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Research Article

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A Lymphokine Regulates Expression of Alpha-1-Proteinase Inhibitor in Human Monocytes and Macrophages

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Abstract

Biosynthesis and secretion of alpha-1-proteinase inhibitor (α_1PI) has been demonstrated in primary cultures of human mononuclear phagocytes, making it possible to study regulation of α_1PI in normal (PiMM) and homozygous-deficient (PiZZ) individuals. In this study, expression of α_1PI by blood monocytes, bronchoalveolar, and breast milk macrophages decreased during 1 wk in culture whereas expression of other secreted proteins increased. The addition of crude supernatants from mitogen-stimulated peripheral blood mononuclear cells to confluent monolayers of mononuclear phagocytes after 1 wk in culture resulted in a 2- to 2.5-fold increase in α_1PI expression. The increase in α_1PI expression was dose- and time-dependent, and involved a mechanism acting at a pretranslational level as shown by an increase in specific messenger RNA content corresponding to the increase in synthesis and secretion of α_1PI. Although α_1PI was expressed in native form and in forms complexed with serine protease by monocytes early in culture, it was expressed in its native form alone when monocytes were incubated with the lymphokine after 1 wk in culture. The regulating factor had the characteristics of a polypeptide and was derived from T lymphocytes, but it was not interferon-alpha, -beta, -gamma, or interleukin 2. This lymphokine also stimulated synthesis of α_1PI in monocytes of homozygous-deficient PiZZ individuals, but had minimal effect on secretion, thereby increasing the intracellular accumulation of the inhibitor and exaggerating the defect in secretion of α_1PI in these individuals. Regulation of mononuclear phagocyte α_1PI expression by a lymphokine provides a model for further analysis of the effect of enhanced synthesis on a defect in posttranslational processing/secretion and for analysis of differential regulation of protease and inhibitor expressed in the same cells.

Introduction

Alpha-1-proteinase inhibitor (α_1PI)[1] is thought to play an important role in elastic tissue injury and repair because it rapidly inactivates neutrophil elastase (reviewed in reference 1). It is a 52,000–55,000-mol-wt single-chain glycoprotein encoded by a 10.2-kilobase (kb) gene (2) on human chromosome 14 (3, 4). Several polymorphic variants of this inhibitor (PiZZ and PiSZ) have been associated with moderate to severe reduction in plasma concentration of the inhibitor, chronic liver disease, and pulmonary emphysema.

Liver is the predominant site of synthesis (5, 6), but human monocytes and macrophages also synthesize and secrete α_1PI (7). Furthermore, the cellular defect in homozygous PiZZ α_1PI deficiency, a selective decrease in rate of secretion of α_1PI, is expressed in human monocytes from PiZZ individuals (8). Thus, blood monocytes and tissue macrophages in primary cell culture make it possible to study the expression and regulation of α_1PI in normal and deficient individuals.

Although plasma concentrations of α_1PI are known to increase during acute inflammation, pregnancy, and oral contraceptive administration (9), relatively little information about the regulation of α_1PI is available. In primary cultures of human blood monocytes, bronchoalveolar macrophages, and breast milk macrophages we previously observed a decrease in α_1PI messenger RNA (mRNA) content and a corresponding decrease in synthesis and secretion of α_1PI during the first 10 d in culture (7). This decrease in expression of α_1PI during maturation in culture was not caused by a change in cell viability or total metabolic activity, as total protein synthesis and synthesis of other specific secreted proteins increased during that interval. These observations suggested that α_1PI was regulated by a factor elaborated in vitro or by loss of an in vivo regulating factor. Expression of DR antigen by human monocytes also decreases during the first week in culture but the decrease can be prevented by the addition of a lymphocyte product, interferon-gamma, to the cell culture fluid (10). In the following study, another lymphokine was shown to increase the expression of α_1PI in mononuclear phagocytes, and, as a result, to accentuate the defect in secretion of α_1PI in severely deficient individuals.

