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Expression of Phospholipases A_2 and C in Human Corneal Epithelial Cells

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Abstract

Purpose—To achieve a better understanding of the involvement of phospholipases in the inflammation and wound-healing processes in human corneal epithelial cells (HCECs), expression of phospholipase A_2s (PLA_2s) and phospholipase Cs (PLCs) was examined in the human corneal epithelium.

Methods—Specific primers were designed for RT-PCR amplification of the known secreted (s) PLA_2, cytosolic (c)PLA_2, and PLC mRNAs. Corresponding PCR products were cloned and the DNA sequenced. Immunofluorescence of flatmounted corneal sections and Western blot analyses were used to detect the PLA_2s and PLCs expressed by HCECs.

Results—The mRNAs for the following phospholipases were detected by RT-PCR in the HCECs: sPLA_2GIII, -GX, and -GXIIA; c$PLA_2\alpha$ and -γ; PLCβ1, -β2, -β3, -β4, -γ1, -γ2, -δ1, -δ3, -δ4, and -ε. Immunofluorescence analyses conducted on corneal epithelium cryosections and Western blot on freshly isolated HCECs demonstrated the presence of sPLA_2GIII, -GX, and -GXIIA; c$PLA_2\alpha$ and -γ; and PLCβ2, -β3, -γ1, -γ2, and -δ3.

Conclusions—Many phospholipase isoforms are expressed by HCECs and may play a major role in signal transduction (PLCs) as well as in the release of precursors of potent mediators of inflammation, such as leukotrienes and prostaglandins (PLA_2s). Moreover, the sPLA_2s expressed by the corneal epithelium could be involved in the normal antibacterial activity in the tears and in wound healing.

Corneal wound healing is a complex process that can be seriously impaired by inflammation. Indeed, keratitis or corneal inflammation often result in an opacification of the cornea that can usually be successfully treated but may also lead to the loss of vision or of the eye itself.[1] Phospholipases are essential for cell proliferation, differentiation, transformation, apoptosis,

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[2] and synthesis of potent precursors of mediators of inflammation.[3] Some phospholipases also exhibit antibacterial properties.[4,5] Phospholipases can therefore be considered major components in corneal wound healing and inflammation processes.[6,7]

Phospholipases hydrolyze membrane phospholipids to induce or transmit signals within the cell.[8] There are different families of phospholipases, among which the phospholipase A_2s (PLA_2s) and phospholipase Cs (PLCs) families are physiologically essential. The PLA_2 family is composed of two major subfamilies: secreted (s)PLA_2 and cytosolic (c)PLA_2, group (G)IV. In mammals, many groups of $sPLA_2$s (GIB, GIIA, GIIC, GIID, GIIE, GIIF, GIII, GV, GX, GXIIA, and GXIIB)[9–12] and four types of $cPLA_2$s (α, β, γ, and δ; GIVA–D)[11,13,14] hydrolyze phospholipids to generate a free fatty acid and a lysophospholipid, both of which can act as lipid signaling molecules. Frequently, the free fatty acid produced is arachidonic acid (AA).[15] AA is the precursor of many potent mediators of inflammation, such as thromboxanes, prostaglandins, and leukotrienes. AA metabolites are, among others, responsible for ocular inflammation.[16,17] $sPLA_2$s and $cPLA_2$s are therefore essential enzymes in the management of corneal inflammation. Furthermore, seven $sPLA_2$s demonstrate potent antibacterial properties in vitro against Gram-positive bacteria.[4,5] $sPLA_2$GIIA accounts, at least in part, for the antibacterial properties of the tears[18] and is secreted by both the lacrimal glands and canals.[19,20] The corneal epithelium may also be responsible for part of the antibacterial properties of the tears, if it secretes $sPLA_2$s.

There are 12 known mammalian isoforms of PLC divided into five different types: PLCβ,[2,21,22] -γ,[2,21,22] -δ,[2,21–23] -ε,[24,25] and -ζ.[26,27] Some of these isoforms also undergo alternative splicing. PLCs are associated with the plasma membrane (β, γ, δ, and ε),[2,25] the cytosol (β, γ, δ, ε, and ζ),[2,25–27] or the nucleus (β and γ).[2] After activation by the cell receptors,[2] PLCs then preferentially hydrolyze phosphatidylinositol 4,5-bishosphate from the membrane to produce two potent second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG).[2] IP_3 induces the release of intracellular calcium from the endoplasmic reticulum reserves, and the main cellular targets for DAG belong to the protein kinase C (PKC) family.[2] The increase in intracellular calcium activates a very intricate signaling pathway in ocular tissues.[28] It also activates $cPLA_2$s through its translocation to the membrane.[29,30]

Phospholipases play a major role in signal transduction (PLCs) and in the release of potent precursors of mediators of inflammation (PLA_2s and PLCs). These enzymes thus play central roles in the corneal epithelium, with particular reference to wound healing as well as to the antibacterial properties of the tears. However, no information is available on the expression of phospholipases by the human corneal epithelium. In the present study, the identity of the different PLA_2s and PLCs expressed by human corneal epithelial cells (HCECs) was thus determined at the protein and transcript level by using immunofluorescence of tissue sections, Western blot analyses, and RT-PCR.

