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Research Article

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Interferon β2/Interleukin 6 Modulates Synthesis of α_1-Antitrypsin in Human Mononuclear Phagocytes and in Human Hepatoma Cells

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Abstract

The cytokine IFN β2/IL-6 has recently been shown to regulate the expression of genes encoding hepatic acute phase plasma proteins. INF β2/IL-6 has also been shown to be identical to MGI-2, a protein that induces differentiation of bone marrow precursor cells toward mature granulocytes and monocytes. Accordingly, we have examined the effect of IFN β2/IL-6 on expression of the IL-1- and tumor necrosis factor-unresponsive acute phase protein α_1-antitrypsin (α_1 AT) in human hepatoma-derived hepatocytes and in human mononuclear phagocytes. Purified human fibroblast and recombinant IFN β2/IL-6 each mediate a specific increase in steady-state levels of α_1 AT mRNA and a corresponding increase in net synthesis of α_1 AT in primary cultures of human peripheral blood monocytes as well as in HepG2 and Hep3B cells. Thus, the effect of IFN β2/IL-6 on α_1 AT gene expression in these cells is primarily due to an increase in accumulation of α_1 AT mRNA and can be distinguished from the direct, predominantly translational effect of bacterial lipopolysaccharide on expression of this gene in monocytes and macrophages. The results indicate that IFN β2/IL-6 regulates acute phase gene expression, specifically α_1 AT gene expression, in extrahepatic as well as hepatic cell types.

Introduction

α_1-Antitrypsin (α_1 AT)[1] is a serum glycoprotein that constitutes the principle inhibitor of neutrophil elastase in tissue fluids (reviewed in references 1 and 2). It is a prototype of the serine protease inhibitor (serpin) supergene family which includes a number of other proteins that inactivate destructive enzymes by the formation of covalently stabilized complexes (reviewed in references 3 and 4). A severe deficiency of α_1 AT, characterized by selective intracellular retention of the mutant protein (5–9), is associated with chronic liver disease and premature development of pulmonary emphysema.

α_1 AT is synthesized in human liver cells and macrophages (9–13). Expression of α_1 AT in human blood monocytes and bronchoalveolar macrophages is subject to tissue-specific regulation by endotoxin (14) and by the target enzyme elastase (15). Endotoxin increases synthesis of α_1 AT in macrophages predominantly by increasing the translational efficiency of α_1 AT mRNA (14, 16). Elastase, neutrophilic or pancreatic, elicits a selective increase in steady-state levels of α_1 AT mRNA and a corresponding increase in synthesis of α_1 AT in macrophages, probably through the formation of elastase-α_1 AT complexes in the extracellular fluid (15). Synthesis of α_1 AT in human hepatoma cells is not affected by endotoxin or elastase (14, 15).

Plasma concentrations of α_1 AT increase three- to fourfold during the host response to inflammation/tissue injury and hence it is considered a positive acute phase reactant (17, 18). α_1 AT synthesis in human hepatoma cells is not affected by the acute phase mediators IL-1 or cachectin/tumor necrosis factor (TNF) (19, 20). Several recent studies indicate that another cytokine, IFN β2/IL-6, has distinct effects on many hepatic acute phase reactants and regulates the expression of these proteins in hepatoma cells in a manner characteristic of the acute phase response (21, 22). Subsequent studies have suggested that IFN β2/IL-6 also regulates synthesis of α_1 AT in human hepatoma cells (23–25). IFN β2/IL-6 has a number of other biological activities. It is identical to the B lymphocyte-stimulating factor BSF-2, to the hybridoma/plasmacytoma growth factor, and to MGI-2, a protein identified by its ability to induce differentiation of myeloid cells into granulocytes and monocytes (reviewed in reference 26). In the present study we have not only examined the effect of IFN β2/IL-6 on α_1 AT synthesis in human hepatoma cells but have also evaluated the effects of this cytokine on α_1 AT synthesis in cells from extrahepatic tissues, specifically human mononuclear phagocytes.

Methods

Materials. DME and DME lacking methionine were purchased from Gibco Laboratories, Grand Island, NY. HBSS and Medium 199 were purchased from Microbiological Associates, Walkersville, MD. FCS, L-glutamine, and penicillin-streptomycin were from Flow Laboratories, Inc., McLean, VA. [^{35}S]Methionine was purchased from ICN Radiochemicals, Irvine, CA, [^{32}P]deoxycytidine triphosphate (specific radioactivity ~ 3,000 Ci/mmol) was obtained from New England Nuclear, Boston, MA, and [^{14}C]methylated protein standards were from Amersham Corp., Arlington Heights, IL. Other reagents included IgG-Sorb from Enzyme Center, Cambridge, MA, and guanidine isothiocyanate from Fluka AG, Buchs, Switzerland. Polymyxin B was purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN. Rabbit anti-human α_1 AT and rabbit anti-human α_2-macroglobulin were from Dako Corp., Santa Barbara, CA, and goat anti-human albumin was purchased from Atlantic Antibodies, Scarborough, ME. Lipopolysaccharide (LPS) preparations extracted from *Escherichia coli* serotype 0111:B4 by Westphal phenolic extraction was purchased

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1. *Abbreviations used in this paper:* α_1 AT, α_1-antitrypsin; TNF, tumor necrosis factor.