Methods

Materials. Dulbecco's modified Eagle's medium (DME), DME lacking methionine, and RPMI 1640 were purchased from Gibco, Grand Island, NY, and Hank's balanced salt solution (HBSS) and medium 199 from Microbiological Assoc., Bethesda, MD. Fetal bovine serum, L-glutamine, and penicillin-streptomycin were from Flow Laboratories, McLean, VA. [^{35}S]methionine (specific radioactivity, ~1,000 Ci/mmol) and [^{32}P]deoxycytidine triphosphate (specific radioactivity, ~3,000 Ci/mmol) were obtained from New England Nuclear, Boston, MA, and [^{14}C]methylated protein standards were obtained from Amersham Radiochemicals, Arlington Heights, IL. Other reagents included sodium deoxycholic acid, ethidium bromide, 2-mercaptoethanol, porcine pancreatic elastase, and Hepes from Sigma Chemical Co., St. Louis, MO; Triton X-100 from Mallinckrodt, Inc., St. Louis, MO; sodium dodecyl sulfate (SDS) and acrylamide from Bio-Rad Laboratories, Richmond, CA; IgG-Sorb from Enzyme Center, Cambridge, MA; leupeptin from Peptide Research Institute, Osaka, Japan; pepstatin A from Calbiochem-

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1. *Abbreviations used in this paper:* α_1PI, alpha-1-proteinase inhibitor; Con A, concanavalin A; DME, Dulbecco's modified Eagle's medium; IL-1 and IL-2, interleukins 1 and 2; PWM, pokeweed mitogen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TPCK, *N*-tosyl-L-phenyl-alanine chloromethyl ketone.

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Behring Corp., San Diego, CA, concanavalin A (Con A) from Miles Laboratories, Naperville, IL; Ficoll from Pharmacia AB, Uppsala, Sweden; sodium diatrizoate (Hypaque sodium 50% wt/vol) from Winthrop Laboratories; immobilized *N*-tosyl-L-phenyl-alanine chloromethyl ketone (TPCK) trypsin from Worthington Biochemicals, Freehold, NJ; pokeweed mitogen (PWM) from Burroughs Wellcome, Research Triangle Park, NC; cesium chloride from Bethesda Research Laboratories, Gaithersburg, MD; guanidine isothiocyanate from Fluka AG, Buchs, Switzerland; and sodium *N*-laurylsarcosinate from ICN Pharmaceuticals, Plainville, NY. Rabbit anti-human α_1PI was purchased from Dako Corp., Santa Barbara, CA; goat anti-human α_1PI, goat anti-human lysozyme, goat anti-human factor B, and goat anti-human C3 were obtained from Atlantic Antibodies, Scarborough, ME. Recombinant-generated human interferons were obtained from Roche Institute of Molecular Biology, Nutley, NJ (alpha), Cetus Corp., Emeryville, CA (beta), and Genentech, San Francisco, CA (gamma). The specific activity of interferon-gamma was 1.9×10^7 IU antiviral activity/mg protein. Purified human monocyte interleukin 1 (IL-1) was a gift from C. A. Dinarello (Boston, MA). Supernatants from alloantigen-stimulated $T4^+$ lymphocyte clones were gifts from Dr. R. Geha (Boston, MA).

Separation and culture of monocytes and macrophages. Confluent monolayers of human peripheral blood monocytes were established by adherence of dextran-purified leukocytes on siliconized glass as previously described (11). Bronchoalveolar macrophages were obtained from sterile saline bronchial lavage, and breast milk macrophages were from milk expressed during the first 4 d of lactation. After centrifugation and washing, cells were allowed to adhere to siliconized glass coverslips (12, 13).

Lymphokines. Peripheral blood mononuclear cells (2×10^6/ml) were isolated by Ficoll-Hypaque density gradient centrifugation (14), depleted of monocytes by one adherence step, and then incubated for 2–6 d in RPMI 1640 containing 2% heat-inactivated fetal calf serum, 0.05 mM 2-mercaptoethanol, 5 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine with 2 μg/ml Con A or 10 μg/ml PWM. After centrifugation, supernatants were separated from cells, filtered, and stored at −70°C. Some aliquots were dialyzed against DME lacking methionine so that they could be included in cell culture fluid during biosynthetic labeling. Control culture supernatants were prepared by culturing peripheral blood mononuclear cells in the same medium but supplementing with Con A or PWM only after supernatants were separated from cells. Supernatants were also obtained from Con A-stimulated T lymphocytes and from alloreactive $T4^+$ lymphocyte clones (15). T lymphocytes were collected from peripheral blood specimens by Ficoll-Hypaque centrifugation, enrichment by rosetting with sheep erythrocytes, passage of E+ cells through nylon wool columns, and cultivation of cells not adhering to siliconized glass surfaces. In specified experiments crude lymphokine preparations were incubated at 56°C and 70°C for 60 min or to 100°C for 5 min, adsorbed by immobilized TPCK-treated trypsin in 0.05 M Tris HCl (pH 8.0)/0.05 M NaCl for 60 min at 37°C or adsorbed by 5–500 ng mouse monoclonal anti-human interferon-gamma. The effect of these lymphokine preparations on α_1PI expression by mononuclear phagocytes was then compared to that of the usual preparations.

