Retinol and retinaldehyde specifically increase α 1-proteinase inhibitor in the human cornea

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α 1-Proteinase inhibitor is a serpin and can inhibit most serine proteinases. The cornea is one of several extrahepatic tissues that synthesizes this inhibitor. In the presence of retinol, corneal α 1-proteinase inhibitor levels were increased 3.8-fold. The maximal response was achieved 2 h after the addition of retinol (1 μ M final concentration) to the culture medium. A similar increase in α 1-proteinase inhibitor was observed with retinaldehyde (1 nM final concentration). Concentrations of α 1-proteinase inhibitor in other tested cells (Hep G2, CaCo 2, MCF-7, monocytes and macrophages) remained unchanged in the presence of retinol.

Retinoic acid did not affect α 1-proteinase inhibitor levels in the cornea or the other cells tested. The acute-phase cytokine, interleukin-6, increased α 1-proteinase inhibitor levels in all tested tissues/cells except the cornea. These results demonstrate that α 1-proteinase inhibitor levels are controlled differently in the cornea compared with other tissues/cells. α 1-Proteinase inhibitor is the first protein identified whose levels are regulated by a mechanism supported by retinol and retinaldehyde but not retinoic acid.

INTRODUCTION

Proteinase inhibitors provide protection against extensive proteolytic damage. α 1-Proteinase inhibitor (α 1-PI) is a serpin and a major proteinase inhibitor found in plasma [1]. Although α 1-PI can inhibit most serine proteinases *in vitro*, the main α 1-PI target is neutrophil elastase. Mechanistically, α 1-PI is a suicide inhibitor [2–4].

α 1-Proteinase inhibitor is synthesized mainly by the liver [5] and is secreted into the blood stream. In plasma, α 1-PI is the major proteinase inhibitor responsible for about 90% of the anti-tryptic activity [6,7]. Extrahepatic cells capable of α 1-PI synthesis include the following: blood monocytes and macrophages [8], alveolar macrophages [9], intestinal epithelial cells [10], breast carcinoma cells [11] and those in the cornea [12]. Blood α 1-PI levels increase during pregnancy and steroid usage [13–16]. Being a positive acute-phase protein, plasma levels of α 1-PI are increased in response to an infection [17], inflammation [18] and injury [19]. Interleukin-6 (IL-6), a general inducer of the acute-phase response, elevates α 1-PI in all tissues and cell lines previously tested [20]. In addition, transforming growth factor- β [21], bacterial lipopolysaccharide [22] and elastase [23] also up-regulate α 1-PI. Although retinoic acid is known to up-regulate several serpins [24,25], the regulation of α 1-proteinase inhibitor by retinoids has not been reported.

We have shown previously that the cornea is an extrahepatic site of α 1-PI production [12]. Synthesis of α 1-PI by the cells of the cornea's three major layers (epithelium, stroma and endothelium) was demonstrated using hybridization *in situ*. Additionally, metabolic labelling of corneas using [35 S]methionine showed the presence of newly synthesized α 1-PI in corneal extracts and conditioned medium.

Here we report that retinol and retinaldehyde, but not retinoic acid, increase α 1-PI protein in the human cornea but not in other cells/tissues. Additionally IL-6, which up-regulates α 1-PI in all tissues/cells tested, did not affect levels of α 1-PI in the cornea. Consequently, corneal α 1-PI is controlled differently than in other tissues/cells.

EXPERIMENTAL

Interleukin-6 was purchased from Genzyme (Cambridge, MA, U.S.A.). Retinol and retinoic acid were obtained from J. T. Baker Co. (Phillipsburg, NJ, U.S.A.). Retinaldehyde, E64, PMSF and pepstatin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Culture medium, penicillin, streptomycin and fetal-calf serum were from Life Technologies (Gaithersburg, MD, U.S.A.). All other chemicals used were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All retinoids and retinol-containing solutions were handled under yellow lights.

Retinol purity

Retinol purity was determined by reversed-phase HPLC using two C₁₈ columns (EQC–Whatman; Hillsboro, OR, U.S.A.) in tandem on a 4500-Chromatography System (Dionex, Sunnyvale, CA, U.S.A.) with detection at 350 nm. The mobile phase was ethyl acetate/1% (w/v) ammonium acetate (80:20) with a 0.5 ml/min flow rate. Under these conditions, retinol had a retention time of 18.5 min and retinaldehyde 21.5 min. Samples (25 μ l of a 100 μ M solution) of retinol were analysed and the concentration of retinaldehyde impurities determined. The molar absorption coefficient of 21 220 M⁻¹cm⁻¹ at 350 nm was used to calculate retinaldehyde concentrations [26].