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from Sigma Chemical Co., St. Louis, MO. *E. coli* 0113 LPS from Associates of Cape Cod, Inc., Woods Hole, MA, was also used. Recombinant human IL-1β was kindly provided by Dr. Bill Joy, St. Louis, MO (specific activity in murine thymocyte costimulation assay, 2×10^6 U/mg), and polyclonal antiserum to recombinant human IL-1β was purchased from Cistron Technology, Pine Brook, NJ. Recombinant human TNFα (specific activity in L cell cytotoxicity assay, 4.02×10^7 U/mg) was obtained from Genentech, South San Francisco, CA, and recombinant IFNγ (specific activity in antiviral assay, 1.3×10^7 U/mg) obtained from Biogen Corp., Boston, MA. Preparation of purified human fibroblast IFN β2/IL-6, *E. coli*-derived human IFN β2/IL-6, and rabbit polyclonal antiserum to *E. coli*-derived human IFN β2/IL-6 has been previously described (27). The apparent specific activity of the *E. coli*-derived human IFN β2/IL-6 is $\sim 10^6$ IU/mg protein in assays for antiviral activity and B cell differentiating activity (27).

Cell culture. Confluent monolayers of human peripheral blood monocytes from healthy individuals were established by adherence of dextran-purified leukocytes on charged tissue culture plastic (Primaria; Becton-Dickinson Labware, Lincoln Park, NJ) (15). Each individual had a normal PiMM α_1 AT phenotype as defined by isoelectric focusing and plasma concentrations of α_1 AT. Bronchoalveolar macrophages were obtained from sterile saline bronchial lavage and allowed to adhere to the same tissue culture surfaces. HepG2 and Hep3B cells were maintained in culture as previously described (28).

Metabolic labeling. Confluent monolayers were rinsed and incubated at 37°C in the presence of methionine-free medium containing [^{35}S]methionine, 250 μCi/ml (pulse period). To determine the net synthesis of α_1 AT or control secretory proteins, cells were subjected to a short pulse interval (20 min), and radiolabeled proteins were detected in the cell lysate alone. To determine the rate of secretion of α_1 AT, cells were subjected to a pulse period of 10 min, rinsed, and incubated in serum-free medium containing an excess of unlabeled methionine (chase period). Methods for solubilization of cells and clarification of cell lysates after labeling have been described (28). Total protein synthesis was estimated by TCA precipitation of aliquots of cell lysates and culture fluid (29).

Immunoprecipitation and analytical gel electrophoresis. Aliquots of cell lysate or medium were incubated overnight at 4°C in 1% Triton X-100/1.0% SDS/0.5% deoxycholic acid, with excess antibody. Immune complexes were precipitated with excess formalin-fixed staphylococci-bearing protein A, washed, released by boiling in sample buffer, and applied to 9.0% SDS-PAGE under reducing conditions (30). ^{14}C-Methylated molecular size markers (200,000, 92,500, 68,000, 46,000, 30,000, and 17,000 mol wt) were incubated on all gels. After electrophoresis, gels were stained in Coomassie brilliant blue, destained, impregnated with 2,5-diphenyloxazole (EN^{3}HANCE; New England Nuclear), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY). Laser densitometer 2222 ultrascan XL from LKB Instruments, Inc., Houston, TX, was used for scanning of fluorograms.

Detection of RNA by RNA blot analysis. Total cellular RNA was isolated from adherent monolayers of monocytes, macrophages, or hepatoma cells, by guanidine isothiocyanate extraction and ethanol precipitation (31). RNA was subjected to agarose-formaldehyde gel electrophoresis and transferred to nitrocellulose filters (32). Filters were then hybridized with ^{32}P-labeled cDNA specific for human α_1 AT (15), HSP 90 (kindly provided by Dr. Neil Rebbe, St. Louis, MO), or ubiquitin (33).

Results

IFN β2/IL-6 mediates a specific increase in synthesis of α_1 AT not only in hepatoma cells but also in monocytes and macrophages. The net synthesis of α_1 AT in Hep3B cells increases substantially in the presence of purified human fibroblast IFN β2/IL-6 or recombinant human IFN β2/IL-6, but not in the presence of recombinant IL-1β (Fig. 1, *left*). The effect of IFN β2/IL-6 on synthesis of α_1 AT is specific in that synthesis of albumin decreases in these cells under the same conditions (*right*). IL-1β is biologically active in this experiment as shown by the decrease in albumin synthesis (*right*) and increase in complement factor B synthesis (data not shown). The effect of IFN β2/IL-6 on synthesis of α_1 AT is concentration dependent (data not shown). Synthesis of α_1 AT is affected similarly in the Hep3B and HepG2 cells (Figs. 1 and 2).

