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Local control of α1-proteinase inhibitor levels: regulation of α1-proteinase inhibitor in the human cornea by growth factors and cytokines

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Abstract

Alpha 1-proteinase inhibitor is a major serine proteinase inhibitor in the human cornea involved in the protection of the avascular corneal tissue against proteolytic damage. This inhibitor is upregulated systemically during infection, inflammation and injury. Cytokines that mediate the acute phase response such as IL-1β and IL-2 increased α1-proteinase inhibitor present in corneal organ culture media. This released inhibitor represented mainly newly synthesized protein. However, IL-6, a general inducer of the acute phase response that upregulates α1-proteinase inhibitor in all other tissues and cells tested, failed to alter corneal α1-proteinase inhibitor levels over the tested period of 24 h. In addition to IL-1β and IL-2, α1-proteinase inhibitor levels in the corneal organ culture medium increased following the addition of FGF-2 and IGF-I. The effect of the above growth factors and cytokines was relatively fast with maximal induction observed within the first 5 h. Among the tested growth factors and cytokines, IL-1β was the most potent and increased total corneal α1-proteinase inhibitor levels approximately 2.4-fold in the cornea organ culture medium. Newly, synthesized α1-proteinase secreted into the medium increased 3.9-fold. In addition to the effect on corneal α1-proteinase inhibitor, IL-1β also increased the amount of α1-proteinase inhibitor released by monocytes and macrophages but not by HepG2, CaCo2, and MCF-7 cells within 24 h. These results suggest that the cornea can locally control levels of α1-proteinase inhibitor in response to an inflammatory insult. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: α1-Proteinase inhibitor; Cornea; Interleukin-1β; Interleukin-2; Interleukin-6; Fibroblast growth factor-2

1. Introduction

α1-Proteinase inhibitor (α1-PI) is a member of the serpin family of proteins. It is the major proteinase inhibitor in human plasma and many tissues [1]. Human α1-PI is a 59 kDa secretory glycoprotein synthesized mainly by the liver [2]. From the liver, it is secreted into the blood stream and supplied to tissues. Extrahepatic tissues and cells that synthesize

Abbreviations: α1-PI, α1-proteinase inhibitor; E64, *trans*-epoxysuccinyl-leucylamido-(4-guanidino)butane; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGF-I, insulin like growth factor; IL, interleukin; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; TGF-α, transforming growth factor-α; TNF-α, tumor necrosis factor-α

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this inhibitor include: blood monocytes and macrophages [3], alveolar macrophages [4], intestinal epithelium cells [5], breast carcinoma cells [6] and the cornea [7]. Metabolic labeling and in situ hybridization studies showed all three layers of the cornea synthesize not only α1-PI, but also α1-antichymotrypsin and α2-macroglobulin [7–9]. The ability of the corneal cells to synthesize proteinase inhibitors is critical because of the avascular nature of the cornea.

α1-PI is capable of inhibiting most serine proteinases, however, the major target of this inhibitor is neutrophil elastase [10]. This inhibitor plays an important role in protection of tissues against extensive proteolytic damage. This is true during infection, inflammation and/or injury when proteolytic enzymes are released from the affected tissue and migrating neutrophils. In response, α1-PI synthesis is increased in hepatocytes and distributed to the tissues via the blood supply [11].

During the acute phase response, levels of cytokines such as IL-1β, IL-2 and IL-6 are increased [12,13]. However, these cytokines are not elevated simultaneously; IL-1β and IL-2 are increased early in the acute phase response and IL-6 is increased at the end of the acute phase response. IL-6 upregulates the synthesis of α1-PI in most tissues and cells that synthesize this inhibitor [14]. In contrast, IL-1β increases α1-PI in MCF-7 breast [6] but does not affect α1-PI levels in HepG2 cells [15]. The effects of cytokines and growth factors on corneal proteinase inhibitors was not previously studied. The only known molecule reported to increase levels of α1-PI in the cornea was retinol [16]. In this study, we showed that in the cornea α1-PI levels were increased following the addition of IL-1β and IL-2 but not IL-6. In addition, corneal α1-PI levels were also increased in the presence of FGF-2 and IGF-I.

2. Materials and methods

2.1. Materials

Interleukin-6 was purchased from Genzyme (Cambridge, MA). FGF-2, EGF, IGF-I, TGF-α, TNF-α, IL-1β, and IL-2 were from Promega (Madison, WI). Culture medium, penicillin–streptomycin and fetal calf serum were from Life Technologies (Gaithersburg, MD). All other chemicals used were from Sigma [^{35}S]methionine (labeling grade) was from Amersham (Arlington Heights, IL).