Materials and Methods

This study was conducted in accordance with our institution's guidelines and the Declaration of Helsinki. The protocols were also approved by the institution's Committee for the Protection of Human Subjects.

Isolation of Human Corneal Epithelium

Fresh corneas were isolated from 62 human donor eyes (age range, 39–88 years; average, 69) within 24 hours after death through the Banque d'Yeux Nationale (Sainte-Foy, Québec, Canada). Briefly, corneas were dissected according to the procedure described by Gipson and Grill.[31] Epithelial sheets were then transferred to either a reagent for subsequent total RNA extraction (TriReagent; Sigma-Aldrich, St. Louis, MO), or PBS (150 mM NaCl, 9.1 mM

Na_2HPO_4, and 1.7 mM NaH_2PO_4 [pH 7.4]) containing 1% (vol/vol) protease inhibitor cocktail (Sigma-Aldrich) for subsequent protein extraction.

Reverse Transcription–Polymerase Chain Reaction

Total RNA was extracted and pooled from human corneal epithelial tissues freshly isolated from 16 human eyes (age range, 48–84 years; average, 66) using extraction reagent (TriReagent; Sigma-Aldrich), as described previously.[32,33] Reverse transcription of total RNA was performed with a reverse transcriptase kit (SuperScript II RNase H^-; Invitrogen, Burlington, Ontario, Canada) according to the manufacturer's instructions. cDNA sequences of $sPLA_2$s, $cPLA_2$s, and PLCs were amplified by PCR on a thermocycler (Techgene; Techne, Princeton, NJ). The primers (Table 1) were provided by the Service de Synthèse d'ADN from the Centre de Recherche du CHUL; Sainte-Foy, Québec, Canada) using a high-throughput DNA synthesizer (model 3900; Applied Biosystems, Foster City, CA). The amplification reactions consisted of 35 three-step cycles of denaturation for 50 seconds at 95°C, annealing for 40 seconds at 55°C, and elongation for 60 seconds at 72°C. The PCR products were then separated by electrophoresis on 1% (wt/vol) agarose gels and photographed (Gel-Doc 2000; Bio-Rad, Hercules, CA).

Cloning and DNA Sequencing

The bands containing the PCR products were excised, purified (Ultra-free-DA columns; Millipore, Bedford, MA), and inserted into a cloning vector (pGEM-T Easy; Promega, Madison, WI), according to the manufacturer's instructions. The mixture was transformed into competent *Escherichia coli* DH5α bacterial cells (Invitrogen). The transformed cells were then plated and grown to a stationary phase and plasmid purification was performed (QIAprep Spin Miniprep kit; Qiagen, Mississauga, Ontario, Canada). DNA sequencing of positive clones was performed by the Service d'Analyse et de Synthèse d'Acides Nucléiques at Université Laval (Sainte-Foy), using T7 sequencing primers.

Indirect Immunofluorescence of Corneal Cryosections

Tissue biopsies (corneas) were obtained from the eyes of a 52-year-old human donor. Corneas were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Bayers Canada, Etobicoke, Ontario, Canada), frozen in liquid nitrogen, and stored at −80°C until use. Indirect immunofluorescence assays were performed on acetone-fixed, 5-μm-thick cryosections, as previously reported.[34] Sections were incubated with primary antibodies (Table 2) diluted 1:50. The large-scale production of the $sPLA_2$GIII, -GX, or -GXIIA antibodies has been reported,[35] except for the $sPLA_2$GIII antiserum, which was prepared in rabbits, as described for the other anti-$sPLA_2$s antisera,[35] by using as an antigen the group III $sPLA_2$ domain of $sPLA_2$GIII. A secondary antibody was then added (Table 2). Cell nuclei were also labeled with Hoechst 33258 reagent (Sigma-Aldrich) after immunofluorescence staining. Cryosections were then observed under an epifluorescence microscope (Optiphot; Nikon, Tokyo, Japan) and photographed with a numeric charge-coupled device (CCD) camera (Sensys; Roper Scientific, Trenton, NJ). Negligible background was observed in control experiments in which primary antibodies were omitted. The expression of the PLCδ4 protein could not be tested by immunologic techniques, because specific antibodies for this phospholipase are not yet commercially available.

