Elastase Regulates the Synthesis of Its Inhibitor, α_1-Proteinase Inhibitor, and Exaggerates the Defect in Homozygous PiZZ α_1 PI Deficiency

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Abstract

The net balance of neutrophil elastase, an enzyme that degrades many components of the extracellular matrix, and its inhibitor, alpha-1-proteinase inhibitor (α_1 PI), is thought to be a critical determinant in the development of destructive lung disease, especially in individuals with homozygous α_1 PI deficiency. Synthesis and secretion of α_1 PI has been recently demonstrated in cells of mononuclear phagocyte lineage, including peripheral blood monocytes and tissue macrophages. In this study we show that α_1 PI gene expression in human monocytes and bronchoalveolar macrophages is affected by a novel mechanism, whereby elastase directly regulates the synthesis of its inhibitor. In nanomolar concentrations, neutrophil or pancreatic elastase mediates a dose- and time-dependent increase in steady state levels of α_1 PI mRNA and in the rate of synthesis of α_1 PI in human monocytes and bronchoalveolar macrophages. Antisera to neutrophil elastase or pretreatment of elastase with the serine proteinase inhibitor diisopropylfluorophosphate abrogates the effect of elastase on α_1 PI expression. Elastase also stimulates the synthesis of α_1 PI in monocytes from homozygous PiZZ α_1 PI-deficient individuals, but has no effect on the rate of secretion; hence, the enzyme mediates an effect on α_1 PI that increases the intracellular accumulation of inhibitor and exaggerates the intrinsic defect in secretion of α_1 PI that characterizes the homozygous PiZZ α_1 PI deficiency.

Introduction

Homozygous PiZZ alpha$_1$-proteinase inhibitor (α_1 PI)[1] deficiency is an autosomal recessive disorder resulting in low serum concentrations of functionally active α_1 PI. It is associated with neonatal hepatitis, chronic liver disease, hepatoma, and early development of pulmonary emphysema (1, 2).

Several studies have directly demonstrated a selective defect in secretion of α_1 PI in PiZZ individuals, probably affecting the transport of α_1 PI from the endoplasmic reticulum to the Golgi apparatus (3–6). There is a single amino acid substitution in the Z variant, (Glu^{342} to Lys^{342} [7–9]), encoded by a single nucleotide substitution (10), but the relationship between this substitution and the defect in secretion of the gene product is not defined. A second amino acid substitution in the Z variant, Val^{213} to Ala^{213}, is thought to be a "silent" polymorphic variant since it is found in 23% of the most common normal α_1 PI haplotype M1 (11).

The pathophysiology of lung injury in PiZZ α_1 PI deficiency is thought to involve a perturbation in the local elastase-antielastase balance, as might occur from the decrease in absolute concentration of α_1 PI and/or from a decrease in the functional activity of α_1 PI during cigarette smoking (1). Several theoretical explanations for liver injury in deficient individuals have been discussed in the literature. In one theory accumulation of α_1 PI in endoplasmic reticulum of liver cells is thought to be directly related to liver injury (2). Evidence supporting the "accumulation theory" of liver injury includes the absence of histologic changes in liver in the rare Pi null variant, a defect in transcription of the α_1 PI gene (12).

There is wide variability in the incidence of tissue damage among individuals with homozygous PiZZ α_1 PI deficiency. A prospective study of infants born in Sweden indicates that 40–50% of PiZZ infants develop serum transaminase elevations during the first year of life, but only 15–20% are affected subsequently by significant liver disease (13). There is also evidence for wide variability in incidence and extent of destructive lung disease in the PiZZ α_1 PI-deficient population even when the absolute serum concentration of α_1 PI and cigarette smoking are taken into consideration. In fact, a number of smoking PiZZ individuals do not develop clinical lung involvement or pulmonary function abnormalities (13, 14; Pierce, J. A., personal communication). Factors that affect this variability have not been identified. Synthesis and secretion of α_1 PI has recently been demonstrated in cells of mononuclear phagocyte lineage, including tissue macrophages (3, 5, 15), raising the possibility that local bronchoalveolar macrophage as well as hepatic Kupffer cell α_1 PI expression and regulation contribute to the variability in lung and liver injury in PiZZ α_1 PI deficiency. Accordingly, we have attempted to define the factors that regulate expression of α_1 PI in cells of the mononuclear phagocyte lineage (16). In this study we show that α_1 PI gene expression in monocytes and macrophages is regulated by serine elastase and therein by the net balance of elastase and antielastase.