Biosynthetic labeling. Confluent monolayers were rinsed and incubated at 37°C for 3 h in the presence of methionine-free medium containing [^{35}S]methionine (500 μCi/ml). Methods for solubilization of cells and clarification of cell lysates after labeling have been described (7). Total protein synthesis was estimated by trichloroacetic acid (TCA) precipitation of aliquots of cell lysates and culture fluid (16).

Immunoprecipitation and SDS-polyacrylamide gel electrophoresis (PAGE). For immunoprecipitation, 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 (17). ^{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), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY). Complexing of α_1PI with proteases was examined by treatment of extracellular media with protease in 0.05 M Tris HCl (pH 8.0)/0.05 NaCl at 25°C for 10 min. Reactions were terminated with 100-fold excess phenylmethylsulfonyl fluoride. Proteases included bovine alpha-chymotrypsin and porcine pancreatic elastase.

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 (18). RNA was quantitated by absorbance at 260 nm and solubilized for agarose-formaldehyde gel electrophoresis and transfer to nitrocellulose filters (19). Filters were then hybridized with ^{32}P-labeled α_1PI-specific complementary (c) DNA (20), complement factor B-specific cDNA (21), and complement C3-specific cDNA (22). After hybridization, filters were washed extensively, dried, and exposed to x-ray film for autoradiography.

Results

Effect of crude lymphokine on α_1PI expression by monocytes and macrophages. Peripheral blood monocytes were maintained in culture for 10 d. At specified intervals, monolayers were incubated with supernatants of unstimulated and Con A-stimulated peripheral blood mononuclear cells and then biosynthetically labeled for 3 h (Fig. 1). As shown in previous experiments (7), synthesis and secretion of α_1PI decreased during these 10 d in culture, as total protein synthesis and synthesis of other specific proteins, such as lysozyme, complement proteins C2 and factor B, increased (data not shown). Incubation with the crude lymphokine, however, led to an increase in expression of α_1PI by days 4–6, but even more noticeably by days 7–9 in culture. In-

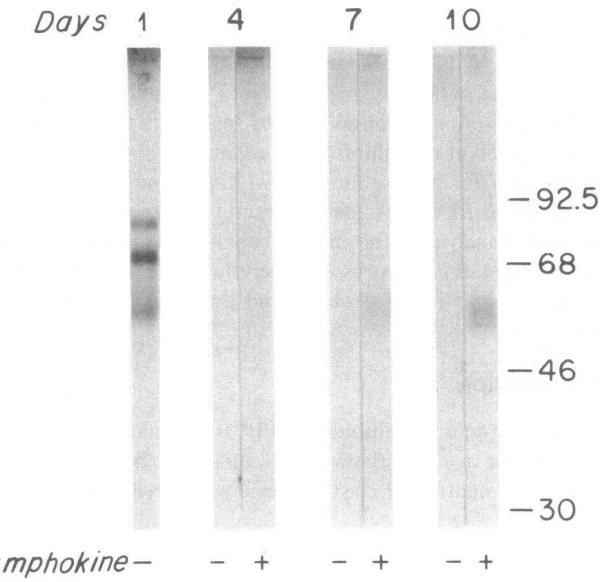

Figure 1. Effect of time in culture on and supernatants of Con A-stimulated peripheral blood mononuclear cells on biosynthesis and secretion of α_1PI by monocytes. Extracellular media from radiolabeled peripheral blood monocytes in culture for 1, 4, 7, and 9 d were immunoprecipitated with anti-human α_1PI. Equivalent volumes of each sample were used for immunoprecipitation despite increases of 3-, 8-, and 10-fold in total TCA-precipitable protein during that time. Monocytes were incubated in control supernatant (−) or supernatant of Con A-stimulated mononuclear cells (+) at 1:10 dilution for 48 h before labeling. Total TCA-precipitable protein was not independently affected by incubation in these supernatants. Molecular mass markers are indicated.

cubation with the crude lymphokine did not affect the expression of α_1PI in freshly isolated monocytes or macrophages (data not shown). These results are consistent with those reported by Boldt et al. (23) for immunohistochemically detected cell-surface α_1PI on unfractionated peripheral blood mononuclear cells incubated with Con A. During the initial 24 h in culture, monocytes secrete α_1PI in the native (55,000 mol wt) form and in forms complexed with serine protease (66,000 and 75,000 mol wt). In contrast, after 7 d in culture, lymphokine-treated monocytes secreted α_1PI in the native (55,000 mol wt) form alone. Native α_1PI secreted by such lymphokine-treated monocytes was not oxidatively inactivated, as demonstrated by the formation of a 75,000-mol wt radiolabeled band after addition of exogenous unlabeled elastase (data not shown).

