Tear Lipocalin: Evidence for a Scavenging Function to Remove Lipids from the Human Corneal Surface

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Purpose. Lipid contamination of the cornea may create an unwettable surface and result in desiccation of the corneal epithelium. Tear lipocalin (TL), also known as lipocalin-1, is the principal lipid-binding protein in tears. TL has been shown to scavenge lipids from hydrophobic surfaces. The hypothesis that TL can remove contaminating fatty acids and phospholipids from the human corneal surface was tested.

Methods. TL was purified from pooled human tear samples by size exclusion and ion exchange chromatographies. Tears depleted of TL were reconstituted from fractions eluted by size exclusion chromatography that did not contain TL. Fresh and formalin-fixed human corneas were obtained from exenteration specimens. Fluorescent analogs of either palmitic acid or phosphatidylcholine were applied to the corneal epithelial surface. Tears, TL, or tears depleted of TL were applied over the corneas, and spectrofluorometry and fluorescent stereomicroscopy were used to monitor the removal of fluorescent lipids. Tears used in the experiments were then fractionated by size exclusion chromatography to determine the component of tears associated with fluorescent lipids.

Results. Significant enhancement of fluorescence for 16AP and NBD C_6-HPC was evident in solutions incubated with whole tears and purified TL but not with tears depleted of TL for fixed and unfixed corneas. After the experiment, size exclusion fractions of tears showed that the fluorescence component coeluted with TL.

Conclusions. TL scavenges lipids from the human corneal surface and delivers them into the aqueous phase of tears. TL may have an important role in removing lipids from the corneal surface to maintain the wettability and integrity of the ocular surface. (*Invest Ophthalmol Vis Sci.* 2005;46:3589–3596) DOI: 10.1167/iovs.05-0569

The human tear film lubricates and moistens the ocular surface. The corneal surface is protected from drying by several anatomic barriers. The epithelium produces glycoproteins (mucins) that span the corneal epithelial membrane to extend into the tear film.[1,2] Soluble mucins are mixed with the aqueous layer of tears. Goblet cells, mainly in the conjunctiva, secrete abundant gel-forming mucins into the tear film. Lipids produced by meibomian glands spread on the tear film surface. Normally, the membrane-anchored mucins form a glycocalyx that protects the apical epithelium and presumably prevents lipid binding to the surface of the cornea.[1] However, the natural processes of apical epithelial shedding and ectoshedding of membrane-associated mucins and the loss of corneal epithelium from minor trauma leave a portion of the corneal surface vulnerable to lipids.[2] Lipids that contaminate the mucin layer or areas devoid of mucins render the corneal surface unwettable and eventually result in desiccation of the corneal epithelium.[3,4] A mechanism to remove meibomian lipids contaminating corneal epithelium is necessary to prevent drying of the ocular surface. There is evidence that the barriers to prevent lipid contamination are impaired in dry eye diseases.[5,6] The mucin covering the cornea may be compromised in dry eye disease, as is suggested by the Rose Bengal staining of epithelial cells.[7] Membrane-associated mucins and secreted mucins are altered by a host of dry eye diseases.[8–10] Epithelial erosions are common in dry eye diseases.[11] Indeed, one of the objective criteria used widely for the diagnosis of dry eye is the presence of fluorescein staining of the cornea in areas in which the epithelium has been disrupted.[12,13] In addition, the low volume of tears in dry eye disease results in thinning of the tear film, abnormal tear breakup time, and vulnerability to lipid contamination.[6]

A plausible mechanism to remove contaminating lipids from the cornea involves tear lipocalin (TL), the major lipid-binding protein in tears. By mass, TL is the third most abundant protein in human tears, with a concentration of approximately 70 μM.[14] TL binds cholesterol and lipids with long alkyl chains, such as stearic acid, which would normally be insoluble in aqueous solution.[15,16] TL has been shown to remove lipids from a variety of surfaces, including glass, quartz, and nonstick resin (Teflon; Dupont, Wilmington, DE).[17] TL is promiscuous and well suited for these insoluble lipids. The solution and crystal structure of TL have been published from separate laboratories, and they show remarkable concordance in the assignment of β strands to form a calyx with a capacious mouth and amino acid residues that sterically permit relatively large lipids to enter the binding cavity.[18,19] Here the hypothesis that TL can scavenge lipids from the corneal surface is tested.

