

# Interferon $\beta$ /Interleukin 6 Modulates Synthesis of $\alpha_1$ -Antitrypsin in Human Mononuclear Phagocytes and in Human Hepatoma Cells

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## Abstract

The cytokine IFN  $\beta$ /IL-6 has recently been shown to regulate the expression of genes encoding hepatic acute phase plasma proteins. INF  $\beta$ /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  $\beta$ /IL-6 on expression of the IL-1- and tumor necrosis factor-unresponsive acute phase protein  $\alpha_1$ -antitrypsin ( $\alpha_1$  AT) in human hepatoma-derived hepatocytes and in human mononuclear phagocytes. Purified human fibroblast and recombinant IFN  $\beta$ /IL-6 each mediate a specific increase in steady-state levels of  $\alpha_1$  AT mRNA and a corresponding increase in net synthesis of  $\alpha_1$  AT in primary cultures of human peripheral blood monocytes as well as in HepG2 and Hep3B cells. Thus, the effect of IFN  $\beta$ /IL-6 on  $\alpha_1$  AT gene expression in these cells is primarily due to an increase in accumulation of  $\alpha_1$  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  $\beta$ /IL-6 regulates acute phase gene expression, specifically  $\alpha_1$  AT gene expression, in extrahepatic as well as hepatic cell types.

## Introduction

$\alpha_1$ -Antitrypsin ( $\alpha_1$  AT)<sup>1</sup> 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  $\alpha_1$  AT, characterized by selective intracellular retention of the mutant protein (5-9), is associated with chronic liver disease and premature development of pulmonary emphysema.

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1. Abbreviations used in this paper:  $\alpha_1$  AT,  $\alpha_1$ -antitrypsin; TNF, tumor necrosis factor.

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$\alpha_1$  AT is synthesized in human liver cells and macrophages (9-13). Expression of  $\alpha_1$  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  $\alpha_1$  AT in macrophages predominantly by increasing the translational efficiency of  $\alpha_1$  AT mRNA (14, 16). Elastase, neutrophilic or pancreatic, elicits a selective increase in steady-state levels of  $\alpha_1$  AT mRNA and a corresponding increase in synthesis of  $\alpha_1$  AT in macrophages, probably through the formation of elastase- $\alpha_1$  AT complexes in the extracellular fluid (15). Synthesis of  $\alpha_1$  AT in human hepatoma cells is not affected by endotoxin or elastase (14, 15).

Plasma concentrations of  $\alpha_1$  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).  $\alpha_1$  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  $\beta$ /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  $\beta$ /IL-6 also regulates synthesis of  $\alpha_1$  AT in human hepatoma cells (23-25). IFN  $\beta$ /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  $\beta$ /IL-6 on  $\alpha_1$  AT synthesis in human hepatoma cells but have also evaluated the effects of this cytokine on  $\alpha_1$  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. [<sup>35</sup>S]Methionine was purchased from ICN Radiochemicals, Irvine, CA, [<sup>32</sup>P]deoxycytidine triphosphate (specific radioactivity ~ 3,000 Ci/mmol) was obtained from New England Nuclear, Boston, MA, and [<sup>14</sup>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  $\alpha_1$  AT and rabbit anti-human  $\alpha_2$ -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

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-1 $\beta$  was kindly provided by Dr. Bill Joy, St. Louis, MO (specific activity in murine thymocyte costimulation assay,  $2 \times 10^6$  U/mg), and polyclonal antiserum to recombinant human IL-1 $\beta$  was purchased from Cistron Technology, Pine Brook, NJ. Recombinant human TNF $\alpha$  (specific activity in L cell cytotoxicity assay,  $4.02 \times 10^7$  U/mg) was obtained from Genentech, South San Francisco, CA, and recombinant IFN $\gamma$  (specific activity in antiviral assay,  $1.3 \times 10^7$  U/mg) obtained from Biogen Corp., Boston, MA. Preparation of purified human fibroblast IFN  $\beta$ /IL-6, *E. coli*-derived human IFN  $\beta$ /IL-6, and rabbit polyclonal antiserum to *E. coli*-derived human IFN  $\beta$ /IL-6 has been previously described (27). The apparent specific activity of the *E. coli*-derived human IFN  $\beta$ /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  $\alpha_1$  AT phenotype as defined by isoelectric focusing and plasma concentrations of  $\alpha_1$  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  $\mu$ Ci/ml (pulse period). To determine the net synthesis of  $\alpha_1$  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  $\alpha_1$  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  $\alpha_1$  AT (15), HSP 90 (kindly provided by Dr. Neil Rebbe, St. Louis, MO), or ubiquitin (33).