2.2. Methods

2.2.1. Cornea organ culture

Human eyes in humid chambers were obtained from the Wisconsin Lions Eye Bank (Milwaukee, WI) kept at 4°C until used. The corneas were dissected from the globe within 18–36 h of death of the donor. The levels of α1-proteinase inhibitor in the organ cultured corneas or the culture mediums was not dependent on the elapsed time between death of the donor and placement in culture. The corneas were weighed and placed in 1 ml of minimum essential medium containing 100 U penicillin and 100 μg streptomycin and cultured for 2 h, at 37°C with 5% CO_2. After a 2-h preincubation period growth factors or cytokines were added and culture continued for an additional 2, 5, 15 or 24 h. The following growth factors and cytokines were used: basic fibroblast growth factor (FGF-2), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), tumor necrosis factor-α (TNF-α), transforming growth factor-α (TGF-α), interleukin-1β (IL-1β), interleukin-2 (IL-2), and interleukin-6 (IL-6). All growth factor and cytokines were used at 5 ng/ml as the final concentration except for TNF-α which was used at the final concentration of 0.2 ng/ml. These levels were chosen based on the reported active levels of the cytokines/growth factors in vitro as specified by the supplier and are within the range of concentrations observed for the cytokines and growth factors found in the tears, aqueous humor or the cornea following injury [17–21]. TNF-α was used at 0.2 ng because of its lower concentration in wounded corneas [20]. Control corneas (contralateral to the experimental corneas) were incubated in the same manner except the growth factors and cytokines were not added. In addition, [^{35}S]methionine (83 μCi/ml) was added to some FGF-2 and IL-1β treated and control corneal cultures to determine whether α1-PI measured by immunological methods represented newly synthesized inhibitor. At 0, 2, 5, 15 and 24 h time

points, the corneas were removed from the medium, washed in phosphate-buffered saline, frozen in liquid nitrogen and freeze fractured. Freeze fractured corneas were homogenized with a glass homogenizer and extracted in 10 mM Tris–Cl buffer, pH 7.2, 140 mM NaCl, 10 mM EDTA, 5 mM E64, 1 μM pepstatin, 10 mM phenylmethylsulfonyl fluoride (PMSF), 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS) and 1% nonidet NP40 (100 ml per 10 mg corneal tissue). The particulate matter was removed by centrifugation at $15\,000 \times g$ for 15 min at 4°C.

2.2.2. Cell culture

The human liver tumor cell line HepG2 and the human breast adenocarcinoma cell line MCF-7 were purchased from American Type Culture Collection (ATCC, Rockville, MD). Both cell lines were cultured in the minimum essential medium supplemented with 10% fetal calf serum according to the ATCC recommended protocol. The human intestinal epithelial cell line, CaCo2, was obtained from Dr. Nancy Dahms (Medical College of Wisconsin) and cultured in Dulbecco's modified Eagle medium supplemented with 20% fetal calf serum. 'Buffy coat' fractions of the human blood were provided by the Blood Center of Southeastern Wisconsin. Leukocytes were isolated from the 'buffy coat' by the method of Carlson and Kaneko [22]. Isolated leukocytes were cultured in the Medium 199 containing 100 U of penicillin and 100 μg streptomycin according to a previously described method [23]. Mononuclear cells were used either after 2 h in culture (monocytes) or after 7 days (macrophages). Cells were preincubated in the minimum essential medium without serum for 2 h and then in the presence or absence of 5 ng/ml IL-1β for additional 5 h. The conditioned medium was collected. Cells were harvested by trypsinization, centrifuged and extracted in the same buffer and by the same procedure used for corneal extraction.