Cornea organ culture

Human eyes were obtained from the Wisconsin Lions Eye Bank. The corneas were dissected from the globe, weighed and placed in 1 ml of minimum essential medium containing 100 U of penicillin and 100 μ g of streptomycin and cultured for 2 h at 37 °C with 5% (v/v) CO₂. After a 2 h preincubation period, IL-6 or retinoids were added and further incubated for 24 h. Stock 100 μ M retinoid solutions in minimum essential medium containing 5 mg/ml of BSA and 50 mg/ml of glucose, were prepared as described by Carillet et al. [27]. Control cultures contained

Abbreviations used: α 1-PI, α 1-proteinase inhibitor; IL-6, interleukin-6; E64, *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane.

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BSA and glucose at the same concentrations as the experimental cultures. All retinoids and solutions containing retinoids were handled under yellow light. After the incubation period, the corneas were removed from the medium, washed in phosphate-buffered saline, frozen in liquid nitrogen and freeze-fractured. Freeze-fractured corneas were homogenized and extracted in 10 mM Tris/HCl buffer, pH 7.2/0.14 M NaCl/10 mM EDTA/5 μ M E64/1 μ M pepstatin/10 μ M PMSF/1 % (w/v) sodium deoxycholate/0.1 % (w/v) SDS/1 % (v/v) Nonidet (100 μ l per 10 mg of corneal tissue). The particulate matter was removed by centrifugation at 15000 g for 15 min at 4 °C. The supernatant fraction and the cornea conditioned medium was stored at -80 °C.

Cell culture

The human liver tumour cell line Hep G2 and the human breast adenocarcinoma cell line MCF-7 were purchased from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). Both cell lines were cultured in the minimum essential medium supplemented with 10 % (v/v) fetal calf serum according to the ATCC recommended protocol. The human intestinal epithelial cell line, CaCo 2, was obtained from Dr. Nancy Dahms (Medical College of Wisconsin) and cultured in Dulbecco's modified Eagle's medium supplemented with 20 % (v/v) fetal calf serum. 'Buffy coat' fractions of 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 [28]. Isolated leukocytes were cultured in Medium 199 containing 100 U of penicillin and 100 μ g of streptomycin according to a previously described method [29]. 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 of one of the following: 1 μ M retinol, 1 μ M or 1 nM retinoic acid, or 100 ng/ml IL-6. The incubation was continued for an additional 2 h for the retinoids, or for 24 h for IL-6. 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.

Western blot assay

The cornea/cell extracts or conditioned media were mixed with an equal volume of 0.1 M Tris, pH 6.8/20 % (v/v) glycerol/2 % (w/v) SDS/0.004 % (w/v) Bromophenol Blue and electrophoresed on 9 % (w/v) SDS/polyacrylamide gels [30]. Following electrophoresis, the proteins were electroblotted to nitrocellulose sheets (BA83, Schleicher and Schuell, Keene, NH, U.S.A.). The nitrocellulose blots were then blocked with 50 ml of 10 % dry milk in TBS-T buffer, pH 7.6 (3.5 mM Tris/137 mM NaCl/0.1 % (v/v) 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, U.S.A.). Monoclonal antibodies raised against human α 1-PI (Medix Biotech), human α 1-antichymotrypsin (Medix Biotech), human β -actin (Sigma Chemical Co.) and rabbit glyceraldehyde-3-phosphate dehydrogenase (Advanced Immuno Chemical, Long Beach, CA, U.S.A.) were used as primary antibodies. Horseradish peroxidase conjugated-goat anti-mouse IgG (Bio-Rad, Hercules, CA, U.S.A.) was used for the secondary antibody. The intensities of the protein chemiluminescent bands were measured on an AMBIS imaging system (San Diego, CA, U.S.A.).

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-blot was washed and probed as described for the Western blots. The intensities of the dot-blot were quantified using an EL380 Microplate Autoreader (Bio-Tek Instruments, Winooski, VT, U.S.A.).