Methods

Cell culture. Confluent monolayers of human peripheral blood monocytes from 18 normal PiMM and 3 symptomatic PiZZ individuals (as defined by isoelectric focusing, serum levels and family studies), were established by adherence of dextran-purified leukocytes on siliconized glass as previously described (17). Bronchoalveolar macrophages were obtained from sterile saline bronchial lavage. After centrifugation and washing, cells were allowed to adhere to siliconized glass coverslips

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1. *Abbreviations used in this paper:* α_1 PI, alpha$_1$ proteinase inhibitor.

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(18). HepG2 and Hep3B cells were maintained in culture as previously described (19). Murine L cells transfected with the cloned human α_1 PI gene (TfAT2) were maintained in selective medium (20). The TfAT2 cell line was derived by cotransfection of murine Ltk- cells with herpes simplex virus thymidine kinase DNA and a genomic DNA clone bearing the entire human α_1 PI gene and 5–6 kb of 5′ and 3′ flanking regions (AT73 [21]) by calcium phosphate precipitation (22). Synthesis and secretion of α_1 PI was demonstrated in this cell line, but not in the parent untransfected Ltk- cell line (unpublished).

Biosynthetic labeling. Confluent monolayers were rinsed and incubated at 37°C in the presence of methionine-free medium containing [^{35}S]methionine, 500 μCi/ml (pulse period). In order to determine the rate of accumulation of α_1 PI or control secretory proteins in the cell, cells were subjected to a short pulse interval (30 min) and radiolabeled proteins were detected in the cell lysate alone. To determine the rate of secretion of α_1 PI, cells were subjected to a pulse period of 30 min, rinsed, and incubated in serum-free medium containing an excess of unlabeled methionine (chase period). Radiolabeled α_1 PI was detected in cell culture fluid and cell lysates at specified intervals of the chase period. To determine the accumulation of α_1 PI in the cell culture fluid, cells were subjected to a long pulse interval (3 h) and radiolabeled α_1 PI identified in cell culture fluid alone. Methods for solubilization of cells and clarification of cell lysates after labeling have been described (15). Total protein synthesis was estimated by trichloroacetic acid precipitation of aliquots of cell lysates and culture fluid (23).

Immunoprecipitation and SDS-PAGE. 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 as described by Laemmli (24). ^{14}C-methylated molecular size markers (200,000, 92,500, 68,000, 46,000, and 30,000 mol wt) were included on all gels. After electrophoresis, gels were stained in Coomassie brilliant blue, destained, impregnated with 2,5-diphenyloxazole (EN^{3}HANCE, New England Nuclear, Boston, MA), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY). Antibodies included rabbit antihuman α_1 PI, rabbit antihuman α_2 macroglobulin, rabbit antihuman α_1 antichymotrypsin from Dako Corp., Santa Barbara, CA, goat antihuman factor B from Atlantic Antibodies, Scarborough, ME, and sheep antihuman complement C2 from Seward Laboratories, London, UK.

Detection of RNA by RNA blot analysis. Total cellular RNA was isolated from adherent monolayers of monocytes and macrophages by guanidine isothiocyanate extraction and ethanol precipitation (25). RNA was quantitated by absorbance at 260 nm and solubilized for agarose-formaldehyde gel electrophoresis and transferred to nitrocellulose filters (26). Filters were then hybridized with ^{32}P-labeled α_1 PI-specific complementary cDNA (27), washed extensively, dried, and exposed to x-ray film for autoradiography.

Effect of elastase on expression of α_1 PI. Cells were incubated for 2–24 h at 37°C in serum-free medium without or with human neutrophil elastase (28), porcine pancreatic elastase, native or oxidized human α_1 PI, or reaction mixtures containing preformed complexes of elastase and α_1 PI. Preparations of porcine pancreatic elastase were obtained from Sigma Chemical Company or Elastin Products (St. Louis, MO), shown to be functionally active and homogeneous by SDS-PAGE. Functional activity was assayed by the rate of hydrolysis of the synthetic substrate Suc-Ala-Ala-Ala-NA (29). One unit per milligram of enzyme represented a change in absorbance at 400 nM of 1.0/min. α_1 PI was oxidized by addition of eight molar equivalents of *N*-chlorosuccinimide to the native protein at pH 9.0, followed by dialysis against 0.5/M Tris HCl/0.05 M NaCl, pH 8.0 (30). Preformed elastase-α_1 PI complexes were generated by an incubation for 90 min at room temperature of elastase 100 ng/ml with native or oxidized α_1 PI, in various concentrations, in 0.05/M Tris HCl. Elastase was inactivated in specified experiments by incubation for 30 min at 30°C in diisopropylfluorophosphate 2 mM dissolved in 80% ethanol/8% propylene glycol. In control experiments cells were incubated in medium supplemented with human neutrophil cathepsin G (31), human complement Cls (32) tosyl phenylchloromethylketone (TPCK)-treated bovine pancreatic trypsin, trypsin, tosyl-lysyl chloromethylketone (TLCK)-treated bovine pancreatic-chymotrypsin (Worthington Biochemicals, Freehold, NJ) in several different concentrations. Cells were also incubated in crude supernatants of alloreactive T_4+ lymphocyte clones as previously described (16).