2.2.3. Immunoassay for α1-PI

Quantification of α1-PI by immunoprecipitation was done as previously described [7]. Briefly, the assay was conducted in 96-well Durapore membrane plates (Millipore; Bedford, MA). First, human α1-PI (Athens Research Technologies; Athens, GA) was bound to the membranes at the bottom of the plate wells by the incubation of 10 μg/well of α1-PI in 100 μl of 50 mM Tris buffer, pH 7.5 containing 150 mM NaCl and 0.02% sodium azide (buffer A) for 1.5 h at room temperature. Nonbound sites on the membrane were blocked using 5 μg bovine serum albumin/well in 200 μl buffer A. The membranes were washed once with 200 μl/ml buffer A and then three times with 200 μl/well of the same buffer containing 0.05% Triton X-100. Next, the membranes were incubated for 1 h with 5.11 μg mouse monoclonal antibodies/well to human α1-PI (Medix Biotech; Foster City, CA) in 200 μl buffer A. The membranes were washed as before. One hundred μl/well of corneal extract, conditioned medium, or standard α1-PI solutions was added to the wells and incubated for 2 h at room temperature. Immunoprecipitated proteins were released from the membrane by incubation with 100 μl of 0.1 M Tris, pH 6.8, 20% glycerol, 4% SDS, 26.7 mM dithiothreitol and 0.05% bromphenol blue for 10 min at 90°C. Proteins were then separated by 10% SDS-PAGE under reducing conditions [24] and either visualized by silver staining (BioRad; Hercules, CA) or when [^{35}S]methionine labeled, processed for autoradiography. For autoradiography, the gels were incubated in Amplify (Amersham, Artlington Heights, IL) for 2 h, dried and exposed to X-Ray film. The bands on the gels or the X-ray film were digitized using AMBIS imaging system (San Diego, CA). The concentration of α1-PI was determined from the standard curve and expressed as a percent of the amount of α1-PI in control corneas.

2.2.4. Western blot assay

The cornea/cell extracts or conditioned mediums were mixed with an equal volume of 0.1 M Tris buffer pH 6.8, 20% glycerol, 2% SDS, 0.004% bromphenol blue and electrophoresed on 9% SDS-polyacrylamide gels [24]. Following electrophoresis, the proteins were electroblotted to nitrocellulose sheets (BA83-Schleicher and Schuell; Keene, NH). The nitrocellulose blots were then blocked with 50 ml 10% dry milk in TBS-T buffer pH 7.6 (3.5 mM Tris, 137 mM NaCl, 0.1% Tween-20). The blots were washed, probed and the bands visualized by chemiluminescence according to the protocol recommended for use with the enhanced chemiluminescence detection system (ECL, Amersham; Arlington Heights, IL). Monoclonal antibodies raised against human α1-

PI (Medix Biotech), human α1-antichymotrypsin (Medix Biotech), human β-actin (Sigma) and rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Advanced Immuno Chemical, Long Beach, CA) were used as primary antibodies. Horseradish peroxidase conjugated-goat anti-mouse IgG (BioRad; Hercules, CA) was used for the secondary antibody. The intensities of the protein chemiluminescent bands were measured on an AMBIS imaging system (San Diego, CA) and the amount of the proteins determined from the standard curves. Human α1-PI (Athens Research Technologies), α1-antichymotrypsin (Athens Research Technologies) and GAPDH (Worthington Biochemical) were used to prepare standard curves.

2.2.5. Dot blot assay

Dot blot assays were done by loading corneal/cell extracts or conditioned medium onto nitrocellulose sheets (BA83-Schleicher and Schuell) in a 96-well Mini-fold dot blot apparatus (Schleicher and Schuell). The dot blots were washed and probed as described for the Western blots. The intensities of the dot blots were quantified using an EL380 Microplate Autoreader (Bio-Tek Instruments; Winooski, VT).

2.2.6. Statistical analysis

The α1-PI levels obtained by immunoprecipitation, Western blot assay and dot blot assay were statistically analyzed by the Student's *t*-test using SigmaStat software (Jandel).