Supernatants from Con A-stimulated mononuclear cells also increased expression of α_1PI by alveolar and breast milk macrophages (Fig. 2, left). The effect of the lymphokine on α_1PI expression was specific, in that total protein synthesis and synthesis of another specific protein, complement component C3 (Fig. 2, right), were not affected.

The effect of this lymphokine on expression of α_1PI in monocytes and alveolar macrophages involved a pretranslational mechanism as demonstrated by an increase in α_1PI mRNA content corresponding to the increase in synthesis and secretion of α_1PI (Fig. 3). There was also an increase in factor B mRNA content but no change in C3 mRNA content in lymphokine-treated macrophages (data not shown).

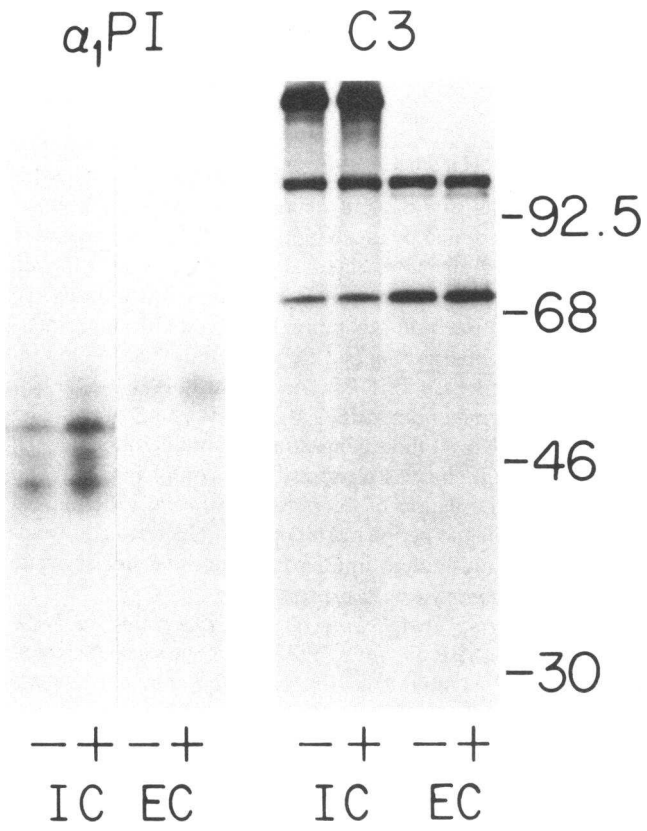

Figure 2. Effect of supernatants of Con A-stimulated peripheral blood mononuclear cells on biosynthesis and secretion of α_1PI and C3 by alveolar macrophages. Intracellular lysates (IC) and extracellular media (EC) from radiolabeled alveolar macrophages after 9 d in culture were immunoprecipitated with anti-human α_1PI (*left*) and then anti-human C3 (*right*). Macrophages were incubated in control supernatant (−) or supernatant of Con A-stimulated peripheral blood mononuclear cells (+) at 1:10 dilution for 48 h before labeling. Total TCA-precipitable protein did not vary significantly between samples. Molecular mass markers are indicated.

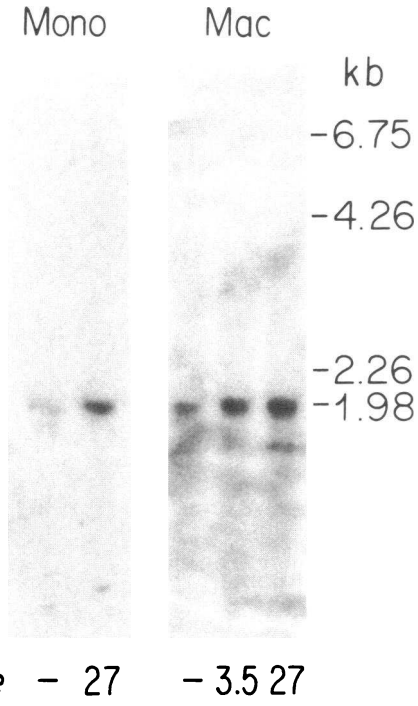

Figure 3. Detection of α_1PI mRNA in blood monocytes and alveolar macrophages after incubation with lymphokine. Equivalent amounts of total cellular RNA from blood monocytes (*left*) and alveolar macrophages (*right*) after 9 d in culture and incubation for 48 h in control (−) or lymphokine supernatants at various relative concentrations were applied to RNA blot analysis. Separate blots were hybridized with radiolabeled cDNA for α_1PI. The number of cells from which RNA was isolated did not vary significantly after incubation with control or lymphokine supernatants. DNA size markers are indicated.