Protein Extraction from HCECs

Freshly isolated human corneal epithelial tissues from 60 eyes (age range, 39–88 years; average, 70) were pooled and lysed in RIPA buffer (150 mM NaCl, 9.1 mM Na_2HPO_4, 1.7 mM NaH_2PO_4 [pH 7.4], 1% [vol/vol] NP40, 0.5% [vol/vol] sodium deoxycholate, 0.1% [vol/vol] SDS, 100 mM $NaVO_3$, and 1% [vol/vol] protease inhibitor cocktail) and then sonicated

for 30 seconds. The homogeneous cell lysate was incubated on ice and then centrifuged. Protein concentration was measured using a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL). The supernatant was kept at −80°C until use.

SDS-PAGE and Western Blot Analyses

Proteins from the human corneal epithelium (60 μg) and from each positive control (50 μg) were separated on a 6% (PLCs), 8% (cPLA$_2$s and PLCδs), or 15% (sPLA$_2$s) (wt/vol) polyacrylamide gel. Prestained broad-range protein molecular weight standards (MBI Fermentas, Burlington, Ontario, Canada) were used for calibration. The proteins from the gel were transferred onto a polyvinylidene difluoride membrane (Amersham Biosciences) for sPLA$_2$s, or onto a nitrocellulose membrane (Bio-Rad) for cPLA$_2$s and PLCs. The membranes were blocked, incubated with primary antibodies (Table 2), washed, and incubated with secondary antibodies (Table 2). The membranes were washed and then soaked in tris-buffered saline (TBS). These immunoconjugates were detected with the chemiluminescent substrate (SuperSignal West Pico; Pierce), and blots were visualized (Fluor-S Max System; Bio-Rad) except for PLCδ3, with which an autoradiographic film was used to reduce background.

Results

PLA$_2$ and PLC mRNAs Expression by HCECs

RT-PCR experiments revealed the expression of mRNAs for sPLA$_2$GIII, -GX, and -GXIIA (Fig. 1A) in the corneal epithelium, with an apparent size of 500, 327, and 723 bp, respectively, as was expected with the primers used (Table 1). As shown in Figure 1B, cPLA$_2\alpha$ and -γ mRNA transcripts were expressed by HCECs, with a respective apparent size of 440 and 1020 bp (Table 1). Many different mRNA transcripts of PLCs were present in HCECs, including PLCβ1, -β2, -β3, -β4, -γ1, -γ2, -δ1, -δ3, -δ4, and -ε(Fig. 1C) with apparent sizes of 690, 620, 550, 500, 924, 882, 458, 716, 468 and 700 bp, respectively, as expected with the primers used (Table 1). sPLA$_2$GIB, -GIIA, -GIID, -GIIE, and -GIIF (Fig. 1A); cPLA$_2\beta$ (Fig. 1B) and -δ (data not shown); and PLCδ2 and -ζ1 (data not shown) mRNA transcripts were not amplified in the corneal epithelium. After cloning and sequencing of the PCR products, identities of the different phospholipases were confirmed by comparison with the known phospholipase sequences using BLASTn (www.ncbi.nlm.nih.gov/BLAST). Additional bands were observed for some of the PLCs—namely, β1, β3, γ1, γ2, and ε(Fig. 1C). Moreover, faint bands were also observed for sPLA$_2$IID and -IIF (Fig. 1A). These bands were excised from the agarose gel, cloned, sequenced, and found to correspond to nonspecific PCR products.

PLA$_2$ and PLC Protein Expression by the Corneal Epithelium

As shown in Figure 2, indirect immunofluorescence analyses conducted on corneal cryosections revealed the presence of and sPLA$_2$GIII, -GX, and -GXIIA (Fig. 2A); cPLA$_2\alpha$ and -γ (Fig. 2B); and PLCβ2, -β3, -γ1, and -γ2 proteins (Fig. 2C). PLCβ1, -β4, -δ1, -δ3, and -ε proteins were not detected in corneal epithelium (data not shown). It can be seen in the micrographs in Figure 2 that all phospholipases were present in the cytoplasm of HCECs. sPLA-GIII and -GXIIA, cPLA$_2\alpha$ and -γ, and PLCγ1 were also present in the nucleus. Whereas PLCβ3 was present uniformly in the cytoplasm of the cells throughout the epithelium, it is also markedly present in the basal region of the basal cells. sPLA$_2$GIII and -GXIIA and cPLA$_2\alpha$ and -γ were markedly present in the apical region of the cornea. It is interesting to note that among all PLA$_2$s and PLCs tested, many are also expressed by stromal fibroblasts, particularly PLCγ2. To further validate these data, Western blot analyses were conducted on crude protein extracts obtained from the corneal epithelium of donor eyes. As shown in Figure 3A, protein bands with apparent molecular masses of 55, 20, and 21 kDa corresponding to sPLA$_2$GIII, -GX, and -GXIIA, respectively, were detected in the human corneal epithelium. Protein bands with apparent molecular masses of 85 and 60 kDa corresponding respectively to cPLA$_2\alpha$ and