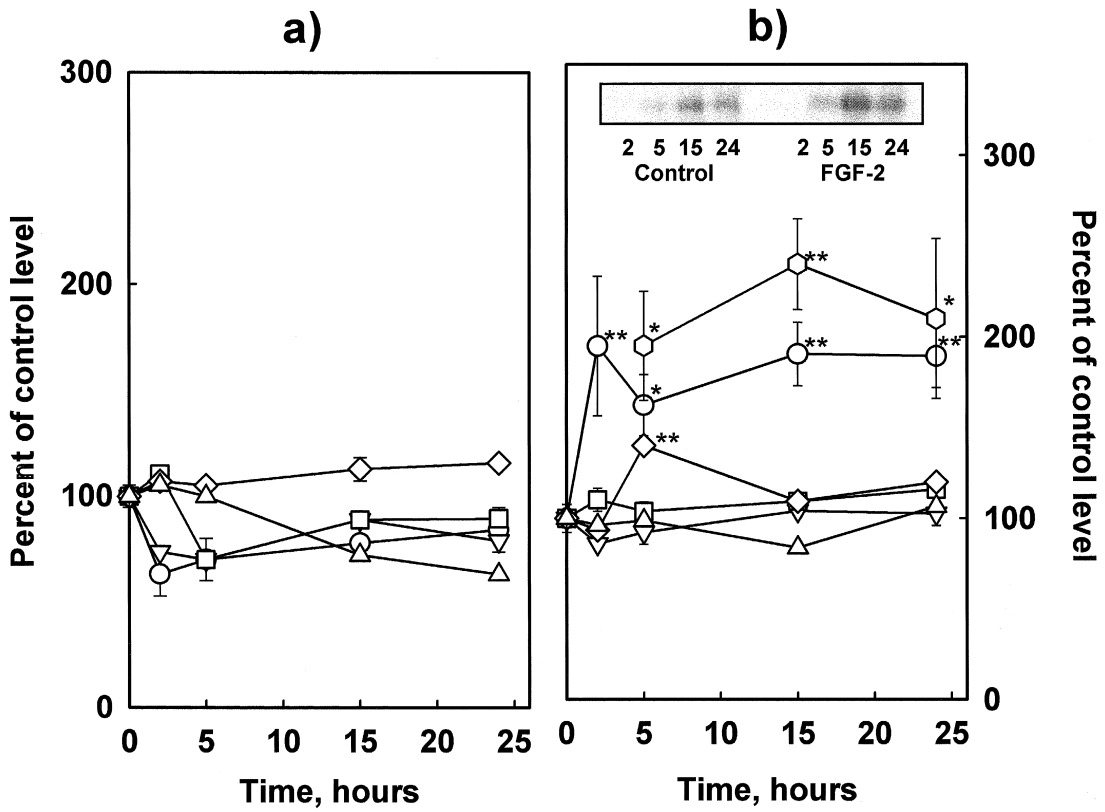

Fig. 1. The effect of growth factors on α1-PI protein levels in the cornea. Levels of α1-PI retained in the cornea (a) and secreted into the medium (b) following the addition of EGF (▽), FGF-2 (○), IGF-I (◇), TGF-α (□), and TNF-α (△), or FGF-2 plus [^{35}S]methionine (octagon) as determined by immunoprecipitation. Growth factors were added to the culture medium at a final concentration of 5 ng/ml, except TNF-α which was added to final concentration of 0.2 ng/ml. Levels of α1-PI in the treated corneas are expressed as percent of the levels of α1-PI in the untreated corneas. All experimental values represent the average of at least three independent measurements in corneas from at least three individuals. The error bars represent the absolute error. Inset: Representative electrophoretic separation of immunoprecipitated ^{35}S methionine labeled α1-PI synthesized by human corneas in the presence or absence of 5 ng/ml FGF-2. The bands were visualized by autoradiography. ∗∗Experimental values significantly different from control value ($p < 0.001$). ∗Experimental values significantly different from control value ($p < 0.005$).

3. Results

3.1. Effect of growth factors on corneal α1-PI

The effects of growth factors (FGF-2, EGF, IGF-I, TNF-α, and TGF-α) on α1-PI levels in the corneal tissue and conditioned medium were analyzed by time course experiments (Fig. 1). Following a 2-h preincubation in serum-free minimal essential medium, the above mentioned growth factors were added to the culture medium and the amounts of α1-PI protein were determined immunologically at 2, 5, 15, and 24 h following their addition. None of the tested growth factors were able to significantly alter α1-PI protein levels retained in the corneal tissue (Fig. 1a). However, FGF-2 and IGF-I increased α1-PI protein levels secreted into the conditioned medium over that secreted by the control corneas (Fig. 1b). A 2-fold increase in secreted α1-PI was observed 2 h following addition of FGF-2 to the cultured medium. At subsequent time points, 5, 15 and 24 h, α1-PI levels were also significantly higher than control levels. IGF-I increased α1-proteinase levels 1.4-fold at 5 h after the addition of the growth factor but not at 2, 15 or 24 h. EGF, TGF-α, and TNF-α did not affect α1-PI levels.