Reverse zymograms

Corneal extracts and the conditioned media were mixed with an equal volume of 0.1 M Tris, pH 6.8/20 % (v/v) glycerol/2 % (w/v) SDS/0.004 % (w/v) Bromophenol Blue, and proteins were immediately resolved on 9 % (w/v) polyacrylamide gels that contained co-polymerized casein (0.1 %, w/v). The gels were washed in 2.5 % (v/v) Triton X-100 for 1 h and then incubated overnight at 37 °C in 100 ml of 50 mM Tris, pH 8.0, containing 5 mM CaCl_2 /0.02 % (w/v) sodium azide/20 ng/ml trypsin (Worthington Biomedical, Freehold, NJ, U.S.A.). Protected bands were visualized by Coomassie Blue staining.

RESULTS

Retinol effect on the α 1-PI level in the cornea

In the presence of 1 μ M retinol, α 1-PI levels increased quickly and peaked at 2 h in both the corneal tissue and the conditioned medium (Figure 1). At the 2 h time point, α 1-PI levels were about 3.8 times higher than that in the control corneas. After 2 h, α 1-PI levels declined but remained at least 2-fold greater than the control levels for the entire 24-h test period. The other retinoids tested, retinaldehyde at 1 μ M or retinoic acid at 1 μ M, failed to alter α 1-PI levels under the same conditions (Figures 1a and 1b). None of the tested retinoids affected α 1-antichymotrypsin or β -actin levels (results not shown).

When different retinol concentrations were tested, corneal α 1-PI was increased across the entire range tested (10^{-10} to 10^{-5} M) (Figure 2). The effect was maximal when retinol was present at 1 μ M. Retinol increased the α 1-PI levels both retained in the cornea (Figure 2a) and secreted into conditioned medium (Figure 2b). α 1-Antichymotrypsin and β -actin protein levels were not significantly altered under the same experimental conditions (results not shown).

To determine whether the increase in the α 1-PI levels, measured by immunological methods, correlated with a corresponding increase in α 1-PI inhibitory activity, the ability of α 1-PI to inhibit trypsin was analysed using reverse zymography (Figure 3). Trypsin was used because it cleaves casein at lower concentrations than neutrophil elastase and is inhibited by α 1-PI. The 59 kDa protected bands in the corneal extracts and the cornea conditioned medium co-migrated with an α 1-PI standard (purified from human plasma). Corneal conditioned medium and tissue extracts, which had been treated with retinol contained more trypsin inhibitory activity. This was observed as more intensively stained 59 kDa bands than corresponding α 1-PI inhibitory bands for the control corneas. Thus the increase in α 1-PI activity in corneas incubated in the presence of retinol is related to the increase in the α 1-PI protein.

Retinol effect on α 1-PI level from tissues other than cornea

The effect of retinol on α 1-PI levels was studied in cells derived from other tissues known to synthesize α 1-PI (Table 1). The following cell lines were used: Hep G2, MCF-7, CaCo 2 and human peripheral blood monocytes and monocyte-derived macro-

Figure 1 Time course of retinoid effect on α 1-PI levels in the cornea

Corneas were cultured for 2 h in the presence of 1 μ M retinol (○), 1 μ M retinoic acid (□) and 1 μ M retinaldehyde (Δ). α 1-PI levels in corneal tissue (a) and cornea conditioned medium (b) were determined by dot-blot assay as described in the Experimental methods section. The concentrations of these proteins were normalized to glyceraldehyde-3-phosphate dehydrogenase levels for the cornea tissue and to total protein for the conditioned medium. Each value represents the average of three independent measurements.

phages. The cornea was the only tissue/cell line where retinol increased α 1-PI levels. In all other cell lines tested, retinol did not affect α 1-PI levels either in the cell extract or in the conditioned medium (Table 1). Retinol, at 1 μ M, did not significantly change α 1-antichymotrypsin or β -actin protein levels in any of the tissues/cell lines tested (results not shown). Retinoic acid, at 1 nM or 1 μ M, did not significantly alter the levels of α 1-PI, α 1-antichymotrypsin, or β -actin. The effect of IL-6 on α 1-PI in the above mentioned tissues/cells was also studied. As expected, IL-6 increased α 1-PI levels in the conditioned medium of cells tested, except in the cornea. The monocyte-derived macrophages were the most responsive.

