at varying concentrations. Thymocyte-stimulating activity was measured by a previously published protocol (23).

Analysis of TNF Activity

TNF was obtained from Genentech and hydrated in medium without serum at 10⁶ U/ml. Varying concentrations of TNF were added to the supernatant medium of HEP 3B2 cells and removed for cellular secreted protein assay 48 h later.

Results

Proteins Secreted in Response to Stimulation by Monocyte-conditioned Medium

After a 48-h stimulation with conditioned supernatants from LPS-treated monocytes, levels of several proteins increased in the supernatant medium of HEP 3B2. The secreted protein values summarized in Table I are based on duplicate determinations of single samples from four experiments. In each experiment a different monocyte preparation was analyzed. In the presence of monocyte supernatants, fibrinogen increased an average of 12-fold, while AGP and C3 elevated over 20-fold. Haptoglobin and CRP increased from undetectable basal levels to induced amounts of 36 and 53 ng/mg monolayer cell protein, respectively. No change in α -1AT was observed. Albumin levels were consistently lower in the induced cells compared with the untreated ones. Thus, with the exception of α 1AT, the proteins that change in serum con-

Table 1. Proteins Secreted by HEP 3B₂ Induced by Monocyte Supernates ($\mu\text{g}/\text{mg}$ per 24 h)

| Protein | Induced* | Uninduced | Fold increase |
|--------------|-----------------|-----------------|---------------|
| Fib | 0.24 \pm 0.07 | 0.02 \pm 0.01 | 12.0 |
| AGP | 0.92 \pm 0.6 | 0.04 \pm 0.02 | 23.0 |
| C3 | 1.51 \pm 0.49 | 0.06 \pm 0.02 | 23.9 |
| Hapt | 0.04 \pm 0.01 | N.D. | |
| CRP | 0.05 \pm 0.02 | N.D. | |
| α 1AT | 2.74 \pm 0.87 | 2.82 \pm 1.3 | 0.74 |
| Alb | 2.05 \pm 1.0 | 4.11 \pm 1.3 | 0.50 |

Summary of four experiments. Alb, albumin; Fib, fibrinogen; Hapt, haptoglobin. N. D., not detectable.
* \pm SD.

centration during the acute phase response in vivo also change appropriately in the HEP 3B₂ cells.

Time Course of Expression of Acute Phase Proteins

Initial studies of protein and mRNA were assessed at 48 h. To determine the kinetics of induction by monocyte-conditioned medium, the production of serum proteins was measured at various time points over 48 h. Quantities of five secreted proteins are plotted in Fig. 1. Elevations of Fib (Fig. 1 A) and AGP (Fig. 1 B) compared with control cultures were detectable between 8–12 h and continued to increase more rapidly than controls. During the growth cycle of HEP 3B₂, the amount of albumin, α -fetoprotein and α 1AT pro-