In the control corneas (incubated for the same period of time but without growth factors or cytokines), the levels of α1-PI secreted into the conditioned medium were approximately 4-fold higher

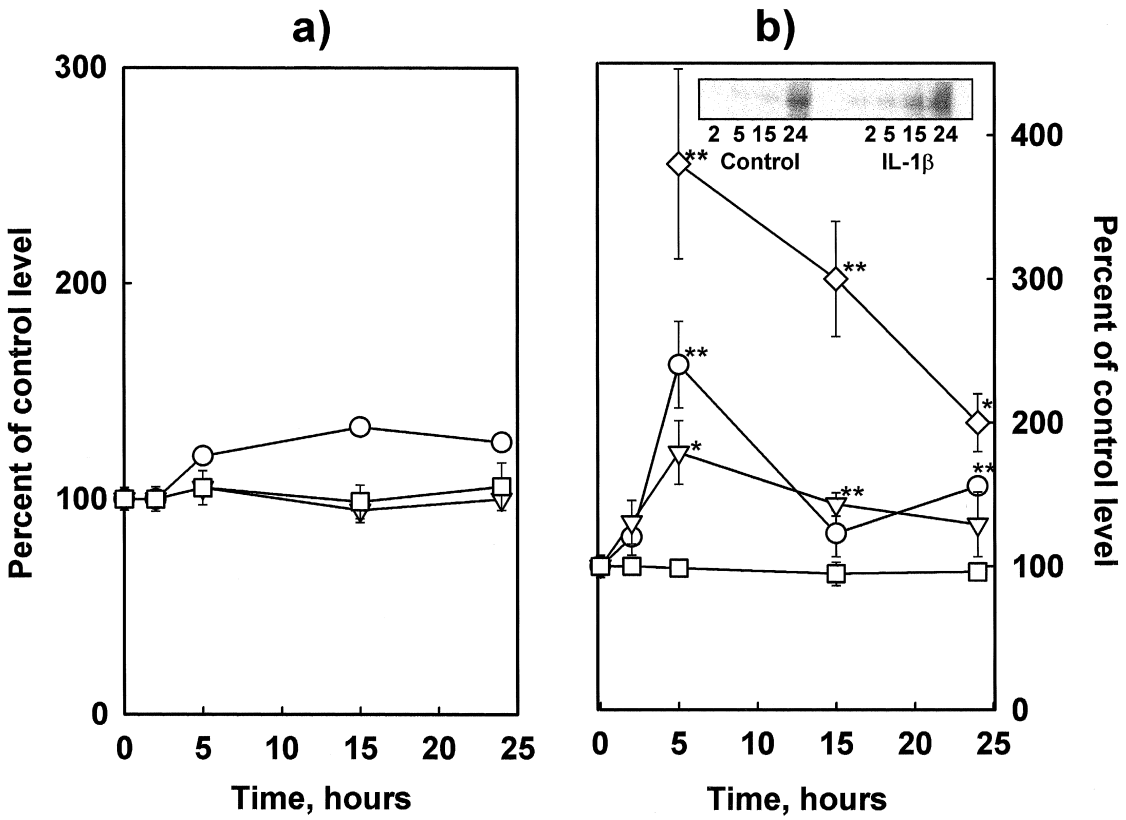

Fig. 2. The effect of cytokines on α1-PI protein levels in the cornea. Levels of α1-PI retained in the cornea (a) and secreted into the conditioned medium (b) following addition of IL-1β (○), IL-2 (▽), and IL-6 (□), or IL-1β plus ^{35}S-methionine (◇) as determined by immunoprecipitation. Cytokines were added to the culture medium at a final concentration of 5 ng/ml. Levels of α1-PI in the treated corneas are expressed as percent of the levels of α1-PI in the untreated corneas. All experimental values represent the average of at least three independent measurements on corneas from at least three individuals. The error bars represent the absolute error. Inset: Electrophoretic separation of immunoprecipitated [^{35}S]methionine labeled α1-PI synthesized by human corneas in the presence or absence of 5 ng/ml IL-1β. The bands were visualized by autoradiography. ∗∗Experimental values significantly different from control value ($p < 0.001$). ∗Experimental values significantly different from control value ($p < 0.005$).

compared to the α1-PI amounts retained in the cornea tissue ($0.19 + 0.01$ μg/mg cornea for the corneal tissue and $0.87 + 0.2$ μg/mg cornea for the conditioned medium) at 5 h. Upon stimulation with FGF-2 for 2 h, the amount of α1-PI released into the medium increased to approximately 11-fold that present in the cornea ($0.14 + 0.02$ μg/mg cornea for the corneal tissue and $1.52 + 0.3$ μg/mg cornea for the conditioned medium). A more modest increase was noted for IGF-I ($0.21 + 0.02$ μg/mg cornea for the corneal tissue and $1.08 + 0.0$ μg/mg cornea for the conditioned medium).