Characteristics of the lymphokine stimulating α_1PI expression. The effect of supernatant from Con A-stimulated mononuclear cells on α_1PI expression was dose- and time-dependent (Fig. 4). Expression of α_1PI increased with increasing relative lymphokine concentration to 270 (dilution 1:10), decreasing slightly at higher relative concentrations (Fig. 4, left). At the optimal relative lymphokine concentration the increase in α_1PI was 200–250% of control, as demonstrated by direct scintillation counting of radiolabeled bands excised from the gels of four separate experiments (Fig. 5). At this relative lymphokine concentration, expression of α_1PI was ~55–60% of that present in the cell culture fluid of unstimulated monocytes during the first 24 h in culture. The effect of the lymphokine on synthesis and secretion of α_1PI reached its maximum between 24 and 48 h of incubation (Fig. 4, right). There was no further change between 48 and 72 h of incubation in lymphokine. The increase in α_1PI expression was not produced by a direct action of Con A or a product of unstimulated peripheral blood mononuclear cells on monocytes (Fig. 6).

Supernatants were also collected from peripheral blood mononuclear cells stimulated with Con A or PWM for varying intervals from 2 to 6 d. α_1PI expression by monocytes increased after incubation in each of these supernatants but increased

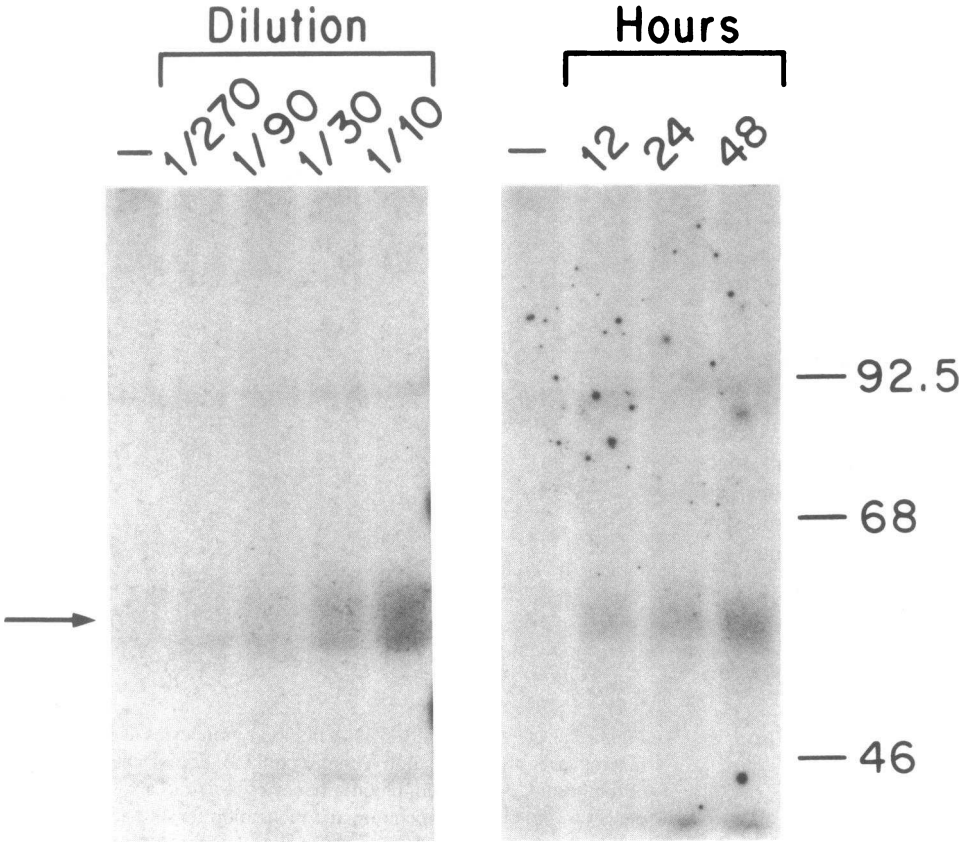

Figure 4. The effect of lymphokine supernatants on synthesis and secretion of α_1PI by monocytes is dose- and time-dependent. Extracellular media from radiolabeled monocytes in culture for 9 d were immunoprecipitated with anti-human α_1PI. Monocytes were incubated with control (−) or lymphokine supernatant at varying dilutions for 48 h (*left*) or lymphokine supernatants at 1:10 dilution for varying intervals (*right*) before labeling. Molecular mass markers are indicated.

maximally in supernatants from peripheral blood mononuclear cells stimulated with Con A for 4 d (data not shown).

