Detection of mRNA for the Cytokines, Interleukin-1α and Interleukin-8, in Corneas From Patients With Pseudophakic Bullous Keratopathy

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Purpose. Cytokines have been implicated in corneal inflammatory diseases. The authors sought to determine if corneal diseases with different patterns of inflammation are accompanied by characteristic patterns of cytokine expression.

Methods. The authors used reverse transcription–polymerase chain reaction to detect mRNA for 12 specific cytokines from patients with Fuch's dystrophy (n = 7), pseudophakic bullous keratopathy (n = 12), or normal corneas (n = 4).

Results. Using Wilcoxon rank sum analysis, mRNA for interleukin (IL)-1α or IL-8 was significantly more abundant in corneas from patients with pseudophakic bullous keratopathy relative to either comparison group. mRNA for the T-cell marker, CD4, and for T-cell derived cytokines, IL-2, IL-4, and interferon γ, could not be detected in any corneal sample. Message for IL-1 receptor antagonist and transforming growth factor-β_1 or -β_2 was readily detectable in most corneas regardless of diagnosis.

Conclusion. The findings indicate that the pattern of cytokine mRNA expression reflects differences in the pathogenesis of these corneal diseases. Invest Ophthalmol Vis Sci. 1995;36:2151–2155.

Cytokines are implicated strongly in the inflammatory process. These proteins interact in complex networks to initiate and regulate the body's response to injury. Cytokines are clearly important within the eye. Several cytokines, such as interleukin (IL)-1 have been specifically investigated with regard to a role in corneal disease. For example, corneal cells synthesize a variety of cytokines and growth factors.[1–3] Interleukin-1 is angiogenic when injected directly into the cornea.[4] Cytokines have been implicated in the pathogenesis of herpetic keratitis.[5–7] Cytokines regulate metalloproteinase activity produced by corneal cells.[8,9]

Fuchs' dystrophy is a corneal dystrophy in which there is premature loss of corneal endothelial cells and the deposition of an abnormal basement membrane.[10] Epithelial and stromal changes also may accompany the thickening of Descemet's membrane.

Pseudophakic bullous keratopathy refers to the corneal edema that may result from chronic trauma to the corneal endothelium subsequent to the placement of an intraocular lens. Inflammation, scarring, and neovascularization often accompany this disease.

We have compared and contrasted the mRNA for specific cytokines that can be detected in the cornea from representative tissue for these two diseases and have hypothesized that different patterns of inflammation are accompanied by characteristic patterns of cytokine expression. We used reverse transcription–polymerase chain reaction (RT–PCR) to detect mRNA for specific cytokines from patients undergoing penetrating keratoplasty. In comparing these mRNA levels from patients with pseudophakic bullous keratopathy, Fuchs' dystrophy, and controls, we find that consistent expression of IL-1α and IL-8 is uniquely associated with pseudophakic bullous keratopathy.

METHODS. Reverse Transcription–Polymerase Chain Reaction. Specific mRNAs were amplified as described.[11] Total RNA was extracted from tissue samples with an acid-phenol procedure.[12] First-strand cDNA synthesis was accomplished with oligo(dT) primed M-MLV reverse transcriptase. Specific cDNA was amplified by 35 PCR cycles using Tfl polymerase and primer pairs specific for human nucleotide sequences. A primer pair for a constitutively expressed gene, cyclophilin, was included in each assay as an internal control. The primer, Mg^{+2}, and nucleotide concentrations were optimized for each primer combination so that results obtained with positive control RNA samples were dependent on input RNA concentration. The PCR products were separated by agarose gel electrophoresis and detected by ethidium bromide staining. Specific primers used are listed in Table 1. Primers were based on published nucleotide sequences and synthesized by Operon Technologies (Alameda, CA), but CD4 primers were purchased from Clontech (Palo Alto, CA). Table 1 lists representative functions associated with each specific cytokine. Each amplification set included a negative control in which RNA was omitted from the RT reaction mixture and a positive control with RNA known to contain the desired mRNA. RNA extracted from synovium obtained from patients with rheumatoid arthritis was used a the positive control for IL-1β, IL-1RA, IL-6, IL-

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TABLE 1. Cytokines and Primers