-γ, were detected in the human corneal epithelium (Fig. 3B). Similarly, protein bands with apparent molecular masses of 140, 150, 155, 120, and 85 kDa, corresponding respectively to PLCβ2, -β3, -γ1, -γ2, and -δ3 (Fig. 3C) were detected in the human corneal epithelium. The disagreement between the indirect immunofluorescence and Western blot analyses for PLCδ3 can be explained by the presence of a very low expression of PLCδ3 in this tissue, since a 1-hour exposure of the membrane was necessary to observe the PLCδ3 band at 85 kDa. The molecular masses of these bands are in good agreement with those obtained with the positive controls provided by the manufacturer (Fig. 3C), except for PLCγ2, which was 20 kDa lower than the positive control. This latter result could be explained by N-terminal proteolysis (epitope mapping at the C terminus), as no splicing has been reported for the PLCγ2 transcript. No protein was detected with PLCβ1, -β4, -δ1, and -ε antibodies (data not shown), in contrast with the data reported by Islam and Akhtar,[36] who observed the presence of PLCβ1 in their cultures of rabbit corneal epithelium. This can be explained either by differences in the expression of PLCs in human and rabbit corneal epithelium or by the treatment of their corneal epithelium with epidermal growth factor (EGF). These data thus suggest that only sPLA$_2$GIII, -GX, and -GXIIA; cPLA$_2\alpha$ and -γ; and PLCβ2, -β3, -γ1, -γ2, and -δ3 are expressed by the human corneal epithelium. Additional bands were observed for three of these phospho, lipases: sPLA$_2$GXIIA, cPLA$_2\alpha$ and PLCγ2. Indeed, four bands of high molecular mass were detected for sPLA$_2$GXIIA which likely correspond to different levels of aggregation of this enzyme. In addition, five and two bands of low molecular weight were detected respectively for cPLA$_2\alpha$ and PLCγ2 which may correspond to protein degradation.

Discussion

No information was available on the expression of phospholipases by the human corneal epithelium. In fact, most of the studies on the expression of ocular phospholipases and their regulation were conducted using rabbit eyes.[8,17,19,36] It is important to investigate the expression of phospholipases in the human eye because of the limitations of extrapolating from animal models. This is especially true of data derived from the rabbit eye,[17] which has a much higher rate of AA metabolism than either the human or bovine eye.[37] In the present study, the identity and localization of the different PLA$_2$s and PLCs expressed by the human corneal epithelium were thus determined.

The antibacterial properties of sPLA$_2$s are well recognized and may be the result of their catalytic action.[4,5] The sPLA$_2$GIIA present in tears [5] originates from lacrimal ducts and glands[19,20] and accounts in part for the antibacterial properties of the tear film. However, sPLA$_2$GIIA is not expressed by the corneal epithelium, which thus does not contribute to the production of this enzyme in the tear film. In vitro, sPLA$_2$GIIA showed the strongest bactericidal activity against Gram-positive bacteria, followed by sPLA$_2$GX, -GV, and -GXIIA.[4] Only sPLA$_2$GXIIA demonstrated a detectable bactericidal activity against the Gram-negative bacteria *Escherichia coli*.[4] We demonstrated an even distribution of sPLA$_2$GX proteins in the cytosol of HCECs and a cytosolic/nuclear distribution of sPLA$_2$GIII and -GXIIA. It would be of interest to determine whether sPLA$_2$GIII, -GX, and -GXIIA proteins can indeed be found in tears and then contribute to the antibacterial properties of the tear film. If this were indeed the case, then sPLA$_2$s activity in tears would originate from lacrimal ducts and glands as well as HCECs, providing even more antibacterial protection to help maintain the sterility of a wound or at least fight against infection after corneal injury.