Effect of retinaldehyde and retinoic acid on α 1-PI in the cornea

Maximal induction of corneal α 1-PI was observed in the presence of 1 μ M retinol in the culture medium; this concentration is

Figure 2 Dose-response curve of retinol effect on α 1-PI levels in the cornea

Corneas were cultured for 2 h in the presence of 10^{-10} to 10^{-5} M retinol. α 1-PI levels in corneal tissue (a) and conditioned medium (b) were determined by dot-blot assays as described in the Experimental methods section. The levels of these proteins were normalized to glyceraldehyde-3-phosphate dehydrogenase levels for the cornea tissue and to total protein for the conditioned medium. Each value represents the average of three independent measurements.

Figure 3 Retinol effect on α 1-PI activity in the cornea

The inhibitory activity of α 1-PI in corneal tissue extracts and conditioned medium incubated in the presence or absence of 1 μ M retinol was determined by reverse zymography using trypsin. The last lane contained 200 ng of human of plasma α 1-PI.

Table 1 Effect of retinol, retinoic acid and IL-6 on α 1-PI levels in tissues and cells that synthesize this inhibitor

Corneas and cells were incubated for 2 h in the presence of 1 μ M retinol, 1 nM or 1 μ M retinoic acid, or for 24 h in the presence of 100 ng/ml IL-6. α 1-PI levels in cells and corneal tissue were determined by dot-blot assay as described in the Experimental methods section. Levels of α 1-PI are percentages of control (untreated) cells and corneas. Each value represents the average of three independent measurements.

	Tissue/cell extract (% of control level)				Conditioned medium (% of control level)			
	Retinol (1 μ M)	Retinoic acid (1 nM)	Retinoic acid (1 μ M)	IL-6 (100 ng/ml)	Retinol (1 μ M)	Retinoic acid (1 nM)	Retinoic acid (1 μ M)	IL-6 (100 ng /ml)
Cornea	386 $\pm$ 27	104 $\pm$ 7	119 $\pm$ 6	93 $\pm$ 3	367 $\pm$ 28	113 $\pm$ 12	113 $\pm$ 12	89 $\pm$ 14
Hep G2	117 $\pm$ 24	82 $\pm$ 15	94 $\pm$ 20	99 $\pm$ 21	90 $\pm$ 33	96 $\pm$ 6	96 $\pm$ 8	278 $\pm$ 29
CaCo 2	104 $\pm$ 13	93 $\pm$ 5	103 $\pm$ 30	112 $\pm$ 24	99 $\pm$ 11	91 $\pm$ 6	96 $\pm$ 8	238 $\pm$ 31
MCF-7	90 $\pm$ 9	111 $\pm$ 22	121 $\pm$ 26	124 $\pm$ 13	101 $\pm$ 4	111 $\pm$ 7	107 $\pm$ 3	296 $\pm$ 6
Monocytes	96 $\pm$ 8	114 $\pm$ 16	116 $\pm$ 27	121 $\pm$ 22	99 $\pm$ 28	125 $\pm$ 32	99 $\pm$ 12	261 $\pm$ 12
Macrophages	87 $\pm$ 12	94 $\pm$ 6	99 $\pm$ 8	231 $\pm$ 33	115 $\pm$ 13	99 $\pm$ 10	104 $\pm$ 5	487 $\pm$ 36

similar to that found in plasma. In contrast, normal physiological plasma concentrations of retinaldehyde and retinoic acid are 2–3 orders of magnitude lower. In the time course experiment described in Figure 1, retinaldehyde and retinoic acid were used at the same concentration as retinol (1 μ M). When the different cell lines were surveyed for their response to retinoids (Table 1), 1 μ M and 1 nM retinoic acid were used. To expand these studies further, a dose–response analysis was performed to determine the effect of different retinaldehyde and retinoic acid concentrations on α 1-PI levels in the cornea and the corneal conditioned medium.