To determine whether the increase in α1-PI secreted into the cornea conditioned medium in the presence of growth factors represented the stimulation of new protein synthesis, selected corneas were metabolically labeled with ^{35}S-methionine in the presence or absence of FGF-2. After the initial 2 h preincubation period, the label was added at the same time as the growth factor. Very little labeled α1-PI was immunoprecipitated after 2 h in the control (Fig. 1b). The amount of labeled α1-PI immunoprecipitated differed from one donor to another (Fig. 1b and Fig. 2b, insets). Because the contralateral cornea was used for the treated corneas, the percent increase due to a given treatment was similar at the various time points between donors. Metabolically-labeled α1-PI concentrations in the conditioned mediums of corneas treated with FGF-2 were increased approximately 2-fold at 5, 15 and 24 h over those in the control

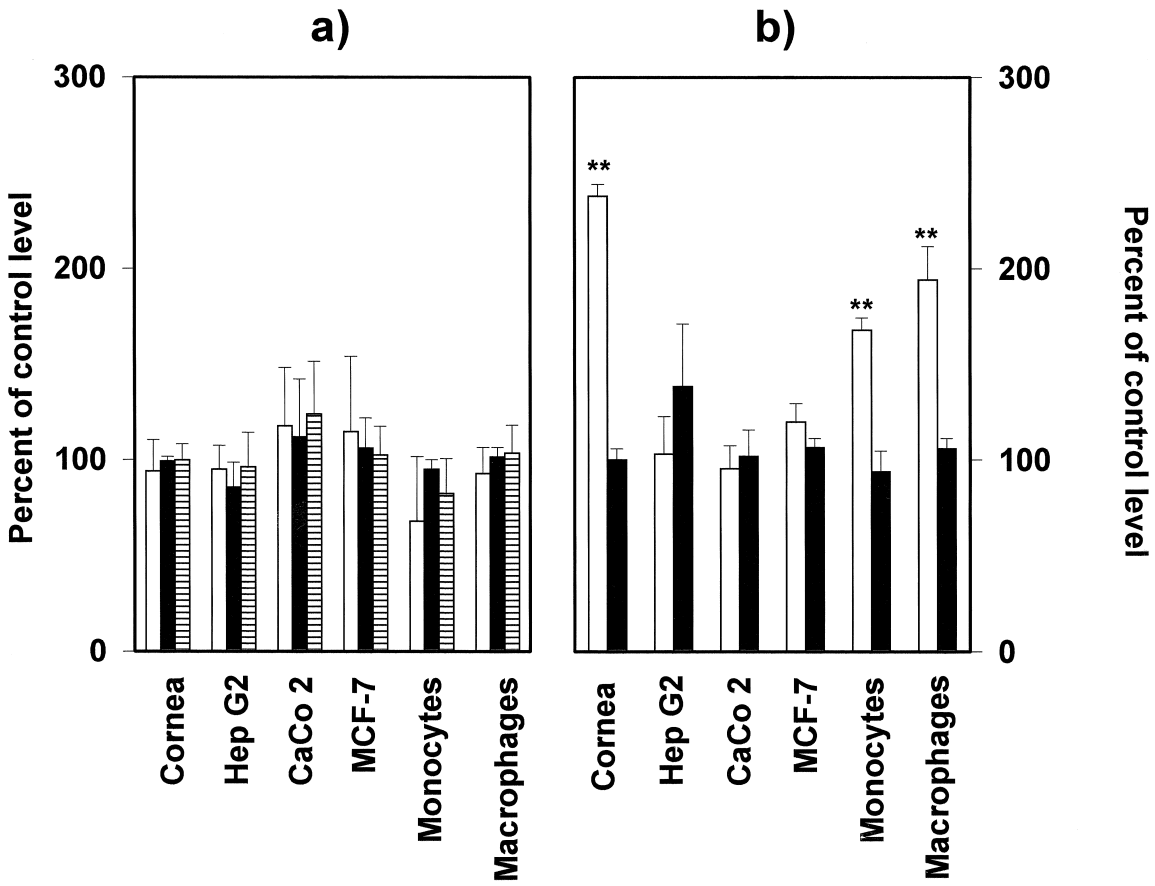

Fig. 3. The effect of IL-1β on α1-PI, α1-antichymotrypsin and β-actin protein levels in the cornea and cells derived from the tissues that synthesize α1-PI. Protein levels of α1-PI (□), α1-antichymotrypsin (■), and β-actin (square with horizontal lines) in the cornea and cell extract (a) and conditioned medium (b) following 5 h incubation in the presence of 5 ng/ml IL-1β. Values were determined by dot blot assay except for β-actin which was determined by Western blot assay. Levels of measured proteins were standardized based on GAPDH levels. All experimental values represent the average of at least three independent measurements. The error bars represent the absolute error. $**$Experimental values significantly different from control value ($p < 0.001$).

conditioned mediums. This increase is similar to that observed for the secreted protein levels in the presence of FGF-2.