Results

The effect of elastase on expression of α_1 PI in mononuclear phagocytes from normal PiMM individuals. Monolayers of human peripheral blood monocytes and bronchoalveolar macrophages were incubated in medium supplemented with porcine pancreatic elastase. Steady state levels of α_1 PI mRNA increased three- to fourfold in monocytes and, in a dose-dependent manner up to eightfold, in macrophages (Fig. 1). Similar amounts of ethidium bromide-stained 18S and 28S ribosomal RNA were visualized in each lane of the RNA blots. Steady state levels of RNA for a control secretory gene product, complement factor B, did not change (data not shown). Human neutrophil elastase had the same effect as pancreatic elastase.

Elastase (neutrophilic and pancreatic) also mediated an increase in the rate of accumulation of α_1 PI in cells after a short interval of pulse labeling and in the rate of accumulation of α_1 PI in the cell culture fluid after a long interval of pulse labeling (Fig. 2). There was a concentration-dependent eightfold increase in α_1 PI in lysates of bronchoalveolar macrophages (*a*), and a concentration-dependent eightfold increase in α_1 PI in culture fluid of bronchoalveolar macrophages (*b* and *c*, alveolar macrophages from two different individuals). At concentrations of pancreatic elastase between 5 and 50 ng/ml, native (55 kD), complexed (75 kD), and modified (51 kD) inhibitor is present in the cell culture fluid. Neutrophil and pancreatic elastase each mediated a concentration-dependent increase in

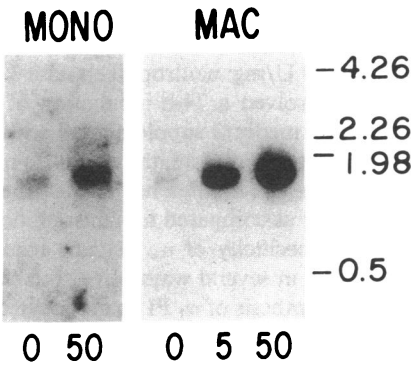

Figure 1. Effect of serine elastase on steady state levels of α_1 PI mRNA. After 24 h in culture, confluent monolayers of peripheral blood monocytes (*MONO*) and bronchoalveolar macrophages (*MAC*) were rinsed and incubated for 24 h in serum-free medium alone (0) or supplemented with porcine pancreatic elastase in the specified concentrations. RNA was then subjected to RNA blot analysis using radiolabeled α_1 PI cDNA as probe. Steady state levels of α_1 PI mRNA increased approximately fourfold in monocytes and eightfold in alveolar macrophages in this experiment as determined by densitometric scanning on LKB laser densitometer 2222 Ultrascan XL. Similar amounts of ethidium bromide stained 18S and 28S RNA was visualized in each lane. DNA size markers are indicated at the right margin.