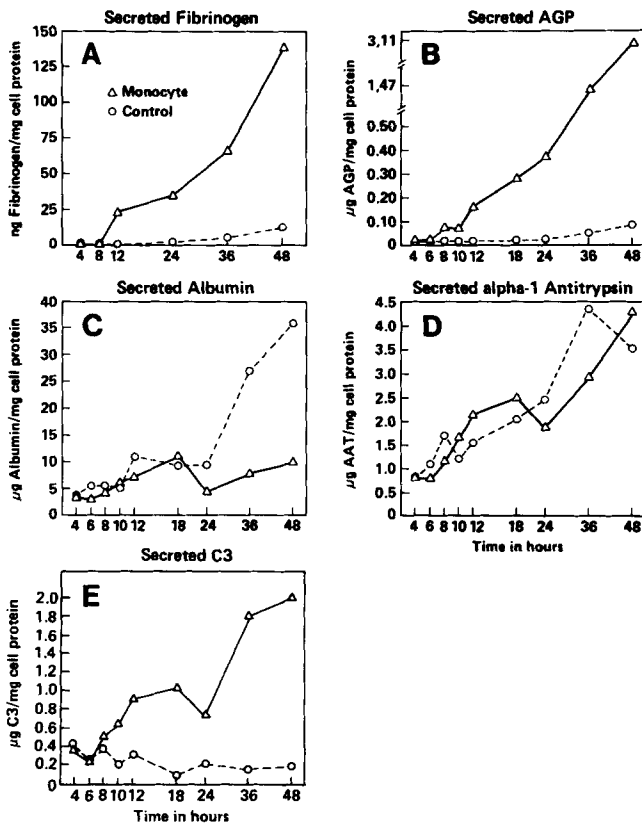


Figure 1. Kinetics of secreted protein production. Proteins secreted by HEP 3B₂ were measured by radioimmunoassay of supernatant medium, from monocyte-treated cells (Δ) and controls (O). Fibrinogen (A), AGP (B), albumin (C), α 1AT (D), and C3 (E).

duced per unit cell protein increases as the density of the culture increases (reference 1, and Darlington, G. J., G. J. Buffone, and J. H. Kelly, manuscript submitted for publication). Albumin (Fig. 1 C) secreted by the monocyte-treated hepatoma cells showed no increase after 18 h, in contrast to the unstimulated control where albumin accumulation continued to rise over time. α 1AT (Fig. 1 D) production was similar for both monocyte-treated and control cultures, increasing over the 48-h period as previously described (Darlington, G. J., G. J. Buffone, and J. H. Kelly, manuscript submitted for publication). C3 (Fig. 1 E) levels elevated in stimulated cells but not in the untreated controls.

Analysis of Total Cellular RNA

Specific mRNAs were measured by hybridization of cDNA probes to serial dilutions of total cellular RNA isolated after 48-h exposure of HEP 3B₂ to monocyte-conditioned medium and bound to nitrocellulose as described in Materials and Methods. Fig. 2 shows the autoradiographic results of this comparison. Densitometric scanning of such samples shows that fibrinogen, AGP, and C3 are the most dramatically increased mRNA populations at 48 h, being 5.4-, 4.3-, and 12.4-fold elevated, respectively. α 1AT and hypoxanthine phosphoribosyl transferase are not different in treated versus control cells, whereas levels of albumin mRNA have decreased at 48 h. Hybridization to pBR322 and

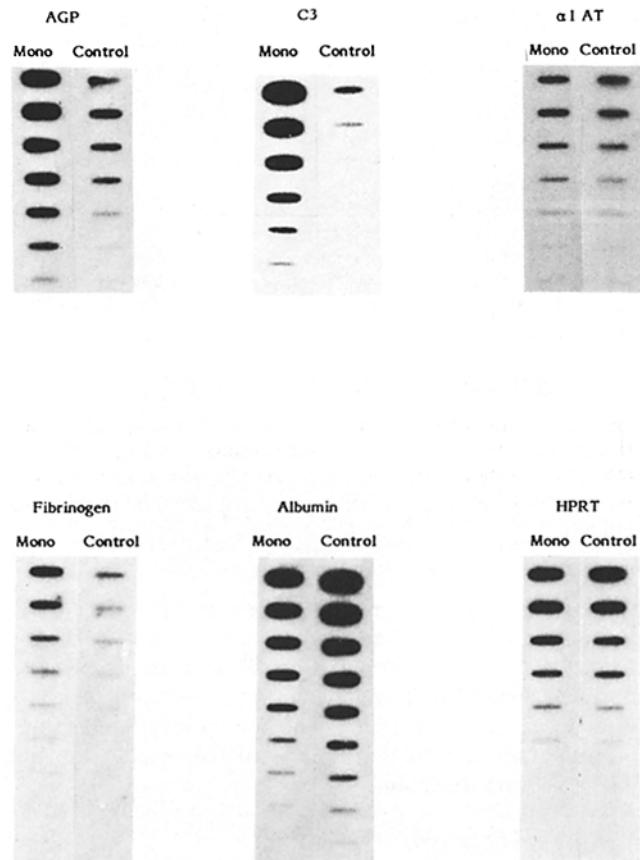


Figure 2. Slot blot analysis of total cellular RNA. HEP 3B₂ RNA was isolated as described in Materials and Methods and applied to nitrocellulose filters in serial twofold dilutions. The RNA was probed with various nick-translated cDNAs and x-ray film exposed to the filter.