3.2. Effect of cytokines on corneal α1-PI

Similar to the effect of the growth factors, no cytokine tested had a significant effect on α1-PI protein levels in the corneal tissue (Fig. 2a), however, two cytokines increased secretion of α1-PI into the conditioned medium. IL-1β and IL-2 were able to elevate α1-PI protein levels secreted into the conditioned medium (Fig. 2b). In the presence of IL-1β, α1-PI levels were increased 2.4-fold over the control level, while addition of IL-2 to the culture medium induced an 1.8-fold increase. The maximal increase in α1-PI levels was observed at 5 h following addition of either IL-1β or IL-2. IL-6 did not significantly alter corneal α1-PI levels in corneal tissue or corneal conditioned medium over the tested period of 24 h.

Corneas incubated in organ culture with IL-1β and metabolically labeled with [^{35}S]methionine secreted 3.9-, 3-and 2-fold more α1-PI at 5, 15 and 24 h, respectively, over those in the control culture (Fig. 2b). At 2 h, metabolically labeled α1-PI was observed in the conditioned medium of corneas incubated with IL-1β but very little was detected in the control conditioned medium. With time, the newly synthesized α1-PI present in the conditioned medium of the control corneas increased faster than in the IL-1β treated corneas resulting in a decrease in the fold stimulation with the cytokine.

3.3. Effect of IL-1β on α1-PI in cells that synthesize this inhibitor

As shown in Fig. 2, IL-1β increased α1-PI levels in corneal conditioned medium. Furthermore, among the growth factors and cytokines tested, IL-1β had the greatest effect on α1-PI protein levels in the cornea. The effect of this cytokine on corneal α1-PI levels was compared to the effect of IL-1β on this inhibitor in other cells that synthesize α1-PI. α1-PI levels were determined 5 h following the addition of IL-1β to the corneal, HepG2, CaCo2, MCF-7, blood monocyte, and blood macrophage cultures (Fig. 3). In addition to the cornea, IL-1β significantly increased α1-PI levels secreted into monocyte and macrophage conditioned mediums, 1.7- and 1.9-fold, respectively (Fig. 3b). After 5 h of culture, IL-1β did not affect α1-PI levels in HepG2, CaCo 2, and MCF-7 cells. This cytokine did not alter the levels of α1-PI retained in cells (Fig. 3a). IL-1β had no significant effect on the levels of α1-antichymotrypsin or β-actin in the cornea or any cells tested (Fig. 3a and b).

4. Discussion

FGF-2, IGF-I, IL-1β, and IL-2 increased the secretion of α1-PI from human corneas in organ culture relative to nontreated control corneas. The increase in secreted α1-PI represents newly synthesized protein. [^{35}S]methionine labeled α1-PI levels increased in response to IL-1β or FGF-2 relative to control levels in a similar manner to that observed for secreted unlabeled α1-PI. The IL-1β and FGF-2 stimulated increase in metabolic labeled α1-PI represented the α1-PI synthesized after the addition of the cytokine or growth factor. In the nonlabeled experiments, α1-PI synthesized and secreted during the 2 h preincubation period was measured in addition to the inhibitor synthesized following the addition of the cytokines or growth factors. Considering this difference, the measured cytokine and growth factor stimulations by both methods are in very good agreement.