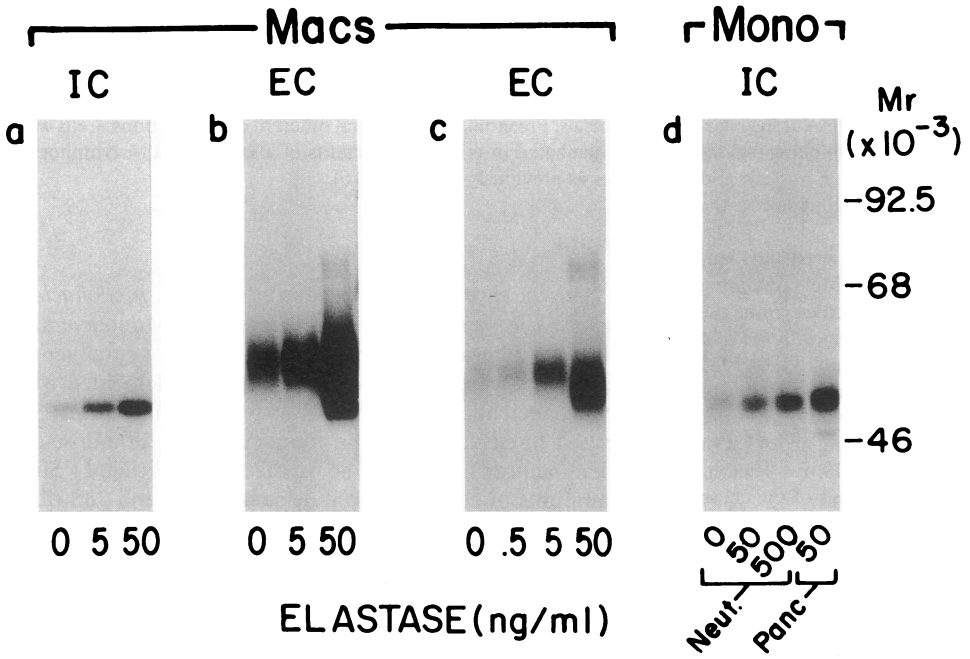

Figure 2. Effect of serine elastase on the accumulation of α_1 PI in cell lysates and cell culture fluid of peripheral blood monocytes and bronchoalveolar macrophages. After 24 h in culture, monocytes or macrophages were studied. For *a*, bronchoalveolar macrophages were incubated for 24 h in serum-free medium alone (0) or pancreatic elastase in the specified concentrations. Cells were rinsed thoroughly and then subjected to pulse radiolabeling for 30 min. α_1 PI was assayed in cell lysates. For *b* and *c*, bronchoalveolar macrophages from two different individuals were incubated for 24 h in serum-free medium alone (0) or medium supplemented with pancreatic elastase in the specified concentrations. Cells were rinsed thoroughly and then subjected to pulse radiolabeling for 3 h. α_1 PI was assayed in cell culture fluid. For *D*, peripheral blood monocytes were incubated for 24 h in serum-free medium alone (0) or medium supplemented with neutrophil elastase (*neut*) or pancreatic elastase (*panc*) in the specified concentrations. Cells were then rinsed thoroughly and then subjected to pulse radiolabeling for 30 min. α_1 PI was assayed in cell lysates. Shorter fluorographic exposure of the lanes in panels b and c show distinct 55- and 51-kD bands that represent native and modified forms of α_1 PI, respectively. Molecular mass markers are indicated at the right margin.

accumulation of α_1 PI in cell lysates of peripheral blood monocytes after a short interval of pulse radiolabeling (Fig. 2 *d*). The magnitude of increases in net α_1 PI accumulation in cell lysates or cell culture fluid of monocytes and macrophages corresponded to the magnitude of increases in steady state levels of α_1 PI mRNA in each case. The effect of pancreatic elastase was evident at 10-fold lower concentrations than that of neutrophil elastase (Figs. 2 *d*; 4 *a*, lanes 1–5), but due to differences in enzymatic activity between preparations, at similar specific elastolytic activities as that of neutrophil elastase (pancreatic elastase 34 U/mg; neutrophil elastase 3.7 U/mg). These experiments involved a 24-h incubation of monocytes or macrophages in medium supplemented with pancreatic or neutrophil elastase. Therefore, the results do not exclude the possibility that there is a difference in the time course of the effect of pancreatic as compared to neutrophil elastase.

The specificity of α_1 PI gene regulation by elastase was examined in several ways. First, the effect of neutrophil elastase on synthesis of α_1 PI in monocytes and macrophages was neutralized by preincubation with antisera to human neutrophil elastase, but not with antibody to the lipid A moiety of endotoxin (kindly provided by Dr. W. C. Bogard, Malvern, PA [33]). The effect of pancreatic or neutrophil elastase was not neutralized by co-incubation of monocytes in polymyxin B in concentrations up to 25 μg/ml (data not shown). Second, α_1 PI synthesis was not affected by the addition of other serine proteinases including cathepsin G, trypsin, chymotrypsin, or Cls (Fig. 3 *a*). Third, total protein synthesis and synthesis of several other monocyte secretory proteins, including complement proteinases C2 and factor B, and proteinase inhibitor α_1-antichymotrypsin, were not affected by pancreatic or neutrophil elastase. In alveolar macrophages there was a twofold increase in synthesis of α_2-macroglobulin, an inhibitor of serine as well as other classes of proteinases (Fig. 3 *b*). Fourth, the effect of elastase was cell specific. Synthesis of α_1 PI in human hepatoma cell lines (HepG2, Hep3B) and mouse fibroblasts L-cells transfected with the cloned human α_1 PI gene (TfAT2) was unaffected (data not shown). The rate of α_1 PI synthesis did increase 2.0- to 2.5-fold in HepG2 and Hep3B cells when incubated in crude supernatants of alloantigen-stimulated T_4+ lymphocyte clones (16).

The effect of elastase on synthesis of α_1 PI in monocytes required enzymatic activity. Pancreatic elastase pretreated with the serine proteinase inhibitor diisopropylfluorophosphate (DFP) did not affect the accumulation of α_1 PI in the cells. (Fig. 4 *a*, lane *6*). In fact, synthesis of α_1 PI decreased in the presence of DFP-treated pancreatic elastase or DFP alone (lane *7*) even though total protein synthesis was unaffected. These results are consistent with the theory that an endogenous serine elastase otherwise affects α_1 PI expression early in monocyte culture (see below). Results were similar when monocytes were incubated with pancreatic or neutrophil elastase that had been pretreated with PMSF or a specific synthetic elastase inhibitor, Me-O-Suc-Ala-Ala-Pro-boraValpinacol (34).