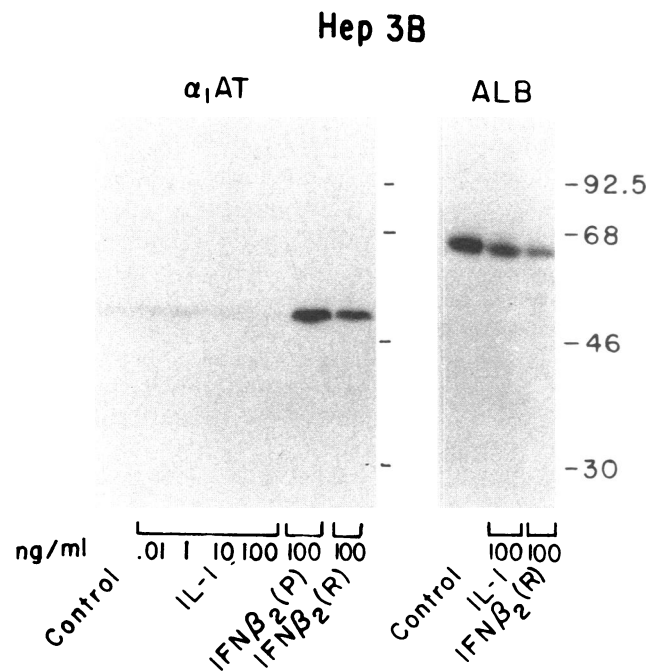
## Results

*IFN  $\beta$ /IL-6 mediates a specific increase in synthesis of  $\alpha_1$  AT not only in hepatoma cells but also in monocytes and macrophages.* The net synthesis of  $\alpha_1$  AT in Hep3B cells increases substantially in the presence of purified human fibroblast IFN  $\beta$ /IL-6 or recombinant human IFN  $\beta$ /IL-6, but not in the

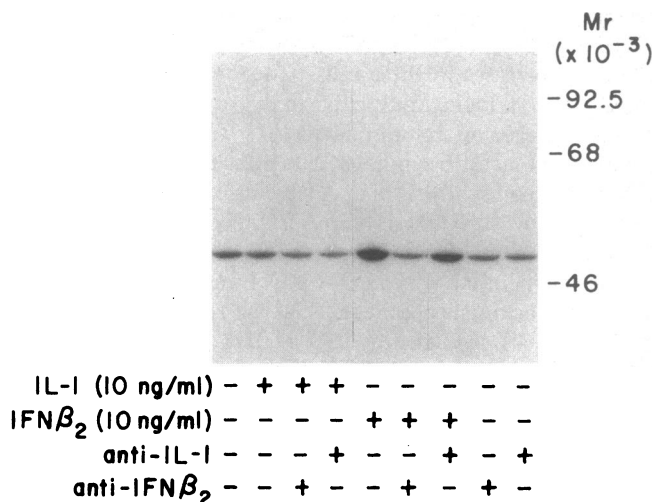
presence of recombinant IL-1 $\beta$  (Fig. 1, left). The effect of IFN  $\beta$ /IL-6 on synthesis of  $\alpha_1$  AT is specific in that synthesis of albumin decreases in these cells under the same conditions (right). IL-1 $\beta$  is biologically active in this experiment as shown by the decrease in albumin synthesis (right) and increase in complement factor B synthesis (data not shown). The effect of IFN  $\beta$ /IL-6 on synthesis of  $\alpha_1$  AT is concentration dependent (data not shown). Synthesis of  $\alpha_1$  AT is affected similarly in the Hep3B and HepG2 cells (Figs. 1 and 2).

Specificity of the IFN  $\beta$ /IL-6 effect was also examined by antibody blocking experiments (Fig. 2). The increase in synthesis of  $\alpha_1$  AT mediated by IFN  $\beta$ /IL-6 is completely neutralized by antibody to IFN  $\beta$ /IL-6 but is not affected by antibody to IL-1 $\beta$ . Antibody to IFN  $\beta$ /IL-6 by itself does not affect the baseline synthesis of  $\alpha_1$  AT.