In contrast to the α1-PI levels in the corneal conditioned medium, the α1-PI level in the cornea did not increase in response to the cytokines. Because α1-PI is a secreted protein, these results suggest the corneal epithelial and/or endothelial cells are affected by the cytokines/growth factors to a greater extent than the stromal keratocytes. If stromal keratocyte synthesis of α1-PI was stimulated, an increase in α1-PI would be expected in the cornea due to deposition of this inhibitor in the stromal extracellular matrix. Corneal stromal keratocytes can synthesize α1-PI as shown by the presence of α1-PI mRNA in these cells by in situ hybridization studies and metabolically labeled α1-PI in the stroma [7]. Thus, the lack of an effect on the retained levels of α1-PI may (1) reflect an absence of receptors to the cytokines and growth factors on the stromal cells, (2) decreased access of the cytokines to the stromal keratocytes relative to the epithelial and endothelial cells or (3) a difference in the mechanism of regula-

tion of α1-PI synthesis by the stromal cells. Because IL-1R and FGF-R are present on stromal keratocytes [25], the absence of a response to the cytokines by stromal keratocytes probably is not due to a lack of receptors. Diffusion of the cytokines (7 to 26 kDa) into the stromal matrix may be a factor. Unlike growth factors and cytokines, a smaller molecule, retinol induces increases in the α1-PI retained in the cornea [16]. In addition, there may also be a difference in the mechanism of control of the synthesis of α1-PI in the corneal stromal keratocytes relative to the epithelial and/or endothelial cells.

In the presence of IGF-I, IL-1β and IL-2, α1-PI levels in the cornea conditioned medium peaked at 2 to 5 h relative to the controls and then decreased. This decrease in α1-PI concentration in the presence of the cytokines/growth factors relative to control α1-PI at the later time points was probably due to increased degradation of α1-PI. IL-1β, like IL-1α, stimulates the synthesis of matrix metalloproteinases [26] and plasminogen (unpublished results) by the cornea.

Among the tested growth factors and cytokines, IL-1β, an acute phase cytokine, increased α1-PI levels the most. In addition to the cornea, IL-1β increased α1-PI levels in the blood monocyte and macrophage conditioned mediums but not the HepG2, CaCo2, or MCF-7 cell conditioned mediums. A previous study also showed this cytokine did not affect α1-PI levels in HepG2 cells [27]. Following 54 h incubation, IL-1β treatment upregulated α1-PI in MCF-7 cells [6]. In our study, α1-PI levels in cells and cornea were determined following a 5-h incubation in the presence of IL-1β. Lack of a response of α1-PI to IL-1β by HepG2 and CaCo2 cells was not due to a deficiency of the IL-1 receptor because these cells contain the IL-1R [28,29]. Further, other genes respond to IL-1β in these cells [28–33].

In contrast to IL-1β, IL-6, a general inducer of the acute phase response, did not alter corneal α1-PI levels during the tested period of 24 h. This was true both for 5 ng/ml used in the current experiments and at 100 ng/ml in our previous experiments [16]. IL-6 at 100 ng/ml was able to increase α1-PI levels secreted into the conditioned mediums by HepG2, CaCo2, MCF-7 cells, blood monocytes and macrophages but not cornea [16]. The absence of a response by the cornea is not due to a lack of IL-6 receptors. Corneal epithelial cells can respond to IL-6 to upregulate the synthesis of integrin a5β [33]. The absence of a response of α1-PI to IL-6 in the cornea is probably due to differences in the regulation of the α1-PI gene by IL-6 between tissues.

The observed cytokine/growth factor stimulated secretion of α1-PI may be physiologically important since these cytokines/growth factors are locally available to the cornea either under normal or disease conditions and are available in the same concentration ranges as those used in the current experiments [17–21]. FGF-2, IGF-I, IL-1β, and IL-2 are synthesized by corneal cells [17,25,34–36] and are beneficial in corneal wound healing [37,38]. In organ culture of human corneas, FGF-2 promotes endothelial wound healing predominately by stimulation of cell migration [38]. In the closed wound, the central endothelial cell density is significantly higher in FGF-2 treated corneas than in controls. The time of complete wound closure is also shortened. After excimer laser keratomileusis in a rabbit model, a highly significant acceleration in epithelial wound healing is observed following FGF-2 application to the corneal surface [37]. IL-1β increases the rate of closure of wounds inflicted on rabbit corneal epithelial cell cultures [39]. IL-2 also increases corneal wound healing but, the effect is indirect because IL-2 increases FGF-2 expression in the cornea [40]. The increase in corneal wound healing in the presence of FGF-2, IL-1β and IL-2 can, at least in part, be explained by upregulation of corneal α1-PI in the presence of these extracellular molecules.

The ability of the cornea to increase the synthesis of α1-PI in response to cytokines and growth factors indicates that the cornea can respond to its environment. Corneal synthesis of this inhibitor is probably important under inflammatory conditions to protect the cornea from degradation by neutrophil elastase. The avascular nature of the cornea makes this response critical because of the lack of supply of α1-PI from blood.

Acknowledgements

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