Materials and Methods

Collection of Human Tears and Corneas

Human tears were collected from healthy volunteers and pooled as previously described.[20] Human corneas were obtained either fresh or after fixation in 10% formalin from exenteration specimens; patients had had no ocular symptoms, and the corneas had no visible abnormalities. Formalin was removed from the fixed corneas by six 15-minute washes in 10 mM Tris buffer, pH 7.2, that contained other ions in concentrations present in tears (133 mM NaCl, 24 mM KCl, 0.8 mM, $CaCl_2$, 0.61 mM $MgCl_2$). The research was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was

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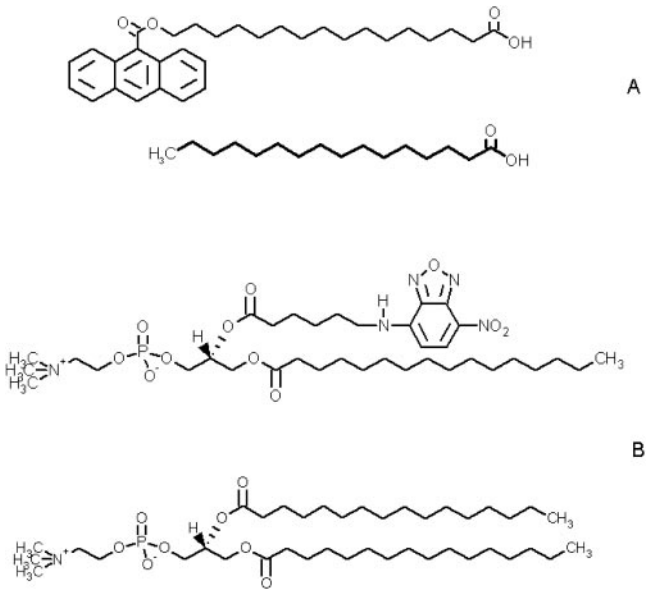

FIGURE 1. Chemical structure of fluorescence-labeled lipids. (**A**) 16AP (*above*) and palmitic acid (*below*). (**B**) NBD C_6-HPC (*above*) and phosphatidylcholine (*below*).

obtained from donors of human tears after explanation of the nature and possible consequences of the study; and the procedures were approved by the institutional review board.

Fluorescence-labeled fatty acid 16-(9-anthroyloxy)palmitic acid (16AP) and fluorescence-labeled phospholipid 2-(6-(7 nitrobenz-2-oxa,1,3-diazol-4-yl)amino)hexanoyl–1-hexadecanoyl-sn-glycero-3-phosphocholine (NBD C_6-HPC) were obtained from Molecular Probes (Eugene, OR; Fig. 1).

Size-Exclusion Chromatography

Size-exclusion chromatography was used for two purposes. First, TL was separated from a portion of the pooled human tears by gel filtration (Sephadex G-100; Sigma-Aldrich, St. Louis, MO) and was further purified as published.[15,17] During this purification, fractions that passed through the DEAE-Sephadex (TL binds with the resin) were collected and combined with the remaining G-100 fractions depleted of TL and reconstituted in the buffer to the initial volume described. Tricine PAGE was performed to confirm that the resultant mixture was depleted of TL, as previously described.[15,20] Whole tears and that portion depleted of TL were tested for their ability to scavenge lipids (see "Application and Removal of Fluorescent Lipids from the Human Cornea").

The second role for size-exclusion chromatography was to detect the protein fraction(s) responsible for lipid removal. Major tear proteins were separated in samples in which tears and tears depleted of TL had been incubated with corneas overlaid with fluorescence-labeled lipid. Fractionation was accomplished by gel filtration (Sephadex G100), as previously described.[15] Absorbance at 280 nm and fluorescence spectra (with parameters described in the next section) were used to correlate protein peaks with labeled lipids (16AP or NBD C_6-HPC). Protein fractions associated with fluorescence were analyzed by tricine PAGE, as indicated. Recombinant TL was expressed and purified as previously described.[18]

Steady State Fluorescence Spectroscopy

Steady state fluorescence measurements were made on a spectrofluorometer (Fluorolog τ-3; Jobin Yvon-SPEX, Edison, NJ). Spectral parameters were chosen for each lipid: 16AP (λ_{ex} = 361 nm; λ_{em} = 400–550 nm) and NBD C_6-HPC (λ_{ex} = 420 nm; λ_{em} = 480–650 nm). Bandwidths for excitation and emission were 2 nm and 3 nm, respectively. Emission measurements were performed in ratio mode to ensure that possible excitation light intensity deviation was not a factor in fluorescence measurements.