Specificity of the IFN β2/IL-6 effect was also examined by antibody blocking experiments (Fig. 2). The increase in synthesis of α_1 AT mediated by IFN β2/IL-6 is completely neutralized by antibody to IFN β2/IL-6 but is not affected by antibody to IL-1β. Antibody to IFN β2/IL-6 by itself does not affect the baseline synthesis of α_1 AT.

We also examined the possibility that IL-1β or glucocorticoids have additive, synergistic, or antagonistic interactions with IFN β2/IL-6 in modulation of α_1 AT synthesis. Separate monolayers of HepG2 cells were incubated with control serum-free medium, medium supplemented with IFN β2/IL-6, IL-1β alone, or medium supplemented with both cytokines. These experiments showed that IL-1β has no additive, synergistic, or antagonistic interaction with IFN β2/IL-6 in regulating α_1 AT; i.e., there was no difference in synthesis of α_1 AT

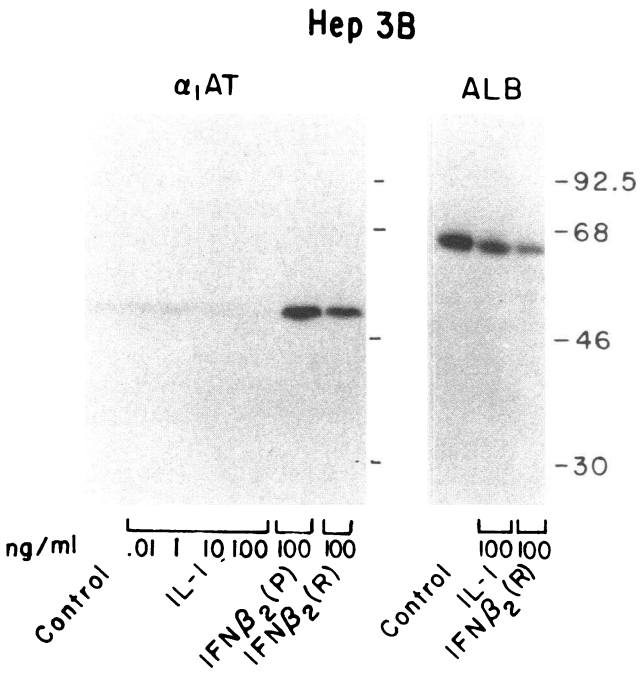

Figure 1. Effect of IFN β2/IL-6 on synthesis of α_1 AT and albumin (*ALB*) in human hepatoma cells. Hep3B cells were incubated for 18 h in serum-free control medium, medium supplemented with recombinant human IL-1β, purified human fibroblast IFN β2/IL-6 (*P*), or recombinant human IFN β2/IL-6 (*R*) in the specified concentrations. Cells were then rinsed thoroughly and incubated for 20 min in [^{35}S]-methionine, 250 μCi/ml DME lacking methionine. Cells were again rinsed and then homogenized. Cell lysates were clarified and subjected to sequential immunoprecipitation with rabbit anti-human α_1 AT and then goat anti-human albumin. Immunoprecipitates were then subjected to 9% SDS-PAGE followed by fluorography. There were no significant differences in the TCA-precipitable radiolabeled proteins under these conditions. Molecular mass markers are indicated at the right margin.

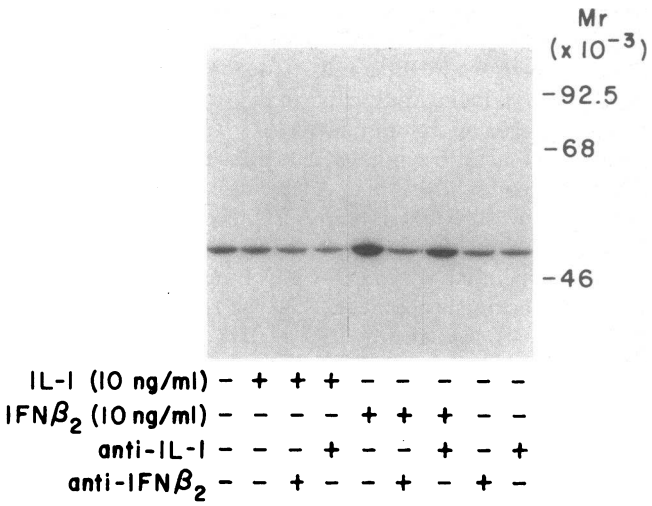

Figure 2. Regulation of α_1 AT synthesis by IFN β2/IL-6 is neutralized by antiserum to recombinant human IFN β2/IL-6. HepG2 cells were incubated for 18 h in control medium, medium supplemented with recombinant IL-1β, IFN β2/IL-6, antiserum to recombinant IL-1β, or antiserum to recombinant IFN β2/IL-6. Separate monolayers were coincubated with IFN β2/IL-6 and each antiserum, or coincubated with IL-1β and each antiserum. Lower effective concentrations of IFN β2/IL-6 (10 ng/ml) were used to facilitate a state of antibody excess. Antisera were used at ~ 10 μg/ml for this experiment. Assay for synthesis of α_1 AT was conducted according to the protocol in Fig. 1 legend. Molecular mass markers are indicated at the right margin.