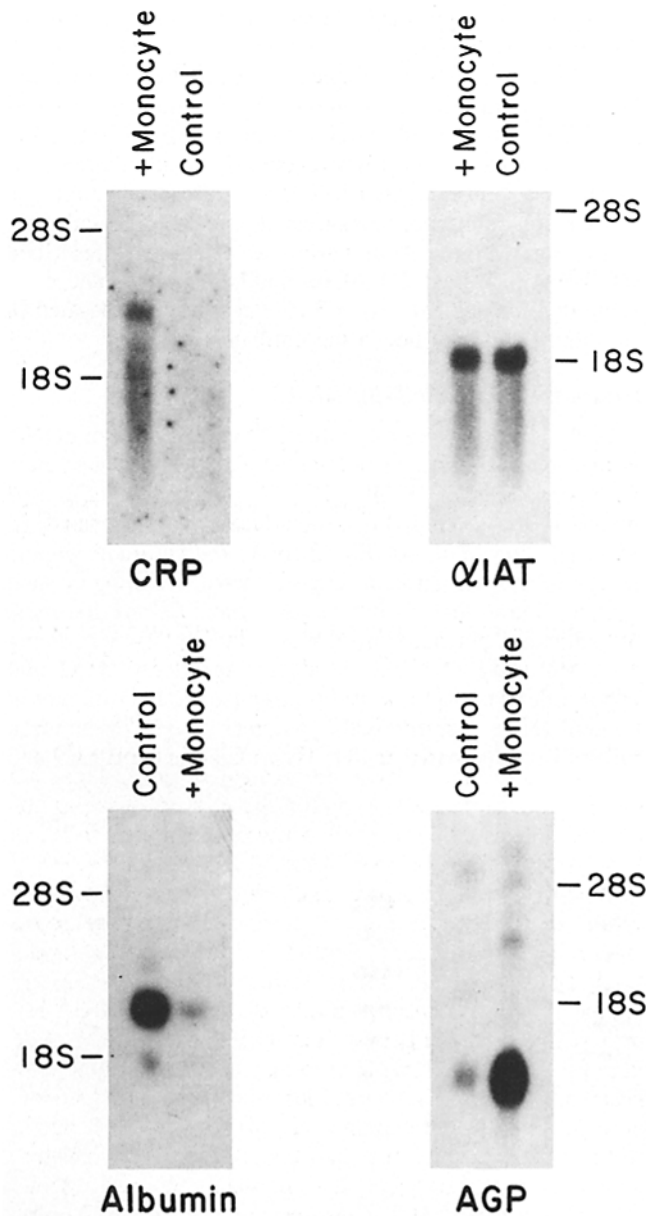


Figure 3. Northern analysis of total cellular RNA. CRP (A), α 1AT (B), albumin (C), and AGP (D). Total cellular RNA (20 μ g) from HEP 3B2 treated for 48 h with monocyte supernates and from control cells was subjected to electrophoresis and blotted to nitrocellulose.

β -globin gave no signal on the slot blots, and β -actin showed no change in stimulated cultures (data not shown). We were interested to see whether α 1AT mRNA might respond to monocyte supernates even though the secreted protein levels were unchanged. Consistent with our observations of unaltered protein production, α 1AT mRNA levels were not affected by the treatment.

Northern Analysis of mRNA

The production of CRP by HEP 3B2 cells as measured by radioimmunoassay was confirmed by Northern analysis of total cellular mRNA. Fig. 3 shows a Northern blot of whole cell RNA from stimulated and unstimulated hepatoma cultures probed with pCRP-5. A CRP mRNA of \sim 2.2 kilobase