cPLA$_2\alpha$ mRNA is ubiquitously expressed in most adult human tissues,[38,39] whereas cPLA$_2\gamma$ mRNA is selectively expressed in some tissues.[38,40,41] In this study, we demonstrated the expression of these two proteins in the cytosol and nucleus of corneal epithelial cells. cPLA$_2\alpha$ has attracted special interest, because it is the only one of numerous PLA$_2$s that selectively release AA over other fatty acids.[42,43] cPLA$_2\alpha$ initiates the immediate

AA release.[3] Its expression is elevated in some tissues in response to pathologic stimuli.[44–47] cPLA$_2\gamma$ properties and regulation are less well known. Asai et al.[48] demonstrated that cPLA$_2\gamma$ remains bound to membranes due to its lipid anchor at its C terminus.[41] The immunofluorescence analyses of corneal tissue sections conducted in the present study demonstrated that this protein was markedly present on the cell membrane of HCECs. By using a cPLA$_2\alpha$ inhibitor, Kang et al.[49] have determined that EGF induces the production of prostaglandins through the activation of this cPLA$_2$ in rabbit corneal epithelial cells. Given that the EGF level increases during corneal wound healing,[50–52] the cPLA$_2\alpha$ expressed by HCECs may be involved in corneal wound healing. Moreover, it has been proposed that increased expression of a cPLA$_2$ in the corneal epithelium takes place after a platelet-activating factor (PAF) stimulus.[53,54] Given that PAF is well known to mediate inflammatory and immune responses, these data suggest that cPLA$_2\alpha$-expressed by the corneal epithelium may be involved in these processes.

Because of their ability to induce numerous effects after hydrolysis of phospholipids,[2] PLCs are believed to be important cell-signaling components during wound healing and inflammation processes. A wide variety of mRNAs coding for PLC isoforms were identified in HCECs (PLCβ1, -β2, -β3, -β4, -γ1, -γ2, -δ1, -δ3, -δ4, and -ε). However, in normal HCECs, only part of these mRNA are translated into proteins (PLCβ2, -β3, -γ1, -γ2, and -δ3). The other ones may then be expressed in pathologic conditions or after a proper stimulation by a growth factor as observed for PLCγ1 in EGF in cultures of rabbit corneal epithelium.[36] Moreover, it is interesting to point out the variation in the localization of PLC proteins within the HCECs. PLCβ2, -β3, -γ1, and -γ2 proteins are all present throughout the cytoplasm of HCECs; PLCβ2 was more concentrated at the cell membrane, whereas PLCβ3 was very much present on the basal side of the basal cell layer of the corneal epithelium. PLCγ1 protein was also present in the nucleus of basal and intermediate cells, whereas PLCγ2 was the only phospholipase strongly detected in stromal fibroblasts among all those examined. This difference in cellular localization reinforces the hypothesis that the different PLC isoforms may play several critical functions within these cells in normal and most probably under pathologic conditions. In this regard, it has been shown that EGF stimulates the expression of PLCγ1.[36,49,55] This PLC may be involved in corneal wound healing, given the involvement of EGF in this process.[50–52] Moreover, by using a specific PLC inhibitor, Huang et al.[56] have shown that the production of bradykinin, which is released during the inflammatory response of the cornea, leads to the activation of a PLC in canine cultured corneal epithelial cells.

The respective function played by each phospholipase (PLA$_2$ and PLC) must be determined, to achieve a better understanding of their involvement in inflammation and wound healing of the corneal epithelium. It is clear that phospholipases play important roles in ocular physiology and pathophysiology and that modulation of their synthesis, sites of action, and inactivation comprise important pharmacological targets for the management of ocular disorders. Such knowledge could lead to new studies to determine which phospholipases represent good therapeutic targets in the establishment of a specific treatment for inflammatory disorders.[57]

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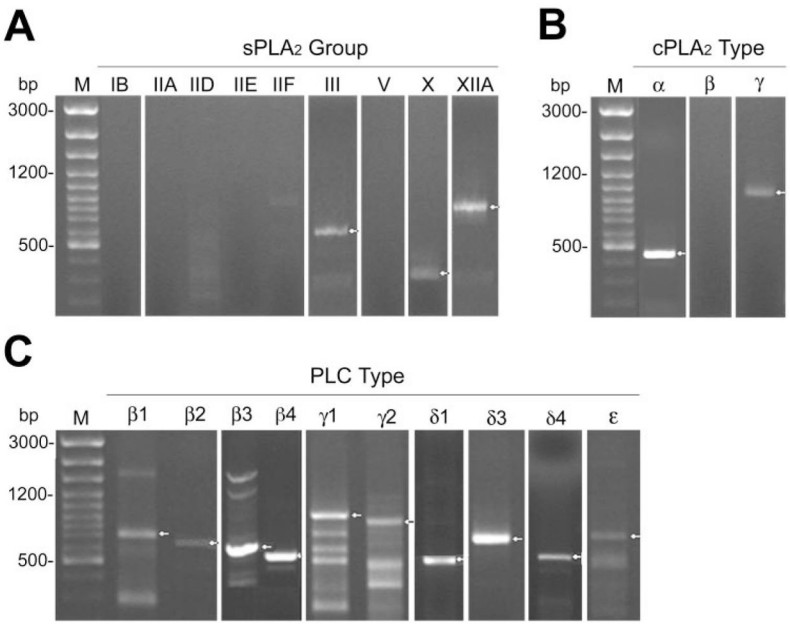