mediated by IFN β2/IL-6 alone as compared with IFN β2/IL-6 together with IL-1β (Table I). In the same experiments it could be shown that IFN β2/IL-6 and IL-1β have an additive effect on synthesis of complement protein factor B (data not shown). Separate monolayers of HepG2 cells were also incubated with INF β2/IL-6 and dexamethasone. In this case there was also no evidence of additive, synergistic, or antagonistic interaction (Table I). Dexamethasone alone did not affect synthesis of α_1 AT in HepG2 cells. In the same experiments IFN β2/IL-6 and dexamethasone had an additive effect on synthesis of α_1 acid glycoprotein (data not shown).

IFN β2/IL-6 also mediates an increase in synthesis of α_1 AT in peripheral blood monocytes (Fig. 3 *A*). This increase is concentration dependent and first evident at an IFN β2/IL-6 concentration of 1 ng/ml (with longer fluorographic exposure of the gel used for Fig. 3 *A*). The magnitude of the effect of IFN β2/IL-6 is less than that of LPS at comparable concentrations (Fig. 3 and data not shown). The effect of IFN β2/IL-6 on monocyte α_1 AT synthesis is also specific in that there is a decrease in synthesis of α_2 macroglobulin in the same cells under these conditions (Fig. 3 *B*). IFN β2/IL-6 has a similar

Table I. Effect of IFN β2/IL-6, IL-1β, and Glucocorticoids on Synthesis of α_1 AT in HepG2 Cells

Effector	Fold increase over control*
IFN β2/IL-6 (100 ng/ml)	3.12±1.07
IL-1β (100 ng/ml)	0.98±0.13
IFN β2/IL-6 (100 ng/ml) + IL-1β (100 ng/ml)	3.29±0.82
Dexamethasone (1 nM)	1.23±0.67
Dexamethasone (0.1 nM)	1.12±0.98
IFN β2/IL-6 (100 ng/ml) + dexamethasone (1 nM)	3.62±1.17
IFN β2/IL-6 (100 ng/ml) + dexamethasone (0.1 nM)	3.17±0.97

* Mean±1 SD for three separate determinations.

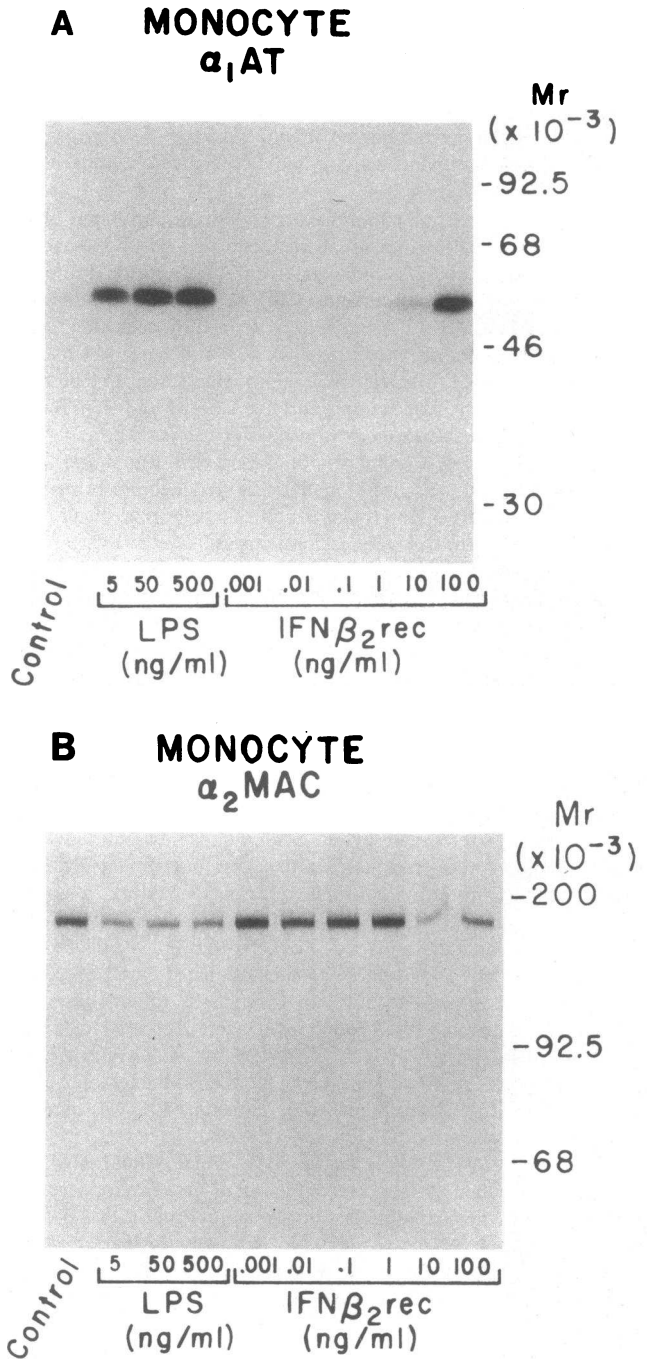

Figure 3. Effect of IFN β2/IL-6 on synthesis of α_1 AT (*A*) and α_2 macroglobulin (*B*) in human peripheral blood monocytes. After 24 h in culture, monocytes were incubated for 18 h with serum-free control medium, medium supplemented with LPS, or recombinant IFN β2/IL-6 (*rec*) in the specified concentrations. Monolayers were then radiolabeled and cell lysates subjected to sequential immunoprecipitation with anti-human α_1 AT and then anti-human α_2 macroglobulin. Molecular mass markers are indicated at the right margin.