An effect on α_1 PI synthesis was also evident when pancreatic elastase was added to monocyte cultures as a preformed complex with α_1 PI (Fig. 4 *b*). Elastase was mixed with native or oxidized human α_1 PI at several different concentrations and the reaction mixtures added to monolayers of blood monocytes after 24 h in culture. When elastase and native α_1 PI were mixed in equimolar concentrations the resulting preformed complexes mediated an increase in α_1 PI synthesis (lane *3*) greater than that of elastase alone (lane *2*) or reaction mixtures in which elastase was in molar excess (lanes *4* and *5*). When elastase was added to oxidized α_1 PI (unable to complex

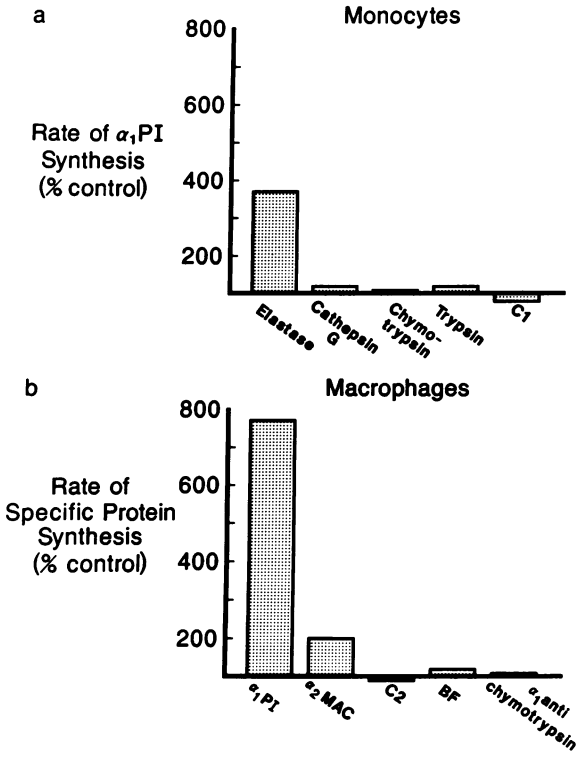

Figure 3. Specificity of effector and response. (*a*) Effector. Confluent monolayers of monocytes were incubated for 24 h in serum-free medium alone or supplemented with human neutrophil elastase, or the other specified enzymes (500 ng/ml). The cells were then rinsed and subjected to pulse radiolabeling for 30 min. Radiolabeled α_1 PI in cell lysates was detected by immunoprecipitation, followed by analytical gel electrophoresis. Rate of accumulation of α_1 PI in cell lysates in each case is expressed as percent control as determined by densitometric scanning of fluorograms on LKB laser densitometer 2222 Ultrascan XL. (*b*) Response. Monolayers of alveolar macrophages were incubated for 24 h in serum-free medium alone or supplemented with human neutrophil elastase (500 ng/ml). The cells were then rinsed and subjected to pulse radiolabeling for 30 min. Radiolabeled α_1 PI, or the other specified proteins, in cell lysates were detected by sequential immunoprecipitation followed by analytical gel electrophoresis. Rate of accumulation in cell lysates of each protein is expressed as percent control as determined by densitometry.