Cytokine	*Representative Function(s)*	*Primers*
IL-1α	Lymphocyte activating factor Mononuclear cell chemoattractant	S: GTCTCTGAATCAGAAATCCTTCTATC A; CATGTCAAATTTCACTGCTTCATCC
IL-1β	Lymphocyte activating factor Mononuclear cell chemoattractant	S: AAACAGATGAAGTGCTCCTTCCAGG A: TGGAGAACACCACTTGTTGCTCCA
IL-1RA	Inhibitor of IL-1	S: GCAAGATGCAAGCCTTCAGAATCTGGG A: GCTGGTCAGCTTCCATCGCTGTCGA
IL-2	T-cell growth factor	S: GCCCAAGAAGGCCACAGAACTGAAAC A: TGTTGAGATGATGCTTTGACA
IL-4	Stimulates B cells Inhibits macrophages	S: ACGGACACAAGTGCGATATCACC A: CTCTCTCATGATCGTCTTTAGCC
IL-6	Activates acute phase response B-cell stimulant	S: CCTTCTCCACAAGCGCCTTC A: GGCAAGTCTCCTCATTGAATC
IL-8	Neutrophil chemoattractant	S: CTGTGTGTAAACATGACTTCCAAGCTGG A: TCCAGACAGAGCTCTCTTCCATCAG
IL-10	Inhibits macrophages and T cells Stimulates B cells	S: GCTTCGAGATCTCCGAGATGCCTTCA A: GGCTTTGTAGATGCCTTTCTCTTGGAGC
TNFα	Activates inflammatory cells Induces hemorrhagic tumor necrosis	S: GCCTGCTGCACTTTGGAGTGATCGG A: GCTCTTGATGGCAGAGAGGAGG
TGFβ_1	Pluripotent cell regulator Immunosuppressor	S: CTGCAAGTGGACATCAACGGGTTCACTA A: ACTTGCAGGAGCGCACGATCATGTTGGACA
TGFβ_2	Pluripotent cell regulator Immunosuppressor	S: GCCCACTTTCTACAGACCCTACTTCAGAAT A: AGTGGACTTTATAGTTTTCTGATCACCACTGG
IFNγ	Antiviral	S:GGTTCTCTTGGCTGTTACTGCCAGG A: AGCTGCTGGCGACAGTTCAGCCATC
CD4	Marker for T-helper cells	S: GTGAACCTGGTGGTGATGAGAGC A: GGGGCTACATGTCTTCTGAAACCGGTG
Cyclophilin	Constitutive cyclosporin binding protein	S: TGTTCTTCGACATTGCCGTCGAC A: GCATTTGCCATGGACAAGATGCCAGGA

8, IL-10, TGFβ_1, TGFβ_2, and CD4. RNA extracted from stimulated human peripheral blood lymphocytes was generously provided by Melissa Brown, Oregon Health Sciences University, and was used as the positive control for IL-2, IL-4, tumor necrosis factor (TNF) α, and interferon (IFN) γ. For the IL-1α positive control, RNA was extracted from cultured human retinal pigment epithelial cells that had been stimulated with human IL-1α for 6 hours as described.[13] Analysis of restriction endonuclease digests of RT–PCR products from positive control samples confirmed that the expected sequences were amplified with each primer pair.

Tissues. Corneas were obtained from patients undergoing penetrating keratoplasty, from eyes donated to the Oregon Eye Bank ($n = 2$), or from eyes undergoing enucleation with normal corneas ($n = 2$). Tissue was frozen in liquid nitrogen within 30 minutes of surgical removal. Seven patients had a clinical diagnosis of Fuchs' dystrophy (2 men, 5 women; mean age, 69 years). Twelve patients had a clinical diagnosis of pseudophakic bullous keratopathy (3 men, 9 women; mean age, 77 years). As described above, there were 4 corneas studied from eyes with no clinical corneal disease (3 men, 1 woman; mean age, 68 years).

Statistical Analysis. Polymerase chain reaction products were quantitated by densitometric scanning of photographic negatives of the ethidium bromide-stained gels. For each sample analyzed, the ratio of cytokine signal to cyclophilin signal was calculated. Detectability of specific mRNAs was compared by application of the nonparametric Wilcoxon rank sum test to these ratios.

RESULTS. We characterized the expression of 12 different cytokines, one marker for T lymphocytes (CD4), and one constitutive mRNA (cyclophilin). Initially, signals were classified as detectable or nondetectable. Figure 1A shows three examples of cytokines, transforming growth factor β_1 (TGFβ_1), TGFβ_2, and IL-1 receptor antagonist (IL-1RA), whose mRNAs were generally detectable in all corneas. Figure 1B shows examples of seven cytokines, IL-1β, IL-2, IL-4, IL-6, IL-10, TNFα, IFNγ, and the T-cell marker CD4. These