We also examined the possibility that IL-1 $\beta$  or glucocorticoids have additive, synergistic, or antagonistic interactions with IFN  $\beta$ /IL-6 in modulation of  $\alpha_1$  AT synthesis. Separate monolayers of HepG2 cells were incubated with control serum-free medium, medium supplemented with IFN  $\beta$ /IL-6, IL-1 $\beta$  alone, or medium supplemented with both cytokines. These experiments showed that IL-1 $\beta$  has no additive, synergistic, or antagonistic interaction with IFN  $\beta$ /IL-6 in regulating  $\alpha_1$  AT; i.e., there was no difference in synthesis of  $\alpha_1$  AT



**Figure 1.** Effect of IFN  $\beta$ /IL-6 on synthesis of  $\alpha_1$  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-1 $\beta$ , purified human fibroblast IFN  $\beta$ /IL-6 (P), or recombinant human IFN  $\beta$ /IL-6 (R) in the specified concentrations. Cells were then rinsed thoroughly and incubated for 20 min in [ $^{35}$ S]-methionine, 250  $\mu$ Ci/ml DME lacking methionine. Cells were again rinsed and then homogenized. Cell lysates were clarified and subjected to sequential immunoprecipitation with rabbit anti-human  $\alpha_1$  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  $\alpha_1$  AT synthesis by IFN  $\beta_2$ /IL-6 is neutralized by antiserum to recombinant human IFN  $\beta_2$ /IL-6. HepG2 cells were incubated for 18 h in control medium, medium supplemented with recombinant IL-1 $\beta$ , IFN  $\beta_2$ /IL-6, antiserum to recombinant IL-1 $\beta$ , or antiserum to recombinant IFN  $\beta_2$ /IL-6. Separate monolayers were coincubated with IFN  $\beta_2$ /IL-6 and each antiserum, or coincubated with IL-1 $\beta$  and each antiserum. Lower effective concentrations of IFN  $\beta_2$ /IL-6 (10 ng/ml) were used to facilitate a state of antibody excess. Antisera were used at  $\sim 10 \mu\text{g/ml}$  for this experiment. Assay for synthesis of  $\alpha_1$  AT was conducted according to the protocol in Fig. 1 legend. Molecular mass markers are indicated at the right margin.

mediated by IFN  $\beta_2$ /IL-6 alone as compared with IFN  $\beta_2$ /IL-6 together with IL-1 $\beta$  (Table I). In the same experiments it could be shown that IFN  $\beta_2$ /IL-6 and IL-1 $\beta$  have an additive effect on synthesis of complement protein factor B (data not shown). Separate monolayers of HepG2 cells were also incubated with IFN  $\beta_2$ /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  $\alpha_1$  AT in HepG2 cells. In the same experiments IFN  $\beta_2$ /IL-6 and dexamethasone had an additive effect on synthesis of  $\alpha_1$  acid glycoprotein (data not shown).

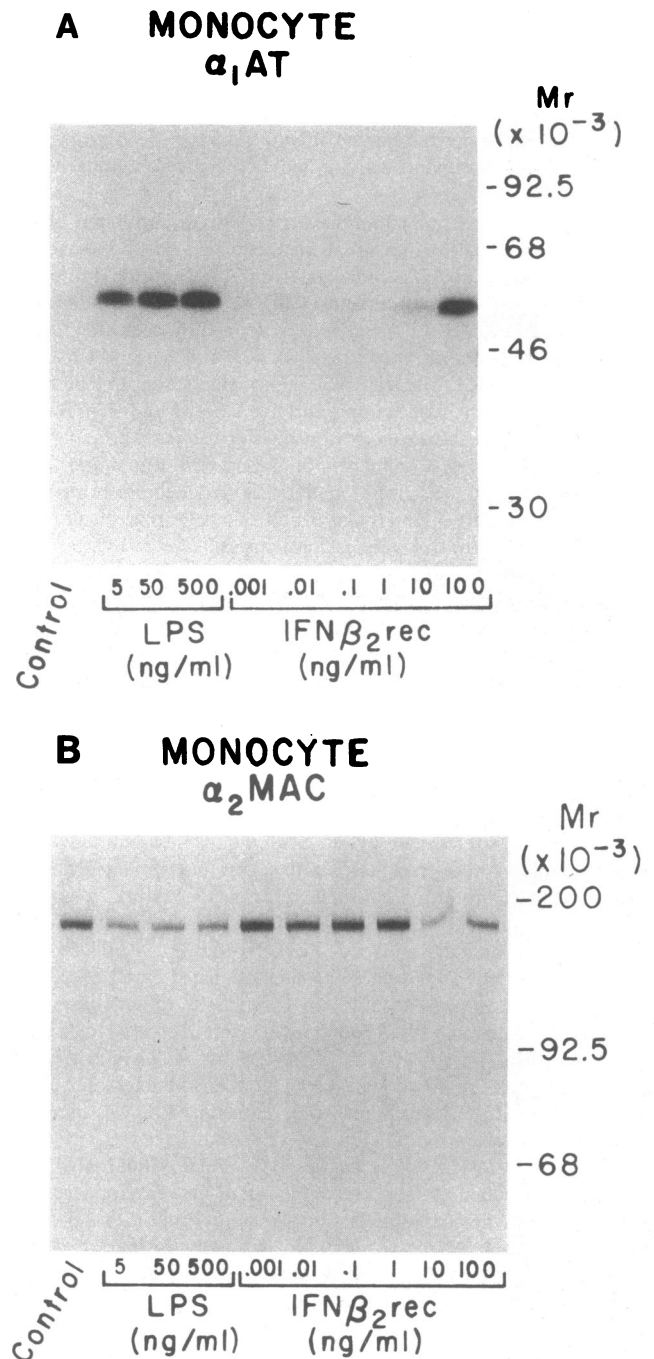
IFN  $\beta_2$ /IL-6 also mediates an increase in synthesis of  $\alpha_1$  AT in peripheral blood monocytes (Fig. 3 A). This increase is concentration dependent and first evident at an IFN  $\beta_2$ /IL-6 concentration of 1 ng/ml (with longer fluorographic exposure