NBD C_6-HPC Binding Experiments, Analysis

A stock solution of NBD C_6-HPC (600 μM) was prepared in ethanol, and concentration was determined using ε_{465} = 21,000 M^{-1} cm^{-1}. The final concentration of ethanol in the titration experiment did not exceed 2%. Fluorescence was measured in mixtures of TL (1 μM) and varying concentrations of NBD C_6-HPC. The NBD C_6-HPC binding data were analyzed with the following formula for one binding site (Origin 7.5 software; OriginLab Corporation, Northampton, MA):

$$\frac{\Delta F}{\Delta F_{max}} = 0.5 \times \left[\left(1 + \frac{K_d}{n \times P} + \frac{L_t}{n \times P} \right) - \sqrt{\left(1 + \frac{K_d}{n \times P} + \frac{L_t}{n \times P} \right)^2 - \frac{4L_t}{n \times P}} \right] + c \times P$$

where ΔF is the change in fluorescence, K_d is the apparent dissociation constant, P is total protein concentration, L_t is the total ligand concentration, c is the parameter that accounts for the contribution of nonspecific binding, and n is stoichiometry.

In competitive displacement experiments, TL (1 μM) was incubated with NBD C_6-HPC (1 μM), and the native phospholipid, L-α-lysophosphatidylcholine (Sigma, St. Louis, MO) was added in increasing concentrations. The IC_{50} parameter was extrapolated from the graph of fluorescence intensity of bound NBD C_6-HPC compared with competitor concentration (Origin 7.5 software; OriginLab Corporation). The final concentration of ethanol in the competitive displacement experiment did not exceed 2%.

Application and Removal of Fluorescent Lipids from the Human Cornea

For these experiments, NBD C_6-HPC was prepared with Tween 20 (Fisher Scientific, Pittsburgh, PA) to prevent the formation of stable bilayers in aqueous solution.[21] NBD C_6-HPC and Tween 20 were dissolved in ethanol, dried under Argon flow to produce a thin film, and vortexed vigorously for 2 minutes in buffer. The final concentration of NBD C6-HPC was 1.12 mM with 20 mole% Tween 20. In general, 6 μL of either 5.3 mM 16 AP or 1.1 mM NBD-C_6-HPC was applied uniformly to the corneal epithelial surface. Excess lipid was removed by gentle rinsing in buffer. Spectra were monitored until there was no detectable fluorescence in the rinse solutions. Two corneal buttons, 3 mm diameter each, were trephined (Sklar Instruments, West Chester, PA) from the same cornea and used for tears and control (tears depleted of TL), respectively. The buttons were placed in 3 × 3-mm quartz cuvettes and overlaid with 200 μL solution, buffer, tears, TL (70 μM), or tears depleted of TL. Gentle mixing was performed before each measurement of fluorescence. A plot was made comparing fluorescence with time. Measurements were made for approximately 90 minutes or until saturation was achieved.

Fluorescence Microscopy

To visualize the removal of fluorescent lipids from the cornea and simultaneously to quantitate fluorescence of the labeled lipid above the cornea, a 3-mm-diameter chamber was machined from black synthetic fluorine-containing resin (Teflon; Professional Plastics Inc., Fullerton, CA) on a milling instrument. The chamber for the cornea was made in contiguity with a 6-mm diameter, 200-μL capacity chamber for constant mixing at 50 rpm (Fig. 2). Experiments were performed as indicated except that the corneas remained in the chamber instead of in a cuvette and the fluid in the mixing chamber was sampled at various time points for fluorescence. Corneal buttons were photo-