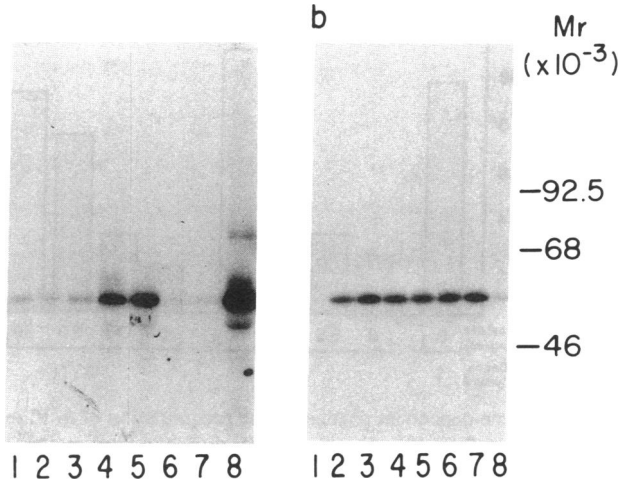

Figure 4. Properties of elastase mediating regulation of α_1 PI synthesis in peripheral blood monocytes. (*a*) Enzymatic activity. After 24 h in culture, monocytes were incubated for 24 h in serum-free medium alone (lane *1*), medium supplemented with human neutrophil elastase 5 ng/ml (lane *2*), 50 ng/ml (lane *3*), or 500 ng/ml (lane *4*), or porcine pancreatic elastase 50 ng/ml (lane *5, 6, 8*). Porcine pancreatic elastase was incubated with diisopropylfluorophosphate 2 mM dissolved in 80% ethanol/8% propylene glycol (lane *6*) or with an equivalent amount of 80% ethanol/8% propylene glycol (lane *8*) for 30 min at 30°C before addition to cell monolayers. Monolayers of monocytes were also incubated in medium supplemented with DFP in ethanol/propylene glycol, but not with pancreatic elastase (lane *7*). Cells were then rinsed and radiolabeled for 30 min. (*b*) Contribution of elastase in preformed complex. After 24 h in culture, monocytes were incubated in control medium (lane *1*), medium supplemented with pancreatic elastase 100 ng/ml (lane *2*), with a mixture of elastase, 100 ng/ml, and native α_1 PI, 100 ng/ml (lane *3*), a mixture of elastase, 100 ng/ml, and native α_1 PI, 10 ng/ml (lane *4*), a mixture of elastase, 100 ng/ml, and native α_1 PI, 1 ng/ml (lane *5*), with native α_1 PI, 100 ng/ml, alone (lane *6*), with a reaction mixture of elastase and oxidized α_1 PI (lane *7*) and with oxidized α_1 PI alone (lane *8*). This incubation was conducted for 24 h. Cells were rinsed and then radiolabeled for 30 min and newly synthesized α_1 PI in the cell lysates detected exactly as described above. Molecular mass markers are indicated at the right margin.

with elastase) the effect on α_1 PI expression (lane *7*), was similar to the effect of elastase alone (lane *2*). There was an increase in synthesis of α_1 PI in monocytes incubated with native α_1 PI alone (lane *6*), but not in monocytes incubated with oxidized α_1 PI alone (lane *8*). The effect of elastase alone, or elastase-α_1 PI complexes, was evident within 3 h, but the effect of adding α_1 PI alone to the culture medium was only evident after 24 h (data not shown). The latter effect may result from the capacity of exogenous α_1 PI to form complexes with the small amount of endogenous elastase present at this time in culture (35, 36).

A decrease in steady state levels of α_1 PI mRNA and a corresponding decrease in synthesis and secretion of α_1 PI ordinarily accompanies the maturation of human blood monocytes, bronchoalveolar and breast milk macrophages in tissue culture (3). This decrease in expression α_1 PI during mononuclear phagocyte culture is not caused by a change in cell viability or total metabolic activity, as total mRNA content, steady state levels of specific mRNAs, total protein synthesis and synthesis of other specific secreted proteins increases during the same interval (37). This observation, therefore, suggests that expression of α_1 PI in mononuclear phagocytes is regulated by a factor elaborated in vitro or by loss of an in vivo regulating factor. We, therefore, examined the possibility that expression of α_1 PI in monocytes is maintained during maturation in cultures supplemented by exogenous elastase. Steady state levels of α_1 PI mRNA and the rate of accumulation of α_1 PI in the cell (Fig. 5) did not decrease in monolayers of peripheral blood monocytes during the first 3 d in culture if the cell culture fluid was supplemented with exogenous pancreatic elastase. The increase in synthesis of α_1 PI mediated by elastase was quantitatively similar on days 2, 5, and 7 in culture (data not shown). These results suggest that expression of α_1 PI decreases during monocyte/macrophage maturation in vitro because the cells are removed from serine elastase present in vivo, or because there is a decrease in expression of endogenous neutrophilic elastase by cells of the monocyte/ macrophage series occurring even earlier in primary culture (35, 36). The results do not exclude the possibility that other factors are also involved in the decrease in α_1 PI expression during monocyte/macrophage maturation in vitro.

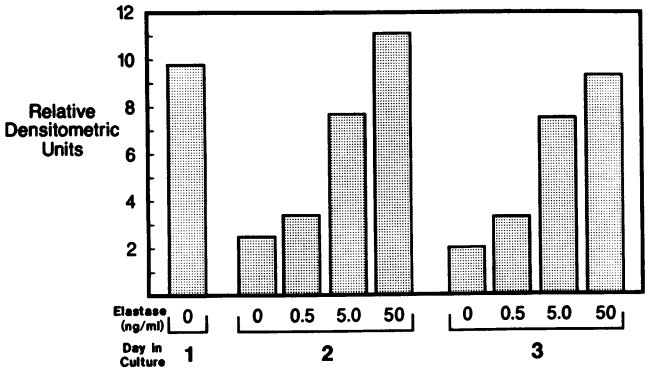

Figure 5. Time-dependent changes in the accumulation of α_1 PI in the cell culture fluid of human monocytes during in vitro maturation. Monolayers of monocytes after 1, 2, or 3 d in culture were incubated for 24 h in serum-free medium alone (0) or medium supplemented with porcine pancreatic elastase in the specified concentrations. Cells were then rinsed and subjected to pulse radiolabeling for 3 h. Accumulation of α_1 PI in native (55 kD) and complexed forms (66 and 75 kD) was determined by immunoprecipitation, gel electrophoresis followed by fluorography and expressed in relative densitometric units.