**Table I.** Effect of IFN  $\beta_2$ /IL-6, IL-1 $\beta$ , and Glucocorticoids on Synthesis of  $\alpha_1$  AT in HepG2 Cells

Effector	Fold increase over control*
IFN $\beta_2$ /IL-6 (100 ng/ml)	3.12 $\pm$ 1.07
IL-1 $\beta$ (100 ng/ml)	0.98 $\pm$ 0.13
IFN $\beta_2$ /IL-6 (100 ng/ml) + IL-1 $\beta$ (100 ng/ml)	3.29 $\pm$ 0.82
Dexamethasone (1 nM)	1.23 $\pm$ 0.67
Dexamethasone (0.1 nM)	1.12 $\pm$ 0.98
IFN $\beta_2$ /IL-6 (100 ng/ml) + dexamethasone (1 nM)	3.62 $\pm$ 1.17
IFN $\beta_2$ /IL-6 (100 ng/ml) + dexamethasone (0.1 nM)	3.17 $\pm$ 0.97

\* Mean $\pm$ 1 SD for three separate determinations.

of the gel used for Fig. 3 A). The magnitude of the effect of IFN  $\beta_2$ /IL-6 is less than that of LPS at comparable concentrations (Fig. 3 and data not shown). The effect of IFN  $\beta_2$ /IL-6 on monocyte  $\alpha_1$  AT synthesis is also specific in that there is a decrease in synthesis of  $\alpha_2$  macroglobulin in the same cells under these conditions (Fig. 3 B). IFN  $\beta_2$ /IL-6 has a similar



**Figure 3.** Effect of IFN  $\beta_2$ /IL-6 on synthesis of  $\alpha_1$  AT (A) and  $\alpha_2$  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  $\beta_2$ /IL-6 (rec) in the specified concentrations. Monolayers were then radiolabeled and cell lysates subjected to sequential immunoprecipitation with anti-human  $\alpha_1$  AT and then anti-human  $\alpha_2$  macroglobulin. Molecular mass markers are indicated at the right margin.

effect on synthesis of  $\alpha_1$  AT and  $\alpha_2$  macroglobulin in bronchoalveolar macrophages (data not shown). The lowest effective concentration of IFN  $\beta_2$ /IL-6 and the magnitude of its effect on  $\alpha_1$  AT synthesis were, in general, similar in hepatoma cells and macrophages.

Since LPS independently mediates an increase in synthesis of  $\alpha_1$  AT and decrease in  $\alpha_2$  macroglobulin synthesis in human monocytes, we examined the possibility that LPS contamination of IFN  $\beta_2$ /IL-6 preparations influences the results of experiments in monocytes. First, the concentration of LPS in recombinant human IFN  $\beta_2$ /IL-6 preps was directly measured by limulus amoebocyte 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  $\beta_2$ /IL-6 in the presence of polymyxin B (Fig. 4). Polymyxin B completely neutralizes the effect of LPS on synthesis of  $\alpha_1$  AT but only partially reduces the effect of IFN  $\beta_2$ /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  $\alpha_1$  AT mediated by IFN  $\beta_2$ /IL-6 in the presence of polymyxin B.