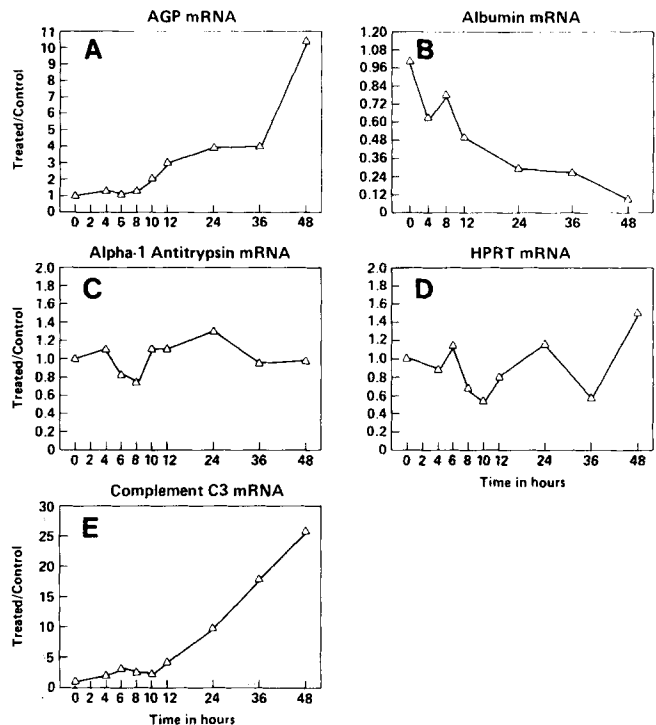


Figure 4. Kinetics of mRNA production. The relative amounts of specific mRNA for AGP (A), albumin (B), α 1AT (C), HPRT (D), and C3 (E) produced over 48 h are plotted as a ratio of treated/control.

(kb) has been described by Tucci et al. (36). We observed a band of the appropriate size for CRP mRNA in stimulated, but not in unstimulated, cultures of HEP 3B2 cells, confirming the protein production data where detectable levels of CRP were found only in treated cells. The level of α 1AT mRNA was not different in treated versus untreated cells by Northern analysis (Fig. 3). It can also be seen in Fig. 3 that albumin mRNA isolated 48 h after addition of monocyte supernates was dramatically reduced in control cells, while AGP was greatly elevated.

Time Course of mRNA Production

The level of mRNA produced by the cells at various time points was assessed by densitometric scanning of autoradiograms of slot blots similar to those shown in Fig. 1. Fig. 4 shows the expression of specific messenger RNAs after exposure to stimulated monocyte supernates. Values are expressed relative to control values. A two-fold elevation in AGP mRNA (Fig. 4 A) could be seen at 10 h, increasing in the presence of monocyte-conditioned medium for the full 48 h. C3 mRNA (Fig. 4 E) was elevated by 12 h and also increased throughout the exposure to monocyte supernates. In contrast, albumin mRNA levels declined as early as 4 h, dropping to only 11% of the control value by 48 h (Fig. 3 B). Because albumin mRNA is relatively stable (9), it seems likely that albumin protein accumulation in monocyte-treated cultures is due to the pool of mRNA synthesized by the hepatoma cell before the addition of monocyte-conditioned medium. The levels of α 1AT (Fig. 3 C) and hypoxanthine phosphoribosyl transferase (Fig. 3 D) mRNAs fluctuated within a twofold range and remained at essentially identical levels with respect to both time and treatment.

Table II. Proteins Secreted by HEP 3B2 in Response to Il-1

| Stimulating Factor | Secreted Proteins | | | | |
|------------------------|-----------------------|------|-------|------|-------|
| | AGP | Fib | CRP | C3 | Alb |
| | <i>µg/ml per 24 h</i> | | | | |
| mIl-1* (U/ml) | | | | | |
| 10,000 | 0.10 | 0.05 | — | 0.98 | 3.10 |
| 1,000 | 0.13 | 0.05 | — | 0.84 | 3.12 |
| 100 | 0.12 | 0.06 | — | 0.73 | 2.95 |
| Control (medium alone) | 0.09 | 0.44 | — | 0.09 | 9.00 |
| hIl-1‡ (U/ml) | | | | | |
| 50 | 0.05 | 0.05 | — | 0.98 | 4.95 |
| 10 | 0.06 | 0.05 | — | 0.94 | 3.30 |
| 2 | 0.06 | 0.07 | — | 0.72 | 3.52 |
| 0.4 | 0.06 | 0.13 | — | 0.43 | 4.22 |
| 0.08 | 0.05 | 0.05 | — | 0.34 | 3.40 |
| 0.016 | 0.05 | 0.23 | — | 0.08 | 6.30 |
| 0.003 | 0.04 | 0.20 | — | 0.16 | 4.39 |
| Monocyte supernate | 0.97 | 1.04 | 0.013 | 2.65 | 1.77 |
| Control (medium alone) | 0.04 | 0.53 | — | 0.10 | 11.25 |