The effect of supernatants from Con A-stimulated peripheral blood T lymphocytes and supernatants from alloreactive T4$^+$ lymphocyte clones was similar to that of supernatants from Con A stimulated peripheral blood mononuclear cells (data not shown). The factor in supernatants of Con A-stimulated mononuclear cells responsible for increasing monocyte α_1PI expression had the characteristics of a polypeptide. The effect of the supernatants was abrogated by incubation at 70°C for 60 min and at 100°C for 5 min or by treatment of supernatants with trypsin (data not shown). Monocyte α_1PI expression was not affected by 24-h incubation with recombinant-generated human interferon-alpha A, interferon-alpha D, interferon-beta, interferon-gamma, or interleukin 2 (IL-2) at concentrations at 1–1,000 U/ml, or with purified human IL-1 at concentrations of 0.5–12.5 U/ml (not shown). At these concentrations, interferon-alpha and interferon-gamma increased expression of complement C2 and factor B (24). Depletion of interferon-gamma in supernatants of Con A-stimulated peripheral blood mononuclear cells by adsorption with anti-human interferon-gamma did not abrogate the effect on monocyte α_1PI expression.

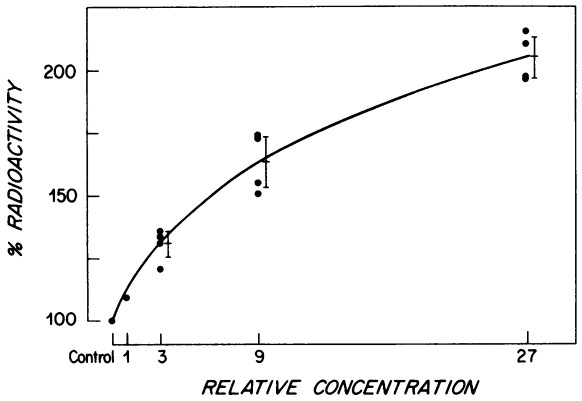

Figure 5. The effect of lymphokine supernatants on synthesis and secretion of α_1PI by monocytes is dose-dependent. After 7 d in culture monocytes were incubated for 48 h with control or lymphokine supernatants, and then radiolabeled. Extracellular media were immunoprecipitated and precipitates subjected to SDS-PAGE and fluorography. The radiolabeled band for native, 55,000-mol-wt α_1PI was excised from the gel, solubilized, and relative radioactivity determined by liquid scintillation counting. Data shown are for four separate experiments. Radioactivity is expressed as percentage as compared with control±1 SD.

Effect of crude lymphokine on α_1PI expression in PiZZ monocytes. Synthesis of α_1PI by PiZZ monocytes also increased in the presence of lymphokine (Fig. 7). Furthermore, the increase in the 52,000-mol wt intracellular form of α_1PI in PiZZ monocytes treated with lymphokine is accentuated when compared with the change in the 55,000-mol wt native extracellular α_1PI. The accentuation of intracellular α_1PI accumulation in PiZZ monocytes by the lymphokine was selective, in that total TCA-precipitable protein and other specific proteins were not similarly affected. Factor B and C3 (Fig. 7, center and right) were increased proportionally in intracellular lysates and extracellular media of lymphokine-treated PiZZ monocytes.