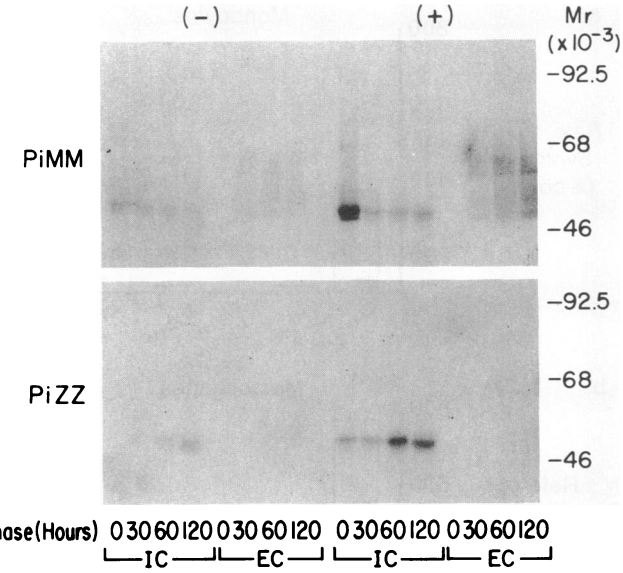

Figure 6. Effect of elastase on the kinetics of secretion of α_1 PI in PiMM and PiZZ monocytes. After 24 h in culture monocytes from PiMM (*upper panels*) and PiZZ individuals (*lower panels*) were incubated in medium alone (−) or medium supplemented with pancreatic elastase 50 ng/ml (+). Monocytes were then subjected to pulse-chase radiolabeling. The synthesis of α_1 PI was lower in the PiZZ than in the PiMM monocytes used in the experiment shown (compare IC time 0 in left upper and left lower panel), but was similar in other experiments here and in previous studies (3, 16). The increase in α_1 PI synthesis in the presence of elastase was approximately 3.7-fold in PiMM monocytes and 4.5-fold in PiZZ monocytes (compare time 0 IC upper left to upper right, and lower left to lower right). The half-time for disappearance of α_1 PI from the intracellular contents of PiMM monocytes was ~ 38 min in the absence and 43 minutes in the presence of elastase as determined by densitometric scanning. In this particular experiment α_1 PI is not detected in the extracellular fluid of PiZZ monocytes. In experiments with longer chase periods (180–360 min) and longer exposure of fluorograms there is secretion of α_1 PI in PiZZ monocytes as previously reported (3, 5, 16). Molecular mass markers are indicated.

The effect of elastase on expression of α_1 PI in monocytes from deficient PiZZ individuals. The cellular defect in homozygous PiZZ α_1 PI deficiency, a selective decrease in rate of secretion of α_1 PI, is expressed in monocytes from PiZZ individuals (15). Thus, blood monocytes in primary cell culture make it possible to study the effect of enhanced synthesis, as mediated by elastase, on a defect in posttranslational processing/secretion. Monocytes from PiMM and PiZZ individuals were separately incubated in control medium or medium supplemented with pancreatic elastase and then subjected to pulse-chase radiolabeling (Fig. 6). The synthesis of α_1 PI increases to a similar extent in PiMM and PiZZ monocytes (compare IC time 0 left upper to right upper and IC time 0 left lower to right lower). Although α_1 PI is already disappearing from the intracellular contents and appearing in the extracellular fluid by 30 min of the chase period in PiMM monocytes, it accumulates in the intracellular contents over the entire 120 min of the chase period in PiZZ monocytes. In the presence of elastase (+), synthesis is increased, but there is no change in the rate of secretion of α_1 PI in PiMM or PiZZ monocytes. Consequently, when elastase mediates an increase in synthesis of α_1 PI, there is more α_1 PI both in the intracellular and extracellular fluid of PiMM monocytes, but only greater intracellular α_1 PI accumulation in PiZZ monocytes.