Alb, albumin; Fib, fibrinogen.

* mIl-1 was measured in the thymocyte stimulation assay at a concentration range between 1.6 and 1,000 U/ml. Thymidine incorporation was stimulated at 40 U/ml and above.

‡ hIl-1 was measured in the thymocyte stimulation assay at a concentration range between 0.01 and 5 U/ml. Thymidine incorporation was stimulated at 0.04 U/ml and above.

Il-1 Partially Stimulates the Acute Phase Response in HEP-3B2 Cells

Table II shows the amounts of various acute phase proteins secreted in response to both mIl-1 and hIl-1. C3 was the only protein stimulated by Il-1. Both mIl-1 (isolated from bacteria as a recombinant DNA product) and hIl-1 induced C3 and had a similar effective range for thymocyte-stimulating activity and the acute phase response of C3. Albumin levels were reduced in cultures treated with Il-1, suggesting that a second gene is regulated by this monokine, but in a negative direction. In contrast to C3 and albumin, concentrations of Il-1 over 1,000-fold greater than those necessary to stimulate thymidine uptake by thymocytes did not stimulate the HEP 3B2 cells to produce AGP, fibrinogen, or CRP. In fact, we have consistently observed inhibition of fibrinogen production by Il-1. Thus Il-1 is not able to elicit the full acute phase response in HEP 3B2, unlike monocyte-conditioned medium.

TNF also Stimulates C3

Table III presents the pattern of protein production by Hep 3B2 in response to TNF. C3 is stimulated by TNF to the same degree as by Il-1 (~10-fold) for the highest concentrations tested. Unlike the response to Il-1, fibrinogen and albumin levels were not significantly affected. In combination, TNF and Il-1 appear to have additive, but not synergistic, effects on C3, although additional concentration combinations need to be tested.

Discussion

We have identified and characterized an interesting and useful in vitro model of the acute phase response. Use of HEP

Table III. Proteins Secreted by HEP 3B2 in Response to TNF

| Stimulating Factor | Secreted proteins | | | |
|--|-----------------------|------|------|------|
| | AGP | Fib | C3 | Alb |
| | <i>µg/ml per 24 h</i> | | | |
| Human TNF (U/ml) | | | | |
| 10,000 | 0.02 | 0.21 | 0.52 | 5.79 |
| 2,000 | 0.02 | 0.28 | 0.68 | 8.53 |
| 1,000 | 0.02 | 0.26 | 0.55 | 6.32 |
| 200 | 0.02 | 0.25 | 0.48 | 7.43 |
| 100 | 0.02 | 0.29 | 0.39 | 6.83 |
| 20 | 0.02 | 0.48 | 0.28 | 8.69 |
| 1,000 U/ml human TNF + 20 U/ml Il-1 | 0.05 | 0.13 | 0.92 | 8.19 |
| Control (medium alone) | 0.03 | 0.48 | 0.06 | 9.81 |

Haptoglobin and CRP were unresponsive to any concentration of TNF tested and were not detectable in any of the samples. Alb, albumin; Fib, fibrinogen.

3B2 cells permits an analysis of the human response at the molecular level in an established, easily maintained cell culture system.

Of the proteins examined, AGP, CRP, fibrinogen, C3, and haptoglobin increased in stimulated HEP 3B2 cells as is observed in vivo. The mRNA corresponding to the first four proteins also increased over a 48-h period as shown by quantitative slot blot analysis or Northern blots. This increase suggested a pretranslational basis for elevated protein levels. Albumin also responded to conditioned medium from monocytes in a manner consistent with the acute phase response in man, as both protein and mRNA decreased with time.