Because LPS enhances the production of IFN  $\beta_2$ /IL-6 in monocytes (22, 27), we examined the possibility that the known effect of LPS on monocyte  $\alpha_1$  AT synthesis results from the autocrine, or paracrine, effect of endogenous IFN  $\beta_2$ /IL-6. Separate monolayers of monocytes were incubated with LPS or IFN  $\beta_2$ /IL-6 in the presence of antibody to IFN  $\beta_2$ /IL-6. Although the effect of IFN  $\beta_2$ /IL-6 on synthesis of  $\alpha_1$  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  $\alpha_1$  AT in monocytes is not at all neutralized by antibody to IFN  $\beta_2$ /IL-6 (Fig. 5). The effect of LPS on synthesis of  $\alpha_1$  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  $\beta_2$ /IL-6 and LPS on monocyte  $\alpha_1$  AT RNA levels (see below), suggest that the effects of LPS and IFN  $\beta_2$ /IL-6 on monocyte  $\alpha_1$  AT gene expression are, at least in part, distinct.

Synthesis of  $\alpha_1$  AT in monocytes is not affected by other cytokines such as IL-1 $\beta$ , TNF $\alpha$ , and IFN $\gamma$  (34). In two sepa-

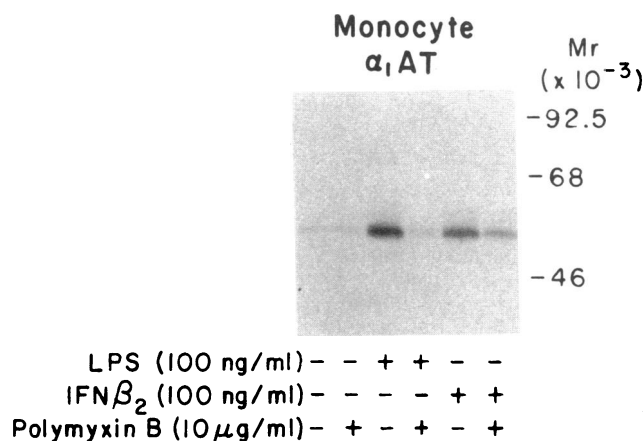


Figure 4. Effect of IFN  $\beta_2$ /IL-6 on monocyte  $\alpha_1$  AT synthesis is independent of LPS. Separate monolayers of monocytes were incubated in control medium, medium supplemented with LPS, or IFN  $\beta_2$ /IL-6, each in the absence or presence of polymyxin B. Synthesis of  $\alpha_1$  AT was determined as described above. Molecular mass markers are indicated at the right margin.

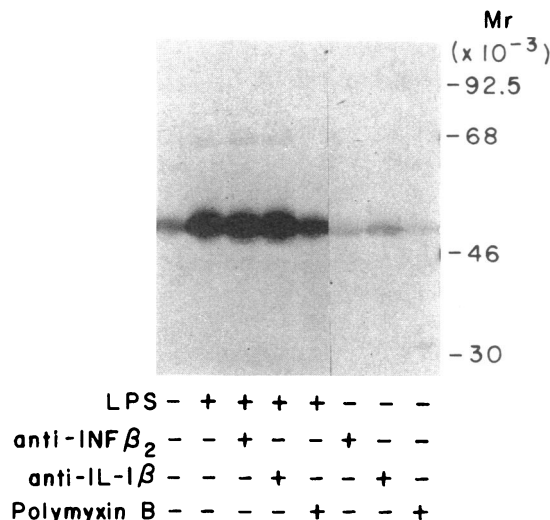


Figure 5. The effect of LPS on monocyte  $\alpha_1$  AT synthesis is distinct from that of IFN  $\beta_2$ /IL-6. After 24 h in culture monocytes were incubated for 18 h with control medium, medium supplemented with LPS (10 ng/ml), antiserum to IL-1 $\beta$  (10 mcg/ml), antiserum to IFN  $\beta_2$ /IL-6 (10 mcg/ml), or polymyxin B (10 mcg/ml). Separate monolayers were coincubated with LPS and anti-IL-1, anti-IFN  $\beta_2$ /IL-6, or polymyxin B. Synthesis of  $\alpha_1$  AT was determined by the methods described above. Molecular mass markers are indicated at the right margin.