Figure 1.
Identification of (**A**) $sPLA_2$, (**B**) $cPLA_2$, and (**C**) PLC transcripts in HCECs by PCR amplification. *Lane M*: 100-bp ladder. *Arrows*: position of each positive phospholipase band.

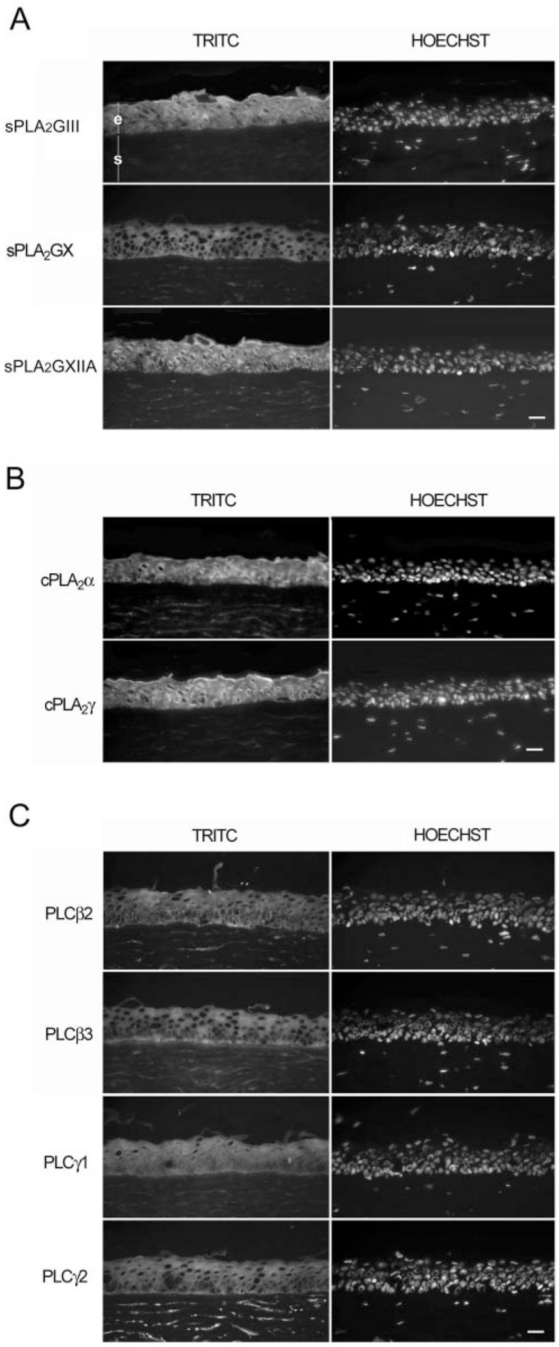

Figure 2.

Immunofluorescence analysis of phospholipases expressed by the human cornea. Immunolocalization of all phospholipases was performed using the (**A**) $sPLA_2$, (**B**) $cPLA_2$, and (**C**) PLC antibodies shown, and further revealed with a secondary antibody labeled with TRITC or Alexa 594 (Table 2). Nuclei were counter-stained with Hoechst 33258 reagent. Negligible background was observed in control experiments, in which primary antibodies were omitted (data not shown). The corneal epithelium (e) and stroma (s) are indicated in (**A**). Scale bar, 100 μm.