effect on synthesis of α_1 AT and α_2 macroglobulin in bronchoalveolar macrophages (data not shown). The lowest effective concentration of IFN β2/IL-6 and the magnitude of its effect on α_1 AT synthesis were, in general, similar in hepatoma cells and macrophages.

Since LPS independently mediates an increase in synthesis of α_1 AT and decrease in α_2 macroglobulin synthesis in human monocytes, we examined the possibility that LPS contamination of IFN β2/IL-6 preparations influences the results of experiments in monocytes. First, the concentration of LPS in recombinant human IFN β2/IL-6 preps was directly measured by limulus amebocyte lysate assay. There is < 10 pg/ml in all undiluted stock preparations used for these experiments. Second, separate monolayers of monocytes were incubated with LPS or IFN β2/IL-6 in the presence of polymyxin B (Fig. 4). Polymyxin B completely neutralizes the effect of LPS on synthesis of α_1 AT but only partially reduces the effect of IFN β2/IL-6 in the same experiment. Densitometric scanning of fluorograms (after longer exposure to the gel) indicates that there is a 3.4-fold increase in synthesis of α_1 AT mediated by IFN β2/IL-6 in the presence of polymyxin B.

Because LPS enhances the production of IFN β2/IL-6 in monocytes (22, 27), we examined the possibility that the known effect of LPS on monocyte α_1 AT synthesis results from the autocrine, or paracrine, effect of endogenous IFN β2/IL-6. Separate monolayers of monocytes were incubated with LPS or IFN β2/IL-6 in the presence of antibody to IFN β2/IL-6. Although the effect of IFN β2/IL-6 on synthesis of α_1 AT is completely neutralized by this antibody in hepatoma cells (Fig. 2) and in monocytes (data not shown), the effect of LPS on synthesis of α_1 AT in monocytes is not at all neutralized by antibody to IFN β2/IL-6 (Fig. 5). The effect of LPS on synthesis of α_1 AT in this experiment, a 6.2-fold increase, can be almost completely neutralized (74%) by polymyxin B (Fig. 5). These data, together with data on the effect of IFN β2/IL-6 and LPS on monocyte α_1 AT RNA levels (see below), suggest that the effects of LPS and IFN β2/IL-6 on monocyte α_1 AT gene expression are, at least in part, distinct.

Synthesis of α_1 AT in monocytes is not affected by other cytokines such as IL-1β, TNFα, and IFNγ (34). In two separate experiments monocytes were coincubated with IFN β2/IL-6 and one of these three other cytokines. There is no additive, synergistic, or antagonistic effect on α_1 AT synthesis by IL-1β, TNFα, or IFNγ (Table II). There is also no additive, synergistic, or antagonistic effect of dexamethasone together with IFN β2/IL-6. IFN β2/IL-6 and LPS have an additive effect on synthesis of α_1 AT in monocytes (Table II).

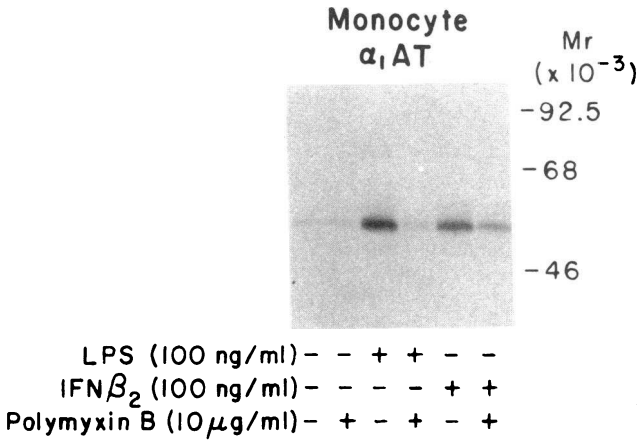

Figure 4. Effect of IFN β2/IL-6 on monocyte α_1 AT synthesis is independent of LPS. Separate monolayers of monocytes were incubated in control medium, medium supplemented with LPS, or IFN β2/IL-6, each in the absence or presence of polymyxin B. Synthesis of α_1 AT was determined as described above. Molecular mass markers are indicated at the right margin.