The dose–response analysis (Figure 4) revealed that 1 nM retinaldehyde elevated α 1-PI levels approximately 3.8-fold in both the corneal tissue (Figure 4a) and the conditioned medium (Figure 4b). When the final concentration of retinaldehyde was between 10^{-10} and 10^{-8} M, α 1-PI levels were significantly higher than the control levels. Higher retinaldehyde concentrations (10^{-7} – 10^{-5} M) did not affect the α 1-PI levels. The magnitude of the increase in the corneal α 1-PI levels obtained with 1 nM retinaldehyde was similar to that observed with 1 μ M retinol. The addition of retinoic acid to the culture medium, over the concentration range of 10^{-10} to 10^{-5} M, did not affect α 1-PI levels. α 1-Antichymotrypsin and β -actin levels were unchanged by retinaldehyde or retinoic acid (results not shown).

The 3.8-fold increase in corneal α 1-PI in the presence of 1 μ M retinol might be due to a 0.1 % contamination by retinaldehyde. To rule this out, reversed-phase HPLC analysis of retinol was carried out. This analysis showed that the retinol used in this study contained 0.000125 % retinaldehyde (1 μ M retinol contained 1.25 pM retinaldehyde). Therefore the effect observed with retinol cannot be assigned to the presence of contaminating retinaldehyde.

DISCUSSION

The present studies showed that α 1-PI in the cornea is regulated differently than in other tissues/cells that synthesize this inhibitor. Being a positive acute-phase protein, α 1-PI was up-regulated by IL-6 in Hep G2, CaCo 2, MCF-7 cells, blood monocytes and macrophages. However, IL-6 did not affect corneal α 1-PI levels. Retinol elevated corneal α 1-PI about 3.8-fold over the control. The increase was observed in both the corneal tissue and the conditioned medium. The retinol-induced increase in α 1-PI was relatively fast. The α 1-PI levels peaked 2 h after retinol addition to the cultured medium. Retinaldehyde,

but not retinoic acid, also stimulated an increase in α 1-PI levels in a similar fashion. Interestingly, retinaldehyde is 1000 times more potent than retinol since the optimal concentration of retinaldehyde was 1 nM versus 1 μ M for retinol. Both retinoids had a maximal effect on α 1-PI when added at concentrations similar to their normal plasma concentrations. Retinoic acid, over the concentration range of 0.1 nM to 10 μ M, failed to change the α 1-PI level. The retinol effect on α 1-PI levels was cornea-specific. In other cells that synthesize α 1-PI (Hep G2, CaCo 2, MCF-7, blood monocytes, and blood macrophages), retinol did not alter the levels of this inhibitor. Additionally, α 1-antichymotrypsin or β -actin levels were unchanged under the conditions that elevated corneal α 1-PI levels.

These experiments show that α 1-PI synthesis in the cornea is under different, and quite possibly unique, control from that in other cells. Because the normal cornea is an avascular tissue, delivery of α 1-PI from the blood is inadequate. The levels of α 1-PI in the tear film and aqueous humour are much lower than that present in the cornea [12]. Thus these fluids that bathe the cornea are poor sources of this inhibitor. Therefore the cornea must rely on its own α 1-PI synthesis to protect itself against elastase released by inflammatory neutrophils.

Retinol serves as a precursor for physiologically active compounds such as retinaldehyde and retinoic acid. When retinol enters the cell, it can be oxidized to retinaldehyde and then further to retinoic acid. This first step in retinol oxidation is reversible. The second step, in which retinaldehyde is oxidized to retinoic acid, is irreversible. Therefore the observation that both retinol and retinaldehyde affect α 1-PI in the cornea, can be explained by interconversion between retinol and retinaldehyde. Some members of the alcohol dehydrogenase family of enzymes can oxidize retinol. The cornea has by far the highest level of retinol-specific alcohol dehydrogenase when compared with other parts of the eye (200 ± 59 m-units/mg of protein in the cornea compared with 0.6 ± 0.2 m-units/mg of protein in the retina) [31]. The high concentration of the retinol-converting enzyme is probably responsible for rapid retinol/retinaldehyde interconversion and the relatively fast increase in α 1-PI levels.