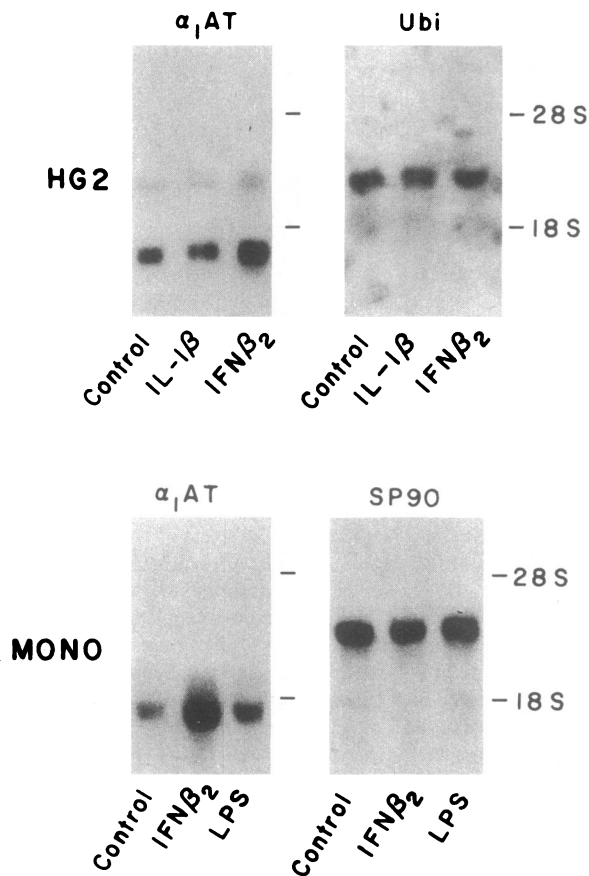
rate experiments monocytes were coincubated with IFN  $\beta_2$ /IL-6 and one of these three other cytokines. There is no additive, synergistic, or antagonistic effect on  $\alpha_1$  AT synthesis by IL-1 $\beta$ , TNF $\alpha$ , or IFN $\gamma$  (Table II). There is also no additive, synergistic, or antagonistic effect of dexamethasone together with IFN  $\beta_2$ /IL-6. IFN  $\beta_2$ /IL-6 and LPS have an additive effect on synthesis of  $\alpha_1$  AT in monocytes (Table II).

The effect of IFN  $\beta_2$ /IL-6 on  $\alpha_1$  AT expression is pretranslational. Steady-state levels of  $\alpha_1$  AT mRNA increase in hepatoma cells (Fig. 6, top) and in blood monocytes (Fig. 6, bottom) incubated in IFN  $\beta_2$ /IL-6-supplemented cell culture fluid. The increase in  $\alpha_1$  AT mRNA levels corresponds to the increase in

Table II. Effect of IFN  $\beta_2$ /IL-6, Other Cytokines, LPS, and Glucocorticoids on Synthesis of  $\alpha_1$  AT in Monocytes

Effector	Fold increase over control*
IFN $\beta_2$ /IL-6 (100 ng/ml)	3.73 $\pm$ 1.02
IL-1 $\beta$ (100 ng/ml)	1.20 $\pm$ 0.21
TNF $\alpha$ (100 ng/ml)	0.97 $\pm$ 0.17
IFN $\gamma$ (100 U/ml)	1.17 $\pm$ 0.21
IFN $\beta_2$ /IL-6 (100 ng/ml) + IL-1 $\beta$ (100 ng/ml)	3.81 $\pm$ 1.31
IFN $\beta_2$ /IL-6 (100 ng/ml) + TNF $\alpha$ (100 ng/ml)	3.30 $\pm$ 1.31
IFN $\beta_2$ /IL-6 (100 ng/ml) + IFN $\gamma$ (100 U/ml)	3.71 $\pm$ 1.80
LPS (10 ng/ml)	3.72 $\pm$ 1.63
IFN $\beta_2$ /IL-6 (100 ng/ml) + LPS (10 ng/ml)	5.36 $\pm$ 0.38
Dexamethasone (0.1 nM)	0.94 $\pm$ 0.27
IFN $\beta_2$ /IL-6 (100 ng/ml) + dexamethasone (0.1 nM)	3.28 $\pm$ 1.00

\* Mean $\pm$ 1 SD for two separate determinations.