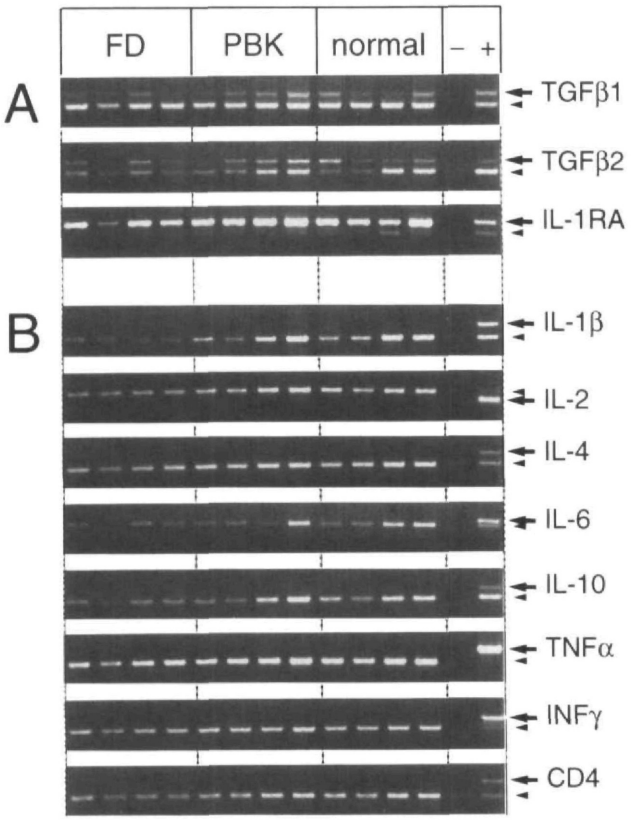

FIGURE 1. Reverse transcription–polymerase chain reaction (RT–PCR) products from four patients in each disease group. The arrowheads indicate the position of the cyclophilin RT–PCR product (292 base pairs). Arrows point to the position of the RT–PCR products of the indicated genes. Amplified product size (base pairs): IL-1β (391), IL-1RA (366), IL-2 (256), IL-4 (371), IL-6 (327), IL-10 (352), TNFα (381), IFNγ (375), TGFβ_1 (472), TGFβ_2 (454), CD4 (438). Negative controls with no RNA added to the RT reaction are in lane −. Products from positive control RNA samples are in lane +. (**A**) Products detected in most samples. (**B**) Products weakly or not detected in nearly all corneas.

mRNAs were generally nondetectable in any tissue studied. Figure 2 shows representative gels for the detection of mRNA for IL-1α and IL-8. These two mRNAs were unique because detectability for each was variable between the sample groups. Because of the semiquantitative nature of the RT–PCR technique used and because of variations in cyclophilin mRNA signals, detectability between groups was evaluated by nonparametric analysis of the ratios of cytokine signal to cyclophilin signal. As shown in Table 2, IL-1α and IL-8 mRNAs were significantly more detectable in corneas from patients with pseudophakic bullous keratopathy than in patients with Fuchs' dystrophy or in controls with no evidence of corneal disease. No significant difference was observed when comparing samples from Fuchs' dystrophy with controls. Signals for TGFβ_1 and TGFβ_2 mRNAs displayed some variation in detectability between samples but did not show any significant difference between patient groups.

DISCUSSION. Our studies demonstrate clearly that RT–PCR can be used to detect mRNA for specific cytokines that are potential contributors to corneal inflammation. Intriguingly, increased expression of mRNA for two cytokines, IL-1α and IL-8, is more likely to be associated with pseudophakic bullous keratopathy than with Fuchs' dystrophy. Pseudophakic bullous keratopathy is associated with chronic inflammation, whereas Fuchs' dystrophy is a hereditary degenerative disorder. mRNA for many of the cytokines, including all of those that are primarily the products of T lymphocytes, were undetectable in most of the samples studied. mRNA for several cytokines—TGFβ_1, TGFβ_2, and IL-1RA—are readily detectable in control as well as diseased tissue. We and others[11,14] have used RT–PCR to study several of these same cytokines in rat corneas and have obtained similar results with regard to mRNAs detectable in normal cornea.

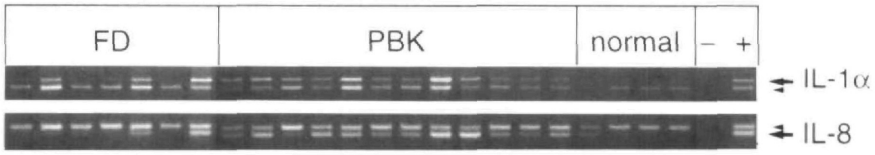

FIGURE 2. Interleukin (IL)-1α and IL-8 reverse transcription–polymerase chain reaction (RT–PCR) products were more readily detected in corneas from patients with pseudophakic bullous keratopathy than in those from controls or patients with Fuchs' dystrophy. The arrowheads indicate the position of the cyclophilin RT–PCR product (292 base pairs). Arrows point to the position of the RT–PCR products of the indicated genes. Amplified product size (base pairs): IL-1α (422), IL-8 (248). Negative controls with no RNA added to the RT reaction are in lane −. Products from positive control RNA samples are in lane +.