In most tissues/cells, retinoic acid isomers (all-*trans*, 9-*cis* and 13-*cis*) readily interconvert [32]. all-*trans*-Retinoic acid applied topically to the cornea can also be isomerized [33]. HPLC analysis showed that approximately 50 % of the retinol taken up by the cornea was isomerized within the first 2 h of the culture period. About 5 % of the retinol has been metabolized to all-*trans* retinoic acid during that time (results not shown). Thus the

Figure 4 Retinaldehyde and retinoic acid effect on α 1-PI levels in the cornea

Corneas were incubated in the presence of 10^{-10} – 10^{-5} M retinoic acid (□) or retinaldehyde (△). α 1-PI levels in corneal tissue (a) and conditioned medium (b) were determined by dot-blot assay as described in the Experimental methods section. The concentrations of these proteins were normalized to glyceraldehyde-3-phosphate dehydrogenase levels for the cornea tissue and to total protein for the conditioned medium. Each value represents the average of three independent measurements.

absence of an effect of all-*trans* retinoic acid on corneal α 1-PI levels cannot be attributed to the inability of the cornea to form the proper retinoic acid isomer.

This is the first example, as far as we know, of protein levels being regulated by retinol and retinaldehyde but not retinoic acid. Most proteins regulated by retinoids utilize all-*trans* retinoic acid and/or 9-*cis*-retinoic acid through binding to retinoic acid receptors. These receptors bind to retinoid response elements on DNA. The mechanism by which retinol and/or retinaldehyde or their metabolites regulate protein levels is not known.

Buck and co-workers showed that retinol and retinaldehyde, but not retinoic acid, increased the survival of B-cells and are required for T-cell activation under serum-free conditions [34]. These experiments and our study used similar experimental conditions: a 2-h culture period, serum-free conditions, and a

retinol concentration of 1 μ M. Similarly to our studies, both T- and B-cell effects are mediated by a narrow retinol concentration range with the maximal effect being observed with 1 μ M retinol. 14-Hydroxy-*retro*-retinol has been identified as an active retinoid in this system. An additional *retro*-retinoid, anhydroretinol, has been characterized [35]. Therefore it is possible that one of these retinoids is also responsible for the effect on corneal α 1-PI.

Regulation of α 1-PI synthesis in the cornea and other extrahepatic tissues should be different from that in the liver. During systemic infection or inflammation, liver production of α 1-PI is elevated, leading to a rise in blood α 1-PI levels and an increase in α 1-PI protein levels in most tissues. The change in α 1-PI synthesis by extrahepatic tissues could be a response to the local changes that may threaten the proteinase-proteinase inhibitor balance. In a localized minor infection, inflammation or injury, independent regulation allows extrahepatic tissues to increase α 1-PI levels for protection from proteolytic damage, without changing α 1-PI levels in other tissues.

We believe that the retinoid-induced increase in corneal α 1-PI synthesis could be part of a general stress response. All experiments described in this study were conducted under serum-free conditions. The importance of 14-hydroxy-*retro*-retinol for growth promotion of HL-60 cells and B-cells [34] was also elucidated under serum-free conditions. It is therefore possible that 14-hydroxy-*retro*-retinol and possibly other retinoids are synthesized and function during a stress response. The cornea is an avascular tissue supplied with oxygen and nutrients from the surrounding fluids. As a result, corneal cells, especially those in the stroma, have lower oxygen and nutrient concentrations than the surrounding cells or the cells from other tissues. Therefore this retinoid-based response could be important *in vivo*, under normal corneal conditions, and also as part of the inflammatory response of the cornea.

This work was supported in part by grants R01 EY-08388, EY-06663 and P30 EY-01931 from the National Eye Institute/National Institutes of Health.