Discussion

The results of these experiments demonstrate that a product of mitogen-stimulated peripheral blood mononuclear cells increases

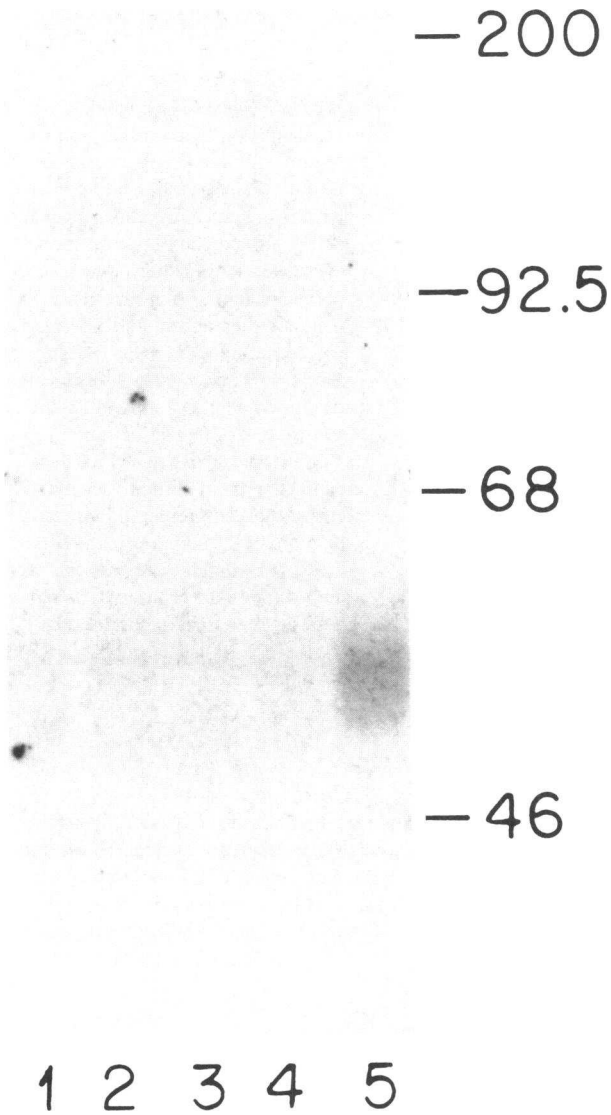

Figure 6. Controls for the effect of lymphokine supernatants on synthesis and secretion of α_1PI by monocytes. Extracellular medium from radiolabeled monocytes after 9 d in culture were immunoprecipitated with anti-human α_1PI. Before labeling, monocytes were incubated for 48 h with medium alone (lane *1*), medium supplemented with Con A 2 μcg/ml (lane *2*), medium supplemented with the supernatant of unstimulated mononuclear cells at 1:10 dilution (lane *3*), medium supplemented with the supernatant of unstimulated mononuclear cells at 1:10 dilution and Con A 2 μcg/ml (lane *4*), and medium supplemented with the supernatant of Con A-stimulated peripheral blood mononuclear cells at 1:10 dilution (lane *5*). Molecular mass markers are indicated.

the expression of α_1PI in human monocytes and macrophages by a pretranslational mechanism. This factor also increases the expression of α_1PI by monocytes from homozygous PiZZ-deficient individuals, and in so doing, accentuates the defect in secretion of α_1PI in these individuals. The regulating factor has the characteristics of a polypeptide and is probably elaborated by T lymphocytes, but it is not an interferon or IL-2.

The expression of α_1PI by monocytes is also regulated by time in culture and by interaction with serine protease (7). During the first 24 h in culture, synthesis and secretion of α_1PI is maximal and α_1PI appears in the cell culture fluid in native and protease-complexed forms. Synthesis and secretion of α_1PI progressively decreases during the first week in culture, but the small amount of α_1PI detectable in cell culture fluid by 7–10 d is almost entirely in native form. These data suggest that the serine protease(s) which interacts with α_1PI early in culture is either passively adsorbed to the cell surface but progressively lost during culture or that *de novo* synthesis of serine protease(s) by monocytes decreases abruptly during the first 4 d in culture. In either case, the factor present in supernatants of mitogen-stimulated peripheral blood mononuclear cells independently regulates the expression of α_1PI, since only the native 55,000-mol wt form increased in cell culture fluid of treated monocytes. It might be argued that serine protease expression was also increased by this lymphokine but not detected because a corresponding increase in release of oxygen radicals resulted in functional inactivation of α_1PI. This possibility was excluded by demonstrating the formation of a complex after the addition of exogenous protease to the cell culture fluid of lymphokine-treated monocytes.