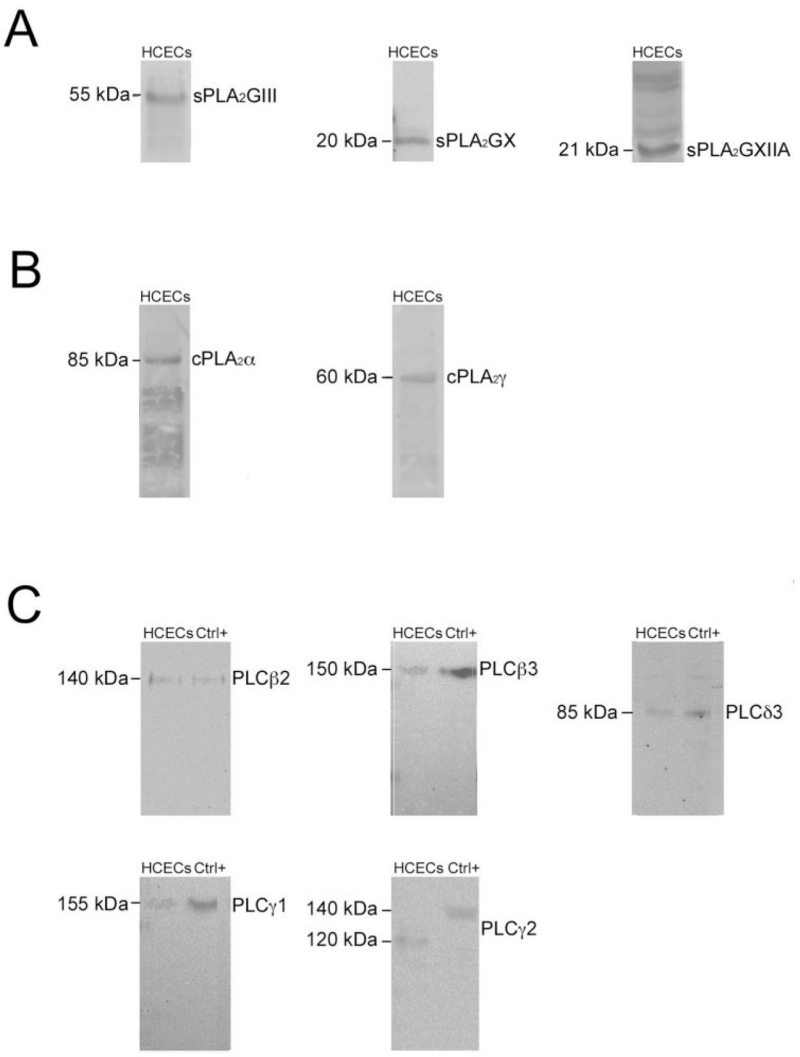

Figure 3.

Western blot analysis of phospholipases in crude extracts from HCECs. Western blot analyses were performed using the (**A**) $sPLA_2$, (**B**) $cPLA_2$, and (**C**) PLC antibodies shown (Table 2). *Left*: molecular mass of the expected proteins. Ctrl+, positive controls RAW 264.7 for PLCs $\beta 2$ and $\delta 3$, A-431 for PLCs $\beta 3$ and $\gamma 1$, and MCF7 for PLC$\gamma 2$.

Table 1

Sequences of the Forward and Reverse Primers Used for the PCR Amplification of Phospholipase Isoforms in Human Corneal Epithelium