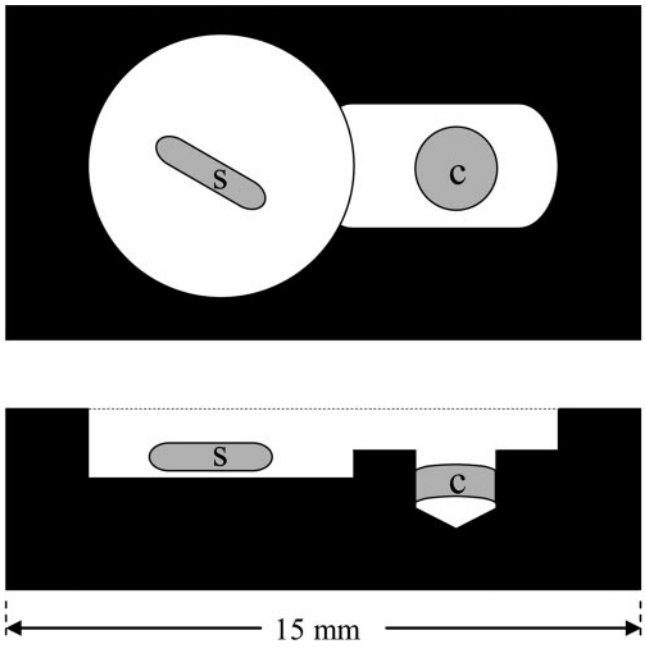

FIGURE 2. Diagram of customized mixing chamber with stir bar (S) adjacent to chamber for the corneal button (C). Top view (*above*) and cross-sectional view (*below*).

graphed with a digital camera (manual mode; Nikon, Tokyo, Japan) mounted on a fluorescence stereomicroscope (excitation filter 480/40 nm and barrier filter >510 nm; MZ FLIII; Leica, Wetzlar, Germany). The time of exposure (0.25 second) and the aperture were kept constant for all photographs.

Quantitation of Lipid

To determine the amount of NBD C_6-HPC scavenged from the corneal surface, the lipid was extracted from the overlying solution with an equal volume of a 2:1 chloroform/methanol mixture. Buffer (300 μL) was also added to the cornea buttons and then extracted with a 2:1 chloroform/methanol mixture to determine the amount of lipid remaining on the cornea. Chloroform fractions were taken for fluorescence measurements.

Statistical Analysis

The differences between fluorescence after incubation with tears and tears depleted of TL were evaluated with analysis of variance. A t test statistic was determined for the difference between two population means. For fatty acid removal from fixed corneas, $n = 6$ for each data point. Hypotheses for testing were formulated as $\mu_1 - \mu_2 = 0$ for H_0 and $\mu_1 - \mu_2 \neq 0$ for H_A, as described.[22]

Histopathology

After the fluorescence measurements were determined, except where quantitation of lipid was performed, corneal buttons were processed for routine histopathology in graded alcohols and xylene and were embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin and periodic acid Schiff stains to verify the presence of corneal epithelium.

RESULTS

Removal of Fatty Acid from the Corneal Surface by TL

These experiments tested the ability of whole tears to remove 16AP from the corneal surface. Because 16AP fluoresces intensely when bound to protein, increased fluorescence is immediately detected as it enters the solution in a complex with TL. Fluorescence data for these experiments were obtained using six corneas that had been previously fixed in formalin (Fig. 3).

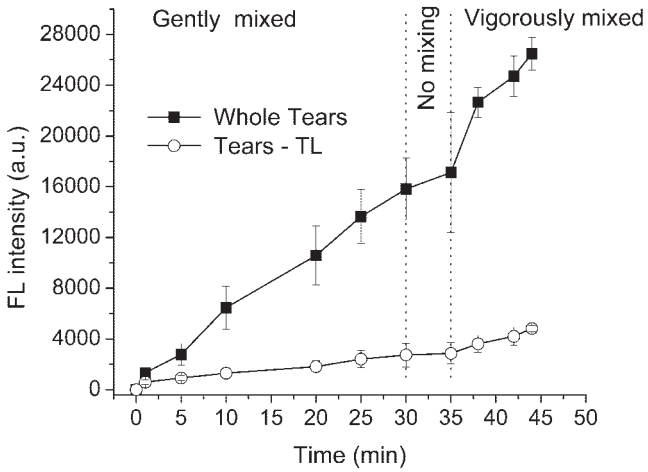

FIGURE 3. Removal of fatty acid analog, 16AP, from the corneal surface (corneas fixed in formalin) by tears and tears depleted of TL. Data are shown as mean fluorescence intensity ± SEM ($n = 6$).