Changes in the expression of α_1PI in monocytes during culture are not simply a reflection of cellular differentiation. Although α_1PI is secreted in native form alone by the "late" monocyte and alveolar macrophage, it is also in complex form in the cell culture fluid of breast milk macrophages. Expression of α_1PI in macrophages of either type decreases over 7–10 d in culture while synthesis of total TCA-precipitable protein and other specific proteins remains unchanged. Finally, the ratio of α_1PI in native to complexed form and the apparent size of complex forms vary between monocyte and breast milk macrophage, suggesting that there is independent regulation of serine protease and α_1PI in different local microenvironments. The expression of several other mononuclear phagocyte secretory proteins is increased by lymphokines. Interferon-gamma mediates an increase in expression of complement proteins C2 and factor B in monocytes and macrophages (24). Crude lymphokine supernatants increase synthesis and secretion of C1 inhibitor (25), plasminogen activator urokinase (26), and procoagulant activity (27) by monocytes. The lymphokine responsible for enhanced monocyte procoagulant activity is also derived from a T lymphocyte of the helper/inducer subset (28). A separate T lymphocyte cell-associated pathway may also increase monocyte procoagulant activity (28–30). Further studies will be necessary to identify the source and identity of the mediator(s) responsible for the increase in expression of α_1PI by monocytes and macrophages.

Most important, however, will be further studies of regulation of mononuclear phagocyte α_1PI expression by lymphokine as a model for the effect of enhanced synthesis on a defect in post-translational processing/secretion, i.e., homozygous PiZZ α_1PI deficiency. If the presence or severity of intracellular α_1PI accumulation is a factor involved in the development of chronic liver disease and/or pulmonary emphysema, differences in the elaboration of this lymphokine and similar mediators in the local environment of the target organs may contribute to the wide variability in disease manifestations among PiZZ individuals.

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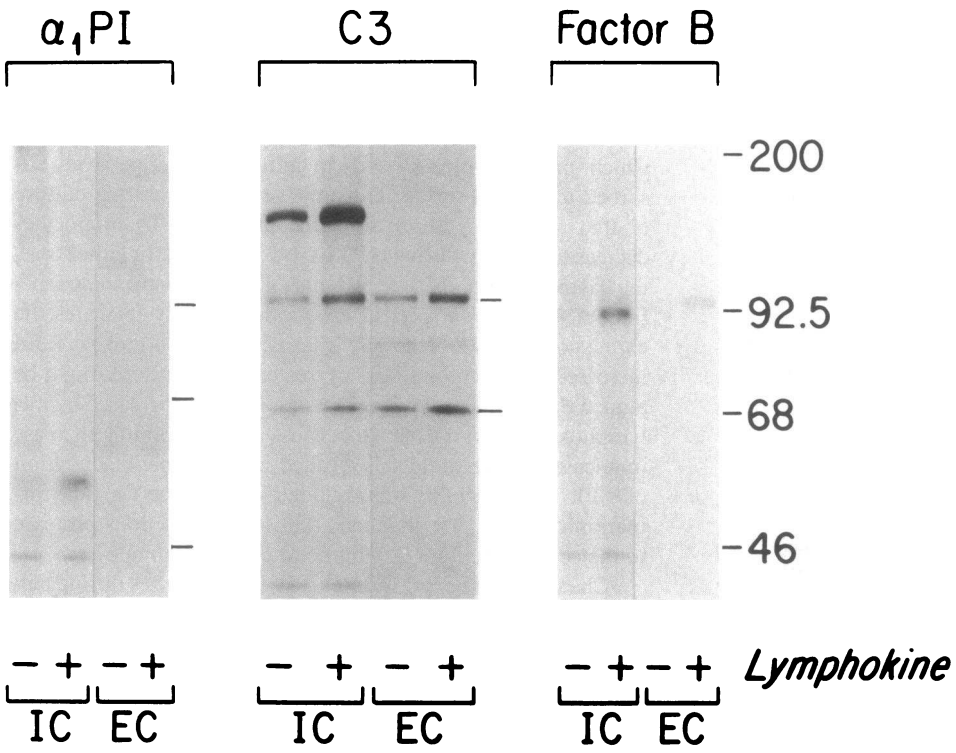

Figure 7. Effect of lymphokine supernatants on synthesis and secretion of α_1PI and C3 by monocytes from PiZZ individuals. Intracellular lysates (IC) and extracellular media (EC) from radiolabeled PiZZ monocytes after 9 d in culture were immunoprecipitated with antihuman α_1PI (*left*) and then anti-human C3 (*center*) or anti-human Factor B (*right*). Monocytes were incubated with control supernatant (−) or lymphokine supernatant (+) at 1:10 dilution for 48 h before labeling. Total TCA-precipitable protein did not vary significantly between samples. Fluorography for lanes containing extracellular media (EC) in left panel was conducted for 18 d as compared with 7 d for lanes containing intracellular lysates (IC). Molecular mass markers are indicated.

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