**Figure 6.** IFN  $\beta$ 2/IL-6 increases steady-state levels of  $\alpha$ <sub>1</sub> 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-1 $\beta$  (100 ng/ml), or with IFN  $\beta$ 2/IL-6 (100 ng/ml). Monocytes were incubated for 18 h in control medium, medium supplemented with IFN  $\beta$ 2/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  $\alpha$ <sub>1</sub> 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  $\alpha$ <sub>1</sub> AT (Figs. 1 and 3) mediated by IFN  $\beta$ 2/IL-6. The effect of IFN  $\beta$ 2/IL-6 on  $\alpha$ <sub>1</sub> 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-1 $\beta$  has no effect on  $\alpha$ <sub>1</sub> AT mRNA levels in hepatoma cells (Fig. 6, *top*) and LPS has a minimal effect (< 1.5-fold) on  $\alpha$ <sub>1</sub> AT mRNA levels in monocytes (Fig. 6, *bottom*; references 14 and 28). The difference in the effects of IFN  $\beta$ 2/IL-6 and LPS on steady-state levels of  $\alpha$ <sub>1</sub> 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  $\alpha$ <sub>1</sub> AT gene expression is mediated indirectly through induction of IFN  $\beta$ 2/IL-6 expression in these cells.

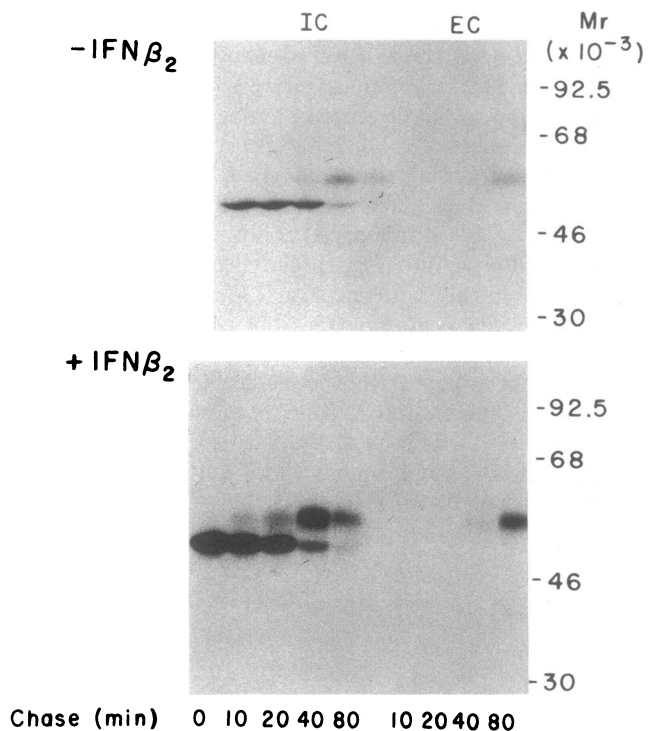
IFN  $\beta$ 2/IL-6 does not affect the kinetics of secretion of  $\alpha$ <sub>1</sub> AT. Human hepatoma cells were subjected to pulse-chase ra-

diolabeling after having been incubated for 18 h in serum-free control medium (Fig. 7, *top*) or medium supplemented with IFN  $\beta$ 2/IL-6 (*bottom*). In this experiment a 52-kD  $\alpha$ <sub>1</sub> AT precursor polypeptide is synthesized and begins to disappear during the chase period. It is converted to the fully glycosylated 55-kD  $\alpha$ <sub>1</sub> 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  $\beta$ 2/IL-6.

IFN  $\beta$ 2/IL-6 also does not affect the kinetics of secretion of  $\alpha$ <sub>1</sub> AT in monocytes (data not shown).

## Discussion

Results of these experiments indicate that IFN  $\beta$ 2/IL-6 mediates a specific increase in expression of  $\alpha$ <sub>1</sub> AT in human hepatocytes and mononuclear phagocytes. In each cell type effec-