When tears depleted of TL were incubated with the cornea, fluorescence-labeled lipid did not appear in the overlying solution to any appreciable extent. When incubated with whole tears (containing TL), fluorescence was evident within 5 minutes (Fig. 3). The result was highly significant ($P < 0.001$ after 20 minutes and $P < 0.0001$ at all points thereafter). 16AP was solubilized throughout the duration of the experiment. The effect of mixing the solution is also evident from Figure 3. Vigorous mixing resulted in a rapid increase in fluorescence with tears but had little effect with tears depleted of lipocalin (Fig. 3). A similar increase in fluorescence was noted with recombinant TL (Fig. 4).

Experiments were also performed with unfixed normal corneas from human eyes obtained immediately after exenterations. Similar results were obtained (Fig. 5). To verify that TL is

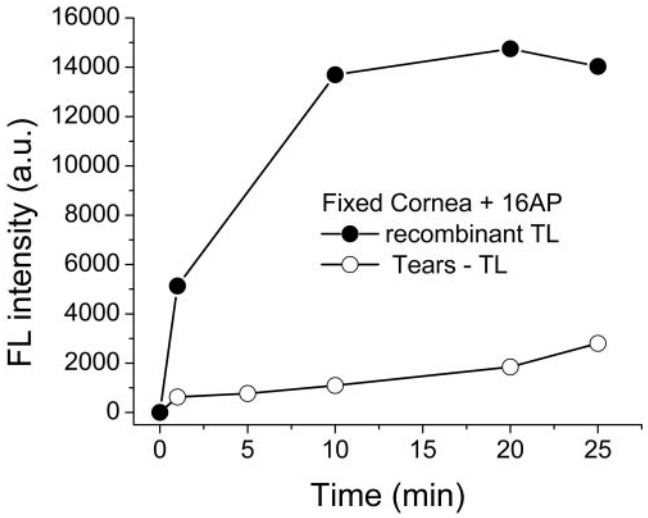

FIGURE 4. Removal of fatty acid analog, 16AP, from the corneal surface (corneas fixed in formalin) by recombinant TL and tears depleted of TL.

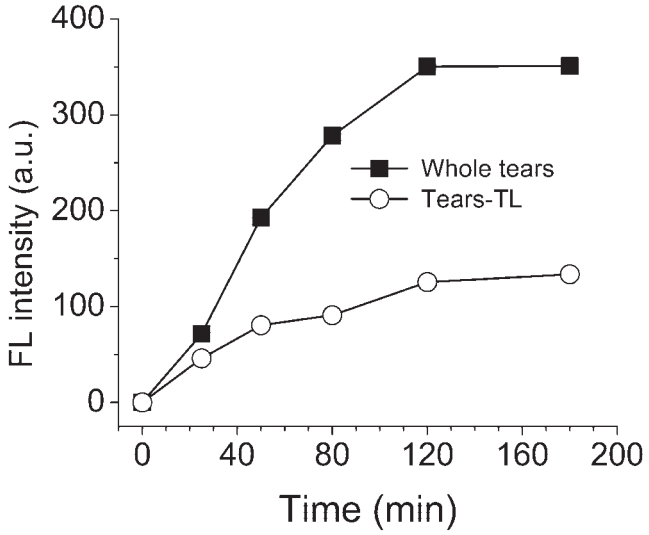

FIGURE 5. Removal of 16AP from fresh, unfixed corneas by tears and tears depleted of TL. Fluorescence intensity is the mean of 2 measurements ($n = 2$).

the component of tears that binds the fluorescent lipid, gel filtration was performed of the solutions used in all lipid-scavenging experiments. It is evident from Figure 6A that a peak of fluorescence coincided with a protein absorbance peak in the elution profile of tears incubated over the cornea.