Discussion

Proteolytic enzymes in a number of biological systems, including the coagulation and fibrinolytic cascades, are known to directly or indirectly regulate the expression of other gene products (38, 41). In this report we describe a novel form of gene regulation by a proteolytic enzyme, whereby the enzyme regulates the synthesis of its own inhibitor. This type of regulation may be particularly important for cells of the mononuclear phagocyte lineage. Mononuclear phagocytes synthesize and secrete collagenase and collagenase inhibitor (42), plasminogen activator and plasminogen activator inhibitor (43), Cl and Cl inhibitor (44). Furthermore, macrophages are situated at sites of tissue injury and inflammation and therein are exposed to proteolytic enzymes released from neutrophils, platelets, activation products of the complement, coagulation and fibrinolytic cascades, damaged tissue and bacteria. These proteinases must ultimately be inactivated in order to prevent incidental destruction of surrounding uninvolved tissue and to ensure the orderly initiation of tissue repair.

Results of our experiments indicate that regulation of α_1 PI expression by serine (neutrophilic and pancreatic) elastase is tissue-specific for cells of the mononuclear phagocyte series. There is an increase in synthesis of α_1 PI by a pretranslational mechanism of action: i.e., there is an increase in steady state levels of α_1 PI mRNA, a corresponding increase in accumulation of newly synthesized α_1 PI in the cell after short intervals of radiolabeling, and in the cell culture fluid after longer intervals of radiolabeling; there is no change in the rate of secretion of α_1 PI in pulse-chase experiments and no change in rate of degradation of α_1 PI in longer intervals of radiolabeling. The response requires enzymatically active elastase (Fig. 4 *a*) and is dependent on the presence of α_1 PI, either endogenous or exogenous in origin (Fig. 4 *b*). Thus, regulation of α_1 PI expression may require the formation of an elastase-α_1 PI complex. The regulatory phenomenon could be mediated by the 4-kD carboxy-terminal fragment cleaved during complex formation, or by an epitope on the 51-kD modified inhibitor newly exposed by virtue of the conformational change that

accompanies complex formation (45–47). Although it is much less likely, the results do not exclude the possibility that the regulatory effect is mediated by an α_2-macroglobulin-elastase complex, since α_2-macroglobulin is also secreted by monocytes and macrophages (48). In either case the results indicate that elastase confers specificity in that other serine proteinases that form complexes with α_1 PI in vitro do not affect the expression of α_1 PI (Fig. 3 *a*). Experiments designed to more precisely elucidate the signal for α_1 PI regulation, its recognition and transduction, will, therefore, require highly purified preparations of the high molecular weight elastase-α_1 PI complex, modified α_1 PI, carboxy and amino terminal fragments of α_1 PI. Moreover, the experimental design will have to take into consideration that the responding cells (monocytes/macrophages) also produce proteinases (35, 36, 49, 50) and antiproteinases (48, 51) each of which affect the net amount of complex formation.

The effect of elastase on expression of α_1 PI in monocytes and macrophages is distinct from that of lipopolysaccharide (LPS [52]). It is neutralized by antisera to neutrophil elastase and enzyme inactivation. The effect of elastase is not neutralized by polymyxin B or monoclonal antibody to the lipid A moiety of LPS, which partially or completely neutralize the effect of LPS on α_1 PI expression (52). The most important distinguishing characteristic, however, is the mechanism of action: the effect of elastase on α_1 PI expression involves a pretranslational mechanism, whereas that of LPS is predominantly translational.

Plasma concentrations of α_1 PI increase three- to fourfold during acute inflammation or tissue injury (53). A number of other human hepatic "acute phase reactants" have now been shown to be regulated in human hepatoma cells (HepG2 and Hep3B) by the monokines IL-1 and cachectin/tumor necrosis factor (TNF [54, 55]). The rate of synthesis of α_1 PI in HepG2 and Hep3B cells is not affected by IL-1, TNF (55) or by supernatants of LPS-stimulated peripheral blood mononuclear cells (56). Synthesis of α_1 PI does increase in HepG2 and Hep3B cells incubated with crude supernatants of alloantigen-stimulated T_4+ lymphocyte clones (16) but the increase is only twofold as compared with the eightfold increase that occurs in alveolar macrophages incubated with elastase (Fig. 2). These observations, therefore, raise the possibility that the acute phase response of α_1 PI involves a different cell type and mediator than that of other hepatic secretory proteins.

Monocytes from α_1 PI-deficient (PiZZ) individuals also recognize and transduce this regulatory signal, but do not deliver additional proteinase inhibitor to the cell culture fluid. Instead, the deficient monocyte/macrophage accumulates more α_1 PI in the intracellular compartment. The deficient individual is, therefore, compromised at several molecular/cellular levels including diminished secretion of inhibitor, inability to deliver to the tissue fluid additional inhibitor when confronted by elastase, and the pathophysiological consequences of intracellular α_1 PI accumulation. Differences in the elaboration of elastase, or other molecules that regulate α_1 PI expression by a pretranslational or translational mechanism, in the local microenvironment of α_1 PI-synthesizing cells of the liver and lung may, therefore, contribute to the wide variability in disease manifestations among PiZZ individuals.

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