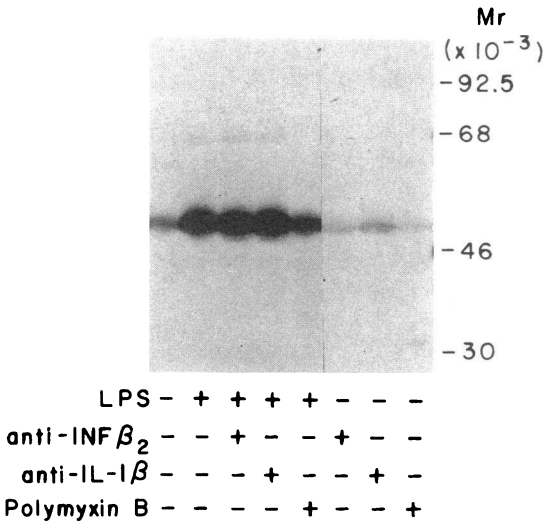

Figure 5. The effect of LPS on monocyte α_1 AT synthesis is distinct from that of IFN β2/IL-6. After 24 h in culture monocytes were incubated for 18 h with control medium, medium supplemented with LPS (10 ng/ml), antiserum to IL-1β (10 mcg/ml), antiserum to IFN β2/IL-6 (10 mcg/ml), or polymyxin B (10 mcg/ml). Separate monolayers were coincubated with LPS and anti-IL-1, anti-IFN β2/IL-6, or polymyxin B. Synthesis of α_1 AT was determined by the methods described above. Molecular mass markers are indicated at the right margin.

The effect of IFN β2/IL-6 on α_1 AT expression is pretranslational. Steady-state levels of α_1 AT mRNA increase in hepatoma cells (Fig. 6, *top*) and in blood monocytes (Fig. 6, *bottom*) incubated in IFN β2/IL-6-supplemented cell culture fluid. The increase in α_1 AT mRNA levels corresponds to the increase in

Table II. Effect of IFN β2/IL-6, Other Cytokines, LPS, and Glucocorticoids on Synthesis of α_1 AT in Monocytes

Effector	Fold increase over control*
IFN β2/IL-6 (100 ng/ml)	3.73±1.02
IL-1β (100 ng/ml)	1.20±0.21
TNFα (100 ng/ml)	0.97±0.17
IFNγ (100 U/ml)	1.17±0.21
IFN β2/IL-6 (100 ng/ml) + IL-1β (100 ng/ml)	3.81±1.31
IFN β2/IL-6 (100 ng/ml) + TNFα (100 ng/ml)	3.30±1.31
IFN β1/IL-6 (100 ng/ml) + IFNγ (100 U/ml)	3.71±1.80
LPS (10 ng/ml)	3.72±1.63
IFN β2/IL-6 (100 ng/ml) + LPS (100 ng/ml)	5.36±0.38
Dexamethasone (0.1 nM)	0.94±0.27
INF β2/IL-6 (100 ng/ml) + dexamethasone (0.1 nM)	3.28±1.00

* Mean±1 SD for two separate determinations.

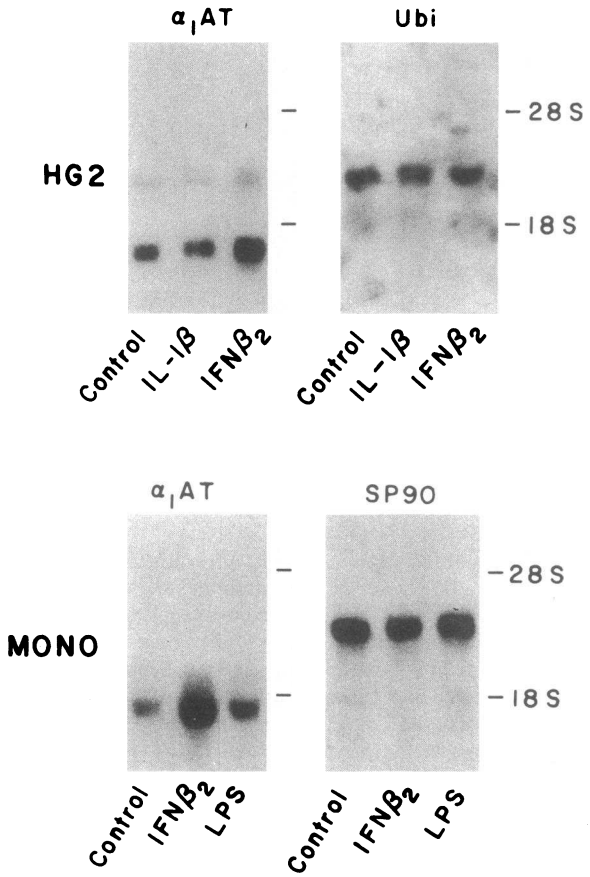

Figure 6. IFN β2/IL-6 increases steady-state levels of α_1 AT mRNA in human hepatoma cells (*top*) and blood monocytes (*bottom*). HepG2 cells were incubated for 18 h in control medium, medium supplemented with IL-1β (100 ng/ml), or with IFN β2/IL-6 (100 ng/ml). Monocytes were incubated for 18 h in control medium, medium supplemented with IFN β2/IL-6 (100 ng/ml), or medium supplemented with LPS (10 ng/ml). Total cellular RNA was then isolated in guanidine isothiocyanate, purified, and subjected to RNA blot analysis with radiolabeled α_1 AT cDNA (*left*) as previously described. Blots were then stripped by boiling in Tris-EDTA so that they could be hybridized with radiolabeled polyubiquitin cDNA (*top, right*) or SP 90 cDNA (*bottom, right*). There was no difference in ethidium bromide–stained ribosomal RNA bands on these blots. 28S and 18S ribosomal RNA markers are indicated at the right margin.