α1AT showed no change in response to factors secreted by monocytes nor was there an increase in α1AT mRNA at any time within the 48-h period examined. We suggest that the increase of α1AT protein in vivo may be due to posttranslational mechanisms such as reduced degradation. In support of this hypothesis, Friedman et al. (14) showed no change in α1AT mRNA in mice that had undergone partial hepatectomy although other acute phase messages increased. However, Chandra et al. (7) measured amounts of α1AT translated in vitro from livers of normal and turpentine-injected baboons and concluded that α1AT mRNA was somewhat increased in the treated animal. Northern analysis of the α1AT mRNA was not done. Other explanations for our observation include the possibility that the α1AT locus in HEP 3B2 has lost the ability to respond to the monocyte factors, or that the monocyte supernates do not contain the necessary factor for α1AT stimulation. Should additional factors be required, the HEP 3B2 cells would likely serve as an effective assay system for these components of the acute phase response. Further studies of protein and mRNA in animal systems should help resolve whether α1AT elevation in vivo is due to increased α1AT mRNA.

Previous reports by MacIntyre et al. (26) using primary rabbit hepatocytes describe the production of CRP after stimulation of the animal by turpentine injection. To our knowledge this paper is the first description of the production of CRP in a human cell culture system, and thereby offers a means of examining the regulation of this interesting protein.

The time course of production of the mRNA population for AGP and C₃ would suggest that mRNA levels increase over the entire 48-h period under study. In our experimental design, the stimulating factors were present throughout the sampling period. It will be of interest to examine the expression of these genes after a pulse with monocyte-conditioned medium. The point at which specific mRNAs increase for each of these two proteins was similar within each experiment (~10 h) and suggested coordinacy of their response. However, the finding that C₃ is regulated by Il-1 whereas AGP is not, points to different mechanisms of induction, albeit temporally coordinate. Albumin mRNA levels showed a decline at the earliest time studied consistent with a reduction of transcription and/or specific mRNA degradation. Preliminary analysis of transcription at the albumin locus using isolated nuclei from stimulated and unstimulated cultures indicates that albumin is down-regulated by a decline in transcription.

Previous reports (33, 35) have ascribed the induction of serum amyloid A production (also an acute phase reactant) and the total response in animals (5) to the action of Il-1. Ramadori et al. (30) have shown Il-1 to be stimulatory to factor B and serum amyloid A in primary murine hepatocytes. We tested the effect of purified preparations of Il-1 on the hepatoma cells and found it to be only partially active as a stimulating factor. While C₃ was induced by Il-1 and albumin repressed, other acute phase reactants were unresponsive. Woloski and Fuller (38) reported that fractions from supernates of monocytic cell lines stimulatory to thymocytes failed to increase fibrinogen production in primary rat hepatocytes, whereas other fractions elevated this protein. Il-1 not only failed to stimulate fibrinogen production in HEP 3B2 cells, but actually reduced the level of this secreted protein. The stimulation of fibrinogen by monocyte supernates must reflect the interaction of at least two factors, Il-1 which was inhibitory, and a second factor (hepatocyte-stimulating factor) that was stimulatory. An analysis of the inhibition of fibrinogen by Il-1 at the mRNA level is underway.

A second monokine, TNF, has a limited effect in eliciting the acute phase response. While TNF plays a role in the inflammatory response, it is not a major one. Interaction between TNF, Il-1, and other stimulatory factors must be tested to determine whether synergistic processes occur.

Our data show that more than one factor is involved in eliciting the full array of acute phase proteins in man. Our observations underscore the usefulness of an in vitro cell system for the analysis of stimulating factors. Koj et al. (20, 21) have described the variation of protein production in different preparations of primary rat hepatocytes that make this analytical system relatively demanding. Measurement of C₃ production in Hep 3B2 in response to Il-1 may prove to be a reproducible, economical, and simple assay for this monocytic product.