REFERENCES

- Carrell, R. and Travis, J. (1985) Trends Biochem. Sci. **10**, 20–22
- Beatty, K., Bieth, J. and Travis, J. (1980) J. Biol. Chem. **255**, 3931–3934
- Johnson, D. A. and Travis, J. (1976) Biochem. Biophys. Res. Commun. **72**, 33–39
- Hubbard, R. C., Ogushi, F., Fells, G. A., Cantin, A. M., Jallat, S., Courtney, M. and Crystal, R. G. (1987) J. Clin. Invest. **80**, 1289–1295
- Hood, J. M., Koep, L. J., Peters, R. L., Schroter, G. P., Weil, R., Redeker, A. G. and Starzl, T. E. (1980) New Eng. J. Med. **302**, 272–275
- Travis, J. and Salvesen, G. S. (1983) Annu. Rev. Biochem. **52**, 655–709
- Patterson, S. D. (1991) Comp. Biochem. Physiol. B **100**, 439–454
- Perlmutter, D. H., Cole, F. S., Kilbridge, P., Rossing, T. H. and Colten, H. R. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 795–799
- White, R., Lee, D., Habicht, G. S. and Janoff, A. (1981) Am. Rev. Resp. Dis. **123**, 447–449
- Perlmutter, D. H., Daniels, J. D., Auerbach, H. S., De Schryver-Kecsckemeti, K., Winter, H. S. and Alpers, D. H. (1989) J. Biol. Chem. **264**, 9485–9490
- Tokes, Z. A., Gendler, S. J. and Dermier, G. B. (1981) J. Supramol. Struct. Cell. Biochem. **17**, 69–77
- Twining, S. S., Fukuchi, T., Yue, B. Y. J. T., Wilson, P. M. and Boskovic, G. (1994) Invest. Ophthalmol. Vis. Sci. **35**, 458–462
- Kueppers, F. (1968) Humangenetik **6**, 207–214
- Laurell, C. B., Kullander, S. and Thorell, J. (1968) Scand. J. Clin. Lab. Invest. **21**, 337–343
- Eriksson, S. (1983) Ann. Clin. Res. **15**, 95–98
- Gadek, J. E., Fulmer, J. D., Gelfand, J. A., Frank, M. M., Petty, T. L. and Crystal, R. G. (1980) J. Clin. Invest. **66**, 82–87
- Gauldie, J., Lamontagne, L. and Stadnyk, A. (1985) Surv. Synth. Pathol. Res. **4**, 126–151
- Carp, H. and Janoff, A. (1980) J. Clin. Invest. **66**, 987–995
- Aronsen, K. F., Ekelund, G., Kindmark, C. O. and Laurell, C. B. (1972) Scand. J. Clin. Lab. Invest., Suppl. **124**, 127–136

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- 20 Perlmutter, D. H., May, L. T. and Sehgal, P. B. (1989) *J. Clin. Invest.* **84**, 138–144
- 21 Mackiewicz, A., Ganapathi, M. K., Schultz, D., Brabenec, A., Weinstein, J., Kelley, M. F. and Kushner, I. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1491–1495
- 22 Barbey-Morel, C., Pierce, J. A., Campbell, E. J. and Perlmutter, D. H. (1987) *J. Exp. Med.* **166**, 1041–1054
- 23 Barbey-Morel, C. and Perlmutter, D. H. (1991) *Pediatr. Res.* **29**, 133–140
- 24 Koj, A., Korzus, E., Baumann, H., Nakamura, T. and Travis, J. (1993) *Biol. Chem. Hoppe-Seyler* **374**, 193–201
- 25 Wang, S. Y. (1992) *J. Biol. Chem.* **267**, 15362–15366
- 26 Furr, H. C., Barua, A. B. and Olson, J. A. (1994) in *The Retinoids* (Sporn, M. B., Roberts, A. B. and Goodman, D. S., eds.), pp. 179–209, Raven Press, New York
- 27 Carillet, V., Morliere, P., Maziere, J. C., Huppe, G., Santus, R. and Dubertret, L. (1990) *Biochim. Biophys. Acta* **1055**, 98–101
- 28 Carlson, G. P. and Kaneko, J. J. (1973) *Proc. Soc. Exp. Biol. Med.* **142**, 853–856
- 29 Einstein, L. P., Schneeberger, E. E. and Colten, H. R. (1976) *J. Exp. Med.* **143**, 114–126
- 30 Laemmli, U. K. (1970) *Nature* **227**, 680–685
- 31 Julia, P., Farres, J. and Pares, X. (1986) *Exp. Eye Res.* **42**, 305–314
- 32 Kojima, R., Fujimori, T., Kiyota, N., Toriya, Y., Fukuda, T., Ohashi, T., Sato, T., Yoshizawa, Y., Takeyama, K., Mano, H., Masushige, S. and Kato, S. (1994) *J. Biol. Chem.* **269**, 32700–32707
- 33 Ubels, J. L. and Edelhauser, H. F. (1985) *Biochem. Biophys. Res. Commun.* **131**, 320–327
- 34 Eppinger, T. M., Buck, J. and Hammerling, U. (1993) *J. Exp. Med.* **178**, 1995–2005
- 35 Buck, J., Grun, F., Derguini, F., Chen, Y., Kimura, S., Noy, N. and Hammerling, U. (1993) *J. Exp. Med.* **178**, 675–680
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Received 17 July 1996/11 October 1996; accepted 1 November 1996