synthesis of α_1 AT (Figs. 1 and 3) mediated by IFN β2/IL-6. The effect of IFN β2/IL-6 on α_1 AT mRNA levels is also specific in that there is no change in other specific mRNAs on the same blots as demonstrated for polyubiquitin mRNA in hepatoma cells (Fig. 6, *top*) and for human stress protein SP 90 in blood monocytes (Fig. 6, *bottom*). IL-1β has no effect on α_1 AT mRNA levels in hepatoma cells (Fig. 6, *top*) and LPS has a minimal effect (< 1.5-fold) on α_1 AT mRNA levels in monocytes (Fig. 6, *bottom;* references 14 and 28). The difference in the effects of IFN β2/IL-6 and LPS on steady-state levels of α_1 AT mRNA in monocytes represents additional evidence that each mediates a distinct form of regulation for this particular gene. These data do not exclude the possibility that part of the effect of LPS on α_1 AT gene expression is mediated indirectly through induction of IFN β2/IL-6 expression in these cells.

IFN β2/IL-6 does not affect the kinetics of secretion of α_1 AT. Human hepatoma cells were subjected to pulse-chase radiolabeling after having been incubated for 18 h in serum-free control medium (Fig. 7, *top*) or medium supplemented with IFN β2/IL-6 (*bottom*). In this experiment a 52-kD α_1 AT precursor polypeptide is synthesized and begins to disappear during the chase period. It is converted to the fully glycosylated 55-kD α_1 AT molecule by 20–40 min of the chase period coincident with secretion of the 55-kD mature protein into the extracellular fluid. The half-times for conversion to the fully glycosylated form, disappearance from the intracellular compartment, and appearance in the extracellular fluid are not significantly different after an approximately threefold increase in net synthesis is induced by IFN β2/IL-6.

IFN β2/IL-6 also does not affect the kinetics of secretion of α_1 AT in monocytes (data not shown).

Discussion

Results of these experiments indicate that IFN β2/IL-6 mediates a specific increase in expression of α_1 AT in human hepatocytes and mononuclear phagocytes. In each cell type effec-

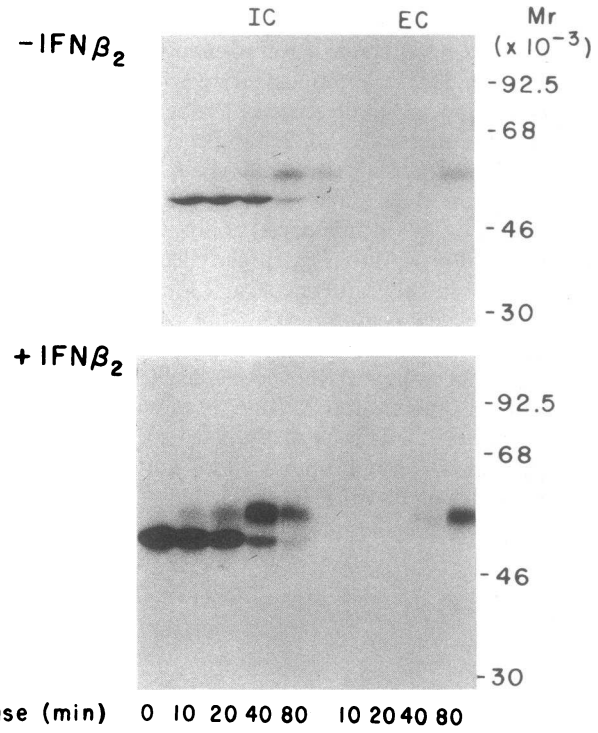

Figure 7. Effect of IFN β2/IL-6 on the kinetics of secretion of α_1 AT in human hepatoma cells. HepG2 cells were incubated for 18 h in serum-free control medium (*−IFNβ2*) or medium supplemented with IFN β2/IL-6 (100 ng/ml) (*+IFNβ2*). Cells were then rinsed thoroughly and incubated for 10 min in [^{35}S]methionine, 250 μCi/ml DME lacking methionine (pulse period). Cells were rinsed again and then incubated in complete DME for intervals up to 80 min. At specified time intervals extracellular medium (*EC*) was harvested and cells were lysed (*IC*). These samples were then subjected to immunoprecipitation and SDS-PAGE, followed by fluorography as described above. Fluorograms were then scanned by densitometry to estimate the kinetics of secretion. Molecular mass markers are indicated at the right margin. The photograph of the pulse chase experiment in the upper panel is slightly smaller in magnification than that in the lower panel but the time points are easily distinguished.