In summary, the existence of a well characterized, transformed human hepatoma line for studies of the coordinate expression of the genes involved in the acute phase response will greatly facilitate the analysis of the factors and mechanism(s) that control these loci. Future studies aimed at identification of specific DNA sequences within or around the acute phase genes that respond to the stimulating factors are being carried out using this in vitro model system in conjunction with gene transfer technologies.

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References

- Aden, D. P., A. Foge, S. Plotkin, I. Damjanow, and B. B. Knowles. 1979. Controlled synthesis of HBsAg in a differentiated human liver carcinomas-derived cell line. *Nature (Lond.)* 282:615-616.
- Baumann, H., G. L. Firestone, T. L. Burgess, K. W. Gross, K. R. Yamamoto, and W. A. Held. 1983. Dexamethasone regulation of α 1-acid glycoprotein and other acute phase reactants in rat liver and hepatoma cells. *J. Biol. Chem.* 258:563-570.
- Baumann, H., W. A. Held, and F. G. Berger. 1984. The acute phase response of mouse liver. *J. Biol. Chem.* 259:566-573.
- Beaudet, A. L., K. J. Matteson, T. S. Su, C. D. Wendt, and W. E. O'Brien. 1982. Construction and screening of a human liver cDNA library. *Pediatr. Res.* 16:189.
- Bornstein, D. L. 1982. Leukocytic pyrogen: a major mediator of the acute phase reaction. *Ann. NY Acad. Sci.* 389:323-337.
- Brennand, J., D. S. Konecki, and C. T. Caskey. 1983. Expression of human and Chinese hamster hypoxanthine-guanine phosphoribosyltransferase cDNA recombinants in cultured Lesch-Nyhan and Chinese hamster fibroblasts. *J. Biol. Chem.* 258:9593-9596.
- Chandra, T., K. Kurachi, E. W. Davie, and S. L. C. Woo. 1981. Induction of α 1-antitrypsin mRNA and cloning of its cDNA. *Biochem. Biophys. Res. Commun.* 103:751-758.
- Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294-5299.
- Clayton, D. F., and J. E. Darnell, Jr. 1983. Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. *Mol. Cell. Biol.* 3:1552-1561.
- Cleveland, D. W., M. A. Lopata, N. J. Cowan, R. J. MacDonald, W. J. Rutter, and M. W. Kirschner. A study of the number and evolutionary conservation of genes coding for α and β tubulin and β and γ cytoplasmic actin using cloned cDNA probes. 1980. *Cell.* 20:95-105.
- DeBruijn, M. H. L., and G. H. Fey. 1985. Human complement component C3: cDNA coding sequence and derived primary structure. *Proc. Natl. Acad. Sci. USA.* 82:708-712.
- Dente, L., G. Ciliberto, and R. Cortese. Structure of the human α 1-acid glycoprotein gene: sequenced homology with other human acute phase protein genes. *Nucleic Acids Res.* 13:3941-3953.
- Fogh, J., and H. Fogh. 1965. Chromosome changes in PPLO-infected FL human amnion cells. *Proc. Soc. Exp. Biol. Med.* 119:233-238.
- Friedman, J. M., E. Y. Chung, and J. E. Darnell, Jr. 1984. Gene expression during liver regeneration. *J. Mol. Biol.* 179:37-53.
- Gewurz, H. 1982. Biology of C-reactive protein and the acute phase response. *Hosp. Pract.* 17:67-81.
- Glisin, V., R. Crkvenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry.* 13:2633-2637.
- Greininger, G., P. W. Plant, T. J. Liang, R. G. Kalb, D. Amrani, M. W. Mosesson, K. M. Hertzberg, and J. Pindyck. 1983. Hormonal regulation of fibrinogen synthesis in cultured hepatocytes. *Ann. NY Acad. Sci.* 408:469-489.
- Higuchi, R., D. Stang, K. Brown, D. Martin, M. Hudt, J. Lipeles, and W. Salsler. 1981. Human ribosomal RNA gene spacer sequences are found interspersed elsewhere in the genome. *Gene.* 15:177-186.
- Kant, J. A., S. T. Lord, and G. R. Crabtree. 1983. Partial mRNA sequences for human A α , B β , and γ fibrinogen chains: evolutionary and functional implications. *Proc. Natl. Acad. Sci. (USA).* 80:3953-3957.
- Koj, A., J. Gauldie, E. Regoeczi, D. N. Sauder, and G. D. Sweeney. 1984. The acute-phase response of cultured rat hepatocytes. *Biochem. J.* 224:505-514.
- Koj, A., J. Gauldie, G. D. Sweeney, E. Regoeczi, and D. N. Sauder. 1985. A simple bioassay for monocyte-derived hepatocyte stimulating factor: increased synthesis of α ₂-macroglobulin and reduced synthesis of albumin by cultured rat hepatocytes. *J. Immunol. Methods.* 76:317-327.
- Kushner, I., H. Gewurz, and M. D. Benson. 1981. C-reactive protein and the acute phase response. *J. Lab. Clin. Med.* 97:739-749.
- Lachman, L. B., L. C. N. Shih, and D. C. Brown. 1985. Interleukin-1 from human leukemic monocytes. *Methods Enzymol.* 116:467-479.
- Lawn, R. M., J. Adelman, S. C. Bock, A. E. Franke, C. M. Hauck, R. C. Najarian, P. H. Seeburg, and K. L. Wion. 1981. The sequence of human serum albumin cDNA and its expression in *E. coli*. *Nucleic Acids Res.* 9:6103-6114.
- 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.)* 312:458-462.