TABLE 2. Statistical Analysis of Densitometric Measurements

Comparison	*IL-1α*	*IL-8*
	P *Value*	
Pseudophakic bullous keratopathy versus normal	<0.005	<0.02
Pseudophakic bullous keratopathy versus Fuchs' dystrophy	<0.05	<0.02
Normal versus Fuchs' dystrophy	>0.1	>0.1

Our observations suggest that the cytokine profile will differ for specific disease entities. A unique cytokine "signature" would argue that a cytokine profile indicates distinctive features in pathogenesis and that this eventually could have some diagnostic implications. The increased detection of mRNA for IL-1α and IL-8 in corneas from patients with pseudophakic bullous keratopathy is compatible with the greater inflammation that characterizes this disease compared to Fuchs' dystrophy. In parallel to our results, a recent study[15] using temporal artery biopsies and RT–PCR found that IFNγ expression distinguished the vessel from patients with temporal arteritis from the vessel from patients with polymyalgia rheumatica. Our observations are somewhat surprising. For example, the overlapping activities of IL-1α, IL-1β, and TNFα suggest that the expression of each is similar. Clearly, this is not the case. The study of temporal artery biopsies also noted a weak correlation for the expression of IL-1, IL-6, and TNFα.[15]

Our data do have a number of limitations. First, RT–PCR as we have used it is not a precisely quantitative technique. Furthermore, a marked difference in the cytokine-to-cyclophilin mRNA ratio in the corneal tissues compared to the positive control samples could result in some false negatives. This technique does, however, indicate relative differences that can be evaluated with nonparametric statistical methods. Second, detecting mRNA is not the same as detecting protein. We cannot be sure that the differences we find in mRNA correlate with differences in functional protein or that the quantity is sufficient for a physiologic effect. Third, in studying human tissue, we generally have access only to tissue that represents the later stage of disease. Oral or topical medications could alter gene expression. We cannot exclude the possibility that mRNA for IL-8, for example, would be readily detectable if tissue from patients with Fuchs' dystrophy had been examined early in the disease process. Finally, the methodology does not precisely localize the cellular source of the RNA. Histologic localization is necessary to determine if increased cytokine mRNA detection is a consequence of gene induction within resident cells, infiltration of inflammatory cells, or both.

Despite these limitations, our data suggest that corneas from patients with pseudophakic bullous keratopathy are more likely than those with Fuchs' dystrophy to have elevated levels of the IL-1α and IL-8 message. This suggests a distinct pathogenesis that may be a clue to the course of the disease. Specific cytokine inhibitors would be logical candidates in the therapy of pseudophakic bullous keratopathy.

Key Words

cornea, cytokines, Fuchs' dystrophy, polymerase chain reaction (PCR), pseudophakic bullous keratopathy

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Axial and Instantaneous Power Conversion in Corneal Topography

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Purpose. To determine if a direct relationship exists between the two common measures of corneal topography—axial and instantaneous powers—that are based on corneal slope or curvature, respectively.

Method. The theoretical relationship between axial and instantaneous powers was derived for unrestricted shapes using equations of basic calculus.

Results. It was found that axial power at any point y on the cornea is exactly equal to the average of the instantaneous powers from the axis to point y.

Conclusions. A simple relationship exists between axial and instantaneous powers that is valid for the intersection of any surface by a meridional plane. This provides a practical means for converting between axial and instantaneous powers for clinical applications of corneal topography. Invest Ophthalmol Vis Sci. 1995;36:2155–2159.

In clinical measures of corneal topography, the shape of the corneal surface is usually interpreted from an array of discrete measures using the paraxial power formula for a single refracting surface, $P = (n - 1)/r$ where n is the index of refraction and r is the radius of curvature. Confusion arises because away from the optical axis, the radius may be measured as either of two values, the instantaneous radius of curvature, r_i (see the mathematical expression in equation 10), or the axial distance, d_a, which is the perpendicular distance from the corneal surface to the optical axis.[1,2] This leads to two expressions of corneal power, namely instantaneous power and axial power.[3] It is recognized that neither axial nor instantaneous power adequately represents corneal refractive power, but each provides shape measures in the form of slope and curvature, respectively. Radius units, rather than power units, might provide a better expression of corneal shape[4]; as will be seen in our derivations, the power units lead to more elegant mathematical expressions.

The most commonly used clinical instrument for measuring corneal topography is the videokeratograph, which traditionally measures axial power, although attempts have been made to measure instantaneous power.[5–8] Other corneal topographers not based on placido disk technology usually provide measurements of the corneal coordinates and generally must convert to a power expression to conform to clinical convention. It is recognized that measurements in terms of either axial or instantaneous power may be preferred for some applications and that an algorithm for converting these powers is of value. Such conversions based on various continuity assumptions have been proposed,[5–9] but this report presents a method for power conversion unlimited by a priori assumptions about corneal shape.

The current analysis considers the curvature and slope of the cornea in a meridional plane containing the reference axis of the measurement system, which is normal to the cornea. Instantaneous power is *defined* as:

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