tive concentrations are well within the range of serum concentrations of IFN β2/IL-6 during acute infections or during in vivo administration of inflammatory mediators (35–37). The effect of IFN β2/IL-6 on α_1 AT gene expression is exclusively pretranslational in that the increase in steady-state levels of α_1 AT mRNA corresponds to the increase in net synthesis of α_1 AT. The effect of IFN β2/IL-6 on this gene is readily distinguished from that of acute phase mediators IL-1β and TNFα since these other cytokines have no effect on α_1 AT synthesis (19). The effect of IFN β2/IL-6 on monocyte/macrophage α_1 AT gene expression is also distinct from that of LPS.

Several well-characterized and highly purified factors have been shown to modulate the expression of the human α_1 AT gene. LPS and elastase mediate distinct positive regulatory effects on α_1 AT synthesis, but these effects are specific for cells of the mononuclear phagocyte lineage (14–16). IFN β2/IL-6 is, therefore, the first well-characterized factor to affect the expression of α_1 AT in human hepatocytes and the first factor capable of modulating the α_1 AT gene in several different cell types. It is also noteworthy that α_1 AT expression in mononuclear phagocytes and hepatocytes is affected by IFN β2/IL-6 in that there are cell-specific differences in constitutive expression of α_1 AT in mononuclear phagocytes and hepatocytes. In fact, there is an alternative start site for transcription of α_1 AT mRNA in macrophages, ~ 2 kb upstream from that governing transcription of α_1 AT mRNA in hepatocytes (38, 39).

Although these data do not directly address the question of whether there is a local extrahepatic acute phase response in vivo, there is a growing body of evidence that local extrahepatic synthesis of acute phase plasma proteins is modulated during tissue injury/inflammation in vivo. There is an increase in serum amyloid A, complement factor B, and C3 mRNA levels in extrahepatic tissues of the mouse after systemic administration of LPS or casein (40–42). Moreover, in mice with lupus glomerulonephritis there is an increase in factor B and C3 expression in the kidney which exceeds the concomitant increase in hepatic factor B and C3 expression (43).

Results of the current study also show that IFN β2/IL-6 and LPS have similar effects on α_1 AT and α_2 macroglobulin expression in monocytes and macrophages, but several lines of evidence argue against the possibility that the effect of LPS is mediated indirectly through induction of IFN β2/IL-6 release by these cells. First, the effect of LPS on synthesis of α_1 AT is not blocked by neutralizing antibody to IFN β2/IL-6. Second, the effect of LPS on α_1 AT expression is predominantly translational, whereas that of IFN β2/IL-6 is predominantly pretranslational with increases in α_1 AT mRNA levels corresponding to increases in α_1 AT synthesis. Finally, the magnitude of the LPS effect on α_1 AT synthesis is several-fold greater than that of exogenous IFN β2/IL-6 [4.5- to 8.7-fold for LPS (14); 2.5- to 3.5-fold for IFN β2/IL-6]. These data do not, however, exclude the possibility that part of the effect of LPS on α_1 AT gene expression in monocytes involves the action of endogenous IFN β2/IL-6. In fact, in some individual monocyte donors LPS may increase α_1 AT mRNA levels 1.5- to 2.5-fold (14). Even in these individuals, however, the predominant effect of LPS on α_1 AT expression involves an IFN β2/IL-6-independent mechanism since LPS increases net synthesis of α_1 AT 4.5- to 8.7-fold (14) and increases the specific translational efficiency of α_1 AT mRNA by greater than 3-fold (16).

We also examined the effect of IFN β2/IL-6 on the kinetics of secretion of α_1 AT. There is no direct effect of this cytokine on the kinetics of α_1 AT secretion in liver cells. Moreover, there is no intracellular accumulation of α_1 AT even though net synthesis of the protein has increased approximately threefold as a result of IFN β2/IL-6 action. Similarly, there is no intracellular accumulation of α_1 AT in monocytes from normal individuals in which synthesis of α_1 AT has increased up to eightfold as a result of endotoxin or elastase action (14, 15). Taken together, these data indicate that the pathway for constitutive secretion of plasma proteins in human liver cells and macrophages is sufficient to accommodate physiologically relevant increases in synthesis of the normal α_1 AT protein. These data do not substantiate the concept that upregulation of α_1 AT synthesis in vivo during acute inflammation, or other altered physiologic states, can be responsible for intrahepatocytic accumulation of α_1 AT in normal (PiMM) individuals (44) or in transgenic mice bearing the normal (PiM) human α_1 AT gene (45, 46). In transgenic animals very high levels of expression may be achieved and species-specific differences in mechanisms of protein secretion may be exaggerated. Thus, the intracellular retention of the mutant α_1 AT protein in homozygous PiZZ α_1 AT deficiency, which is exaggerated by the effect of factors that enhance α_1 AT synthesis (14, 15), involves a fundamentally distinct cell biological mechanism.

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