26. MacIntyre, S. S., D. Schultz, and I. Kushner. 1983. Synthesis and secretion of C-reactive protein by rabbit primary hepatocyte cultures. *Biochem. J.* 210:707-715.
27. Macnab, G. M., J. J. Alexander, G. Lecatsas, E. M. Bey, and J. M. Urbanowicz. 1976. Hepatitis B surface antigen produced by a human hepatoma cell line. *Br. J. Cancer.* 34:509-515.
28. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: a laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
29. Pepys, M. B., and M. L. Balz. 1983. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol.* 34:141-212.
30. Ramadori, G., J. D. Sipe, C. A. Dinarello, S. B. Mizel, and H. R. Colten. 1985. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. *J. Exp. Med.* 162(3):930-942.
31. Ritchie, D. G., and G. M. Fuller. 1983. Hepatocyte-stimulating factor: a monocyte-derived acute phase regulatory protein. *Ann. NY Acad. Sci.* 408:490-502.
32. Rupp, R. G., and G. M. Fuller. 1979. The effects of leukocytic and serum factors on fibrinogen biosynthesis in cultured hepatocytes. *Exp. Cell Res.* 118:23-30.
33. Sipe, J. D., S. W. Vogel, M. B. Sytein, M. Skinner, and A. S. Cohen. 1982. The role of interleukin 1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. *Ann. NY Acad. Sci.* 389:137-150.
34. Stearman, R. S., C. A. Lowell, W. R. Pearson, and J. F. Morrow. 1982. Regulation of synthesis of amyloid A-related protein. *Ann. NY Acad. Sci.* 389:106-115.
35. Sztejn, M. B., S. N. Vogel, J. D. Sipe, P. A. Murphy, S. B. Mizel, J. J. Oppenheim, and P. 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.* 63:164-176.
36. Tucci, A., G. Goldberg, A. S. Whitehead, R. M. Kay, D. E. Woods, and H. R. Colten. 1983. Biosynthesis and postsynthetic processing of human C-reactive protein. *J. Immunol.* 131:2416-2419.
37. Whitehead, A. S., G. A. P. Bruns, A. F. Markham, H. R. Colten, and D. E. Woods. 1983. Isolation of human C-reactive protein complementary DNA and localization of the gene to chromosome 1. *Science (Wash. DC).* 221:69-71.
38. Woloski, B. M. R. N. J., and G. M. Fuller. 1985. Identification and partial characterization of hepatocyte-stimulating factor from leukemia cell lines: comparison with interleukin 1. *Proc. Natl. Acad. Sci. (USA).* 82:1443-1447.