Phospholipase Isoform	Forward Primer	Reverse Primer	Expected PCR Product Size (bp)	GenBank Accession Number
$sPLA_2GIB$	TGGTCATCTCAGTTCTTTTC	TCAACTCTGACAATACTTCT	482	NM_000928
$sPLA_2GIIA$	AGCCACCAAGGAGGAGCAGG	CAGCACTGGGTGGAAGGTTT	309	NM_000300
$sPLA_2GIID$	GCCAGCATCTGCCTCCACT	AGGAACAGGGTAGAGGGTGA	499	NM_012400
$sPLA_2GIIE$	TGCTCCTTGTGCACCTC	CAGCTTGTTGGGATAATGGG	437	NM_014589
$sPLA_2GIIF$	AGAAGTTCTTCACCGT	TTGGTGACTGCAGGTGACCT	480	NM_022819
$sPLA_2GIII$*	TGCCTACAGAATCAGCACGA	TTGAGCAGCTGGAACTCGAT	500	AF_220490
$sPLA_2GV$	TGGATACCAATGTTCCGAC	CCTAGGAGCAGAGGATGTTG	549	NM_000929
$sPLA_2GX$	CGCCTATATGAAATATGGT	CAAGGTAGTCAGTCACACTTG	327	NM_003561
$sPLA_2GXII$	AAGGAGGCGTGGATATGGAGCT	ATCTGTCACTAGCTGTCGGCAT	723	NM_030821
$cPLA_2\alpha$	GAGCTGAAAAAGGATCCTGACT	TGTCCCTAGAGTTTCATCCA	440	M68874
$cPLA_2\beta$†	CTTCATGATGCCAGCTGAGCGCCGCC	CCCGGCCATCAGTGGGGCCTGCGC	2730	AF121908
$cPLA_2\gamma$	CCACAGGCATCTATGTTGAA	ATTACTCTCTGACCGACTTC	1020	XM_055864
$cPLA_2\delta$	AATTATGGAGAGCCTGTCACCTGGG	TATATCAGGTCTGTGCCCATGGAGG	2457	AB090876
PLCβ1	TTGGATGTGGGGAACATCGG	AAATAGTGAGAAAGGGGCTG	690	XM_045636
PLCβ2	GAGCAACTAGATTTCTGGAG	TCACCGGAATCTTCCCTTCA	620	BC009009
PLCβ3	AGCGGTTCCTGAACAAGCTG	AGGTCTTGAAGGCAGTCTCG	550	NM_000932
PLCβ4	CCCAGTGGAAAGAATGATGA	TTCCATCCCAGCAGTCAAGT	500	L41349
PLCγ1	GCTGCCTGCGGATGGGCTGT	GTACCACTCTTTGCTCTCGT	924	NM_002660
PLCγ2	GCTGCCTGCGCATGGGCTGT	GTACCACGGCTTGGACTCGT	882	BC_011772
PLCδ1	TGTCGCTACTCAAGTGAGTC	ATGGAGCCTGAGTGGTGGAT	458	BC050382
PLCδ2	CAGGGTAGGTTACCTATTAA	ATGTTCGTAACAAAACCTAC	369	N/A‡
PLCδ3	AGCCATGCTGTGCGGCCGCT	AGACGGTCGTTGTTGGAGTG	716	NM_133373
PLCδ4	CAGCTCACAGACACAGGAAA	GCTGGTGACAACATCCACCA	468	NM_032726
PLCε§	CAGCAGAAGGTAATGGCT	TCCCTTGGGCTTTGGGAAAT	700	NM_016341
PLCζ1	GAGGGTATGCCAATTACACTT	GGCTGTTTTATTGCGATGCA	447	NM_015184 NM_033123

*Degousee et al.[35]

†Pickard et al.[38]

‡Bovine sequence for PLCδ2 isoform, Meldrum et al.[23]

§Concensus sequence for PLCεisoforms.

Table 2
Primary and Secondary Antibodies

Antibodies	Category	Source
Primary antibodies		
$sPLA_2$GIII (MG-12-02-02-1)	Rabbit polyclonal	Michael H. Gelb
$sPLA_2$GX	Rabbit polyclonal	Michael H. Gelb
$sPLA_2$GXIIA (MG-2-17-01-1)	Rabbit polyclonal	Michael H. Gelb
$cPLA_2\alpha$ (MF-145)	Rabbit polyclonal	Merck Frost Center for Therapeutic Research, Montreal, QC, Canada
$cPLA_2\gamma$	Rabbit polyclonal	Christina C. Leslie, National Jewish Medical and Research, Denver, CO
PLCβ1 (G-12)	Rabbit polyclonal	Santa Cruz Biotechnology, Santa Cruz, CA
PLCβ2 (Q-15)	Rabbit polyclonal	Santa Cruz Biotechnology
PLCβ3 (C-20)	Rabbit polyclonal	Santa Cruz Biotechnology
PLCβ4 (C-18)	Rabbit polyclonal	Santa Cruz Biotechnology
PLCγ1 (E-12)	Rabbit polyclonal	Santa Cruz Biotechnology
PLCγ2 (Q-20)	Rabbit polyclonal	Santa Cruz Biotechnology
PLCδ1 (C-20)	Goat polyclonal	Santa Cruz Biotechnology
PLCδ3 (2A11-D10-D8)	Mouse monoclonal	Steve Roffler, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
PLCε	Goat polyclonal	Santa Cruz Biotechnology
Secondary antibodies (IF)		
Anti-rabbit	Goat-TRITC	Chemicon, Temecula, CA
Anti-goat	Rabbit-TRITC	Jackson IRL, West Grove, PA
Anti-mouse	Goat-Alexa 594	Molecular Probes, Eugene, OR
Secondary antibodies (Western blot)		
Anti-rabbit	Donkey-HRP	Amersham Biosciences, Baie d'Urfé, Québec, Canada
Anti-goat	Bovine-HRP	Santa Cruz Biotechnology
Anti-mouse	Sheep-HRP	Amersham Biosciences, Baie d'Urfé, Québec, Canada

IF, immunofluorescence; TRITC, tetramethylrhodamine isothiocyanate; HRP, horseradish peroxidase.