**Figure 7.** Effect of IFN  $\beta$ 2/IL-6 on the kinetics of secretion of  $\alpha$ <sub>1</sub> AT in human hepatoma cells. HepG2 cells were incubated for 18 h in serum-free control medium (*-IFN $\beta$ 2*) or medium supplemented with IFN  $\beta$ 2/IL-6 (100 ng/ml) (*+IFN $\beta$ 2*). Cells were then rinsed thoroughly and incubated for 10 min in [<sup>35</sup>S]methionine, 250  $\mu$ Ci/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  $\beta$ /IL-6 during acute infections or during in vivo administration of inflammatory mediators (35–37). The effect of IFN  $\beta$ /IL-6 on  $\alpha_1$  AT gene expression is exclusively pretranslational in that the increase in steady-state levels of  $\alpha_1$  AT mRNA corresponds to the increase in net synthesis of  $\alpha_1$  AT. The effect of IFN  $\beta$ /IL-6 on this gene is readily distinguished from that of acute phase mediators IL-1 $\beta$  and TNF $\alpha$  since these other cytokines have no effect on  $\alpha_1$  AT synthesis (19). The effect of IFN  $\beta$ /IL-6 on monocyte/macrophage  $\alpha_1$  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  $\alpha_1$  AT gene. LPS and elastase mediate distinct positive regulatory effects on  $\alpha_1$  AT synthesis, but these effects are specific for cells of the mononuclear phagocyte lineage (14–16). IFN  $\beta$ /IL-6 is, therefore, the first well-characterized factor to affect the expression of  $\alpha_1$  AT in human hepatocytes and the first factor capable of modulating the  $\alpha_1$  AT gene in several different cell types. It is also noteworthy that  $\alpha_1$  AT expression in mononuclear phagocytes and hepatocytes is affected by IFN  $\beta$ /IL-6 in that there are cell-specific differences in constitutive expression of  $\alpha_1$  AT in mononuclear phagocytes and hepatocytes. In fact, there is an alternative start site for transcription of  $\alpha_1$  AT mRNA in macrophages,  $\sim$  2 kb upstream from that governing transcription of  $\alpha_1$  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  $\beta$ /IL-6 and LPS have similar effects on  $\alpha_1$  AT and  $\alpha_2$  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  $\beta$ /IL-6 release by these cells. First, the effect of LPS on synthesis of  $\alpha_1$  AT is not blocked by neutralizing antibody to IFN  $\beta$ /IL-6. Second, the effect of LPS on  $\alpha_1$  AT expression is predominantly translational, whereas that of IFN  $\beta$ /IL-6 is predominantly pretranslational with increases in  $\alpha_1$  AT mRNA levels corresponding to increases in  $\alpha_1$  AT synthesis. Finally, the magnitude of the LPS effect on  $\alpha_1$  AT synthesis is several-fold greater than that of exogenous IFN  $\beta$ /IL-6 [4.5- to 8.7-fold for LPS (14); 2.5- to 3.5-fold for IFN  $\beta$ /IL-6]. These data do not, however, exclude the possibility that part of the effect of LPS on  $\alpha_1$  AT gene expression in monocytes involves the action of endogenous IFN  $\beta$ /IL-6. In fact, in some individual monocyte donors LPS may increase  $\alpha_1$  AT mRNA levels 1.5- to 2.5-fold (14). Even in these individuals, however, the predominant effect of LPS on  $\alpha_1$  AT expression involves an IFN  $\beta$ /IL-6-independent mechanism since LPS increases net synthesis of  $\alpha_1$  AT 4.5- to 8.7-fold (14) and increases the specific translational efficiency of  $\alpha_1$  AT mRNA by greater than 3-fold (16).

We also examined the effect of IFN  $\beta$ /IL-6 on the kinetics of secretion of  $\alpha_1$  AT. There is no direct effect of this cytokine

on the kinetics of  $\alpha_1$  AT secretion in liver cells. Moreover, there is no intracellular accumulation of  $\alpha_1$  AT even though net synthesis of the protein has increased approximately threefold as a result of IFN  $\beta$ /IL-6 action. Similarly, there is no intracellular accumulation of  $\alpha_1$  AT in monocytes from normal individuals in which synthesis of  $\alpha_1$  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  $\alpha_1$  AT protein. These data do not substantiate the concept that upregulation of  $\alpha_1$  AT synthesis in vivo during acute inflammation, or other altered physiologic states, can be responsible for intrahepato-cytic accumulation of  $\alpha_1$  AT in normal (PiMM) individuals (44) or in transgenic mice bearing the normal (PiM) human  $\alpha_1$  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  $\alpha_1$  AT protein in homozygous PiZZ  $\alpha_1$  AT deficiency, which is exaggerated by the effect of factors that enhance  $\alpha_1$  AT synthesis (14, 15), involves a fundamentally distinct cell biological mechanism.

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