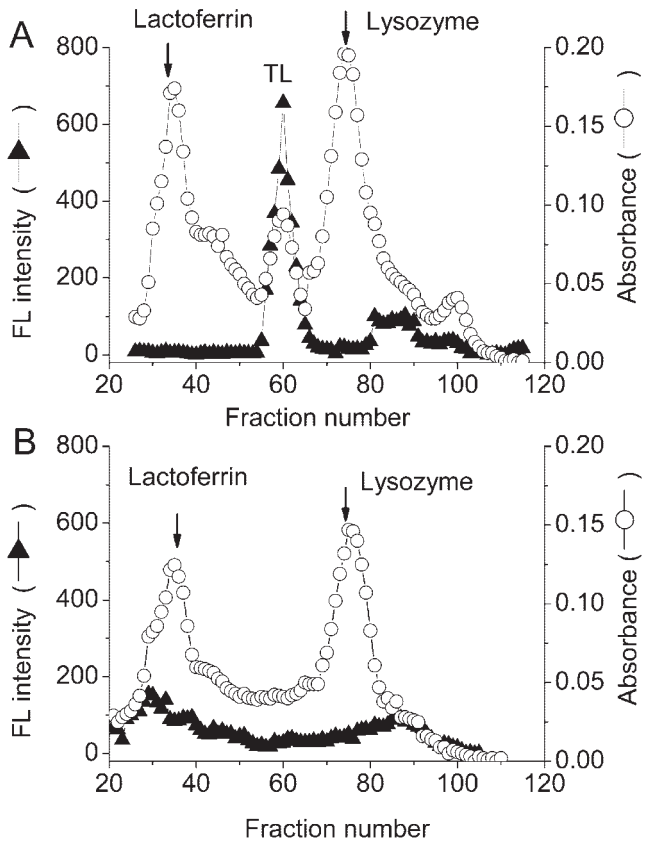

FIGURE 6. Gel-filtration profile of tears (**A**) and tears depleted of TL (**B**) used in experiments that test removal of 16AP from the cornea. Absorbance peaks of lactoferrin, TL, and lysozyme are indicated.

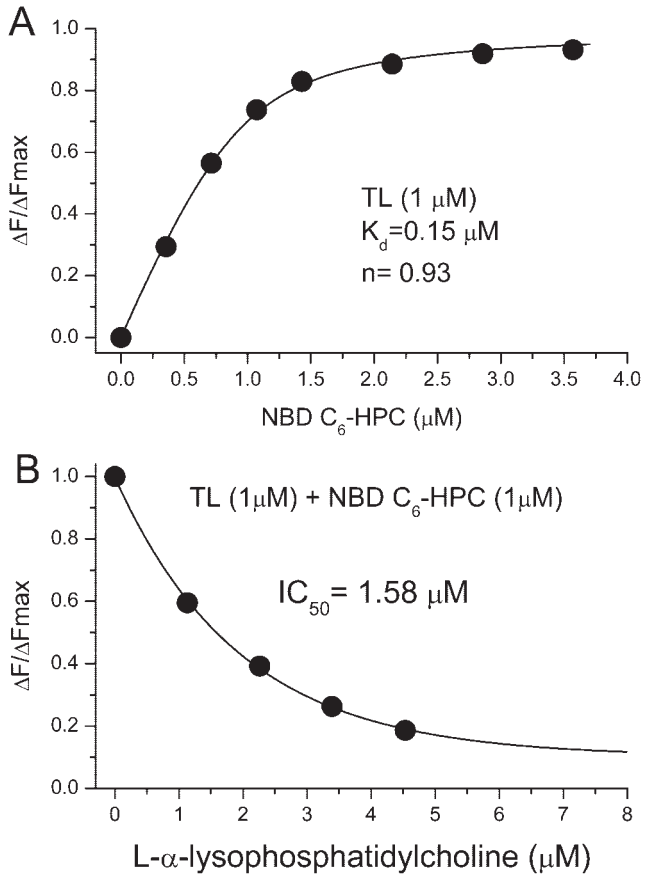

FIGURE 7. (**A**) Binding curve for TL and fluorescence-labeled phospholipid. The dissociation constant (K_d) and stoichiometry (n) are shown. (**B**) Competitive displacement of fluorescent phospholipid analog with native L-α-lysophosphatidylcholine. IC_{50} (concentration of competitor producing 50% inhibition of fluorescence) is shown.

This protein absorbance peak corresponds to TL. However, tears depleted of TL showed neither the fluorescence peak nor the protein absorption peak corresponding to TL. A broad peak was observed that eluted in a high-molecular-weight region that did not correspond precisely to any protein peak (Fig. 6B). In addition, free lipid was observed to elute in a broad band in gel filtration fractions 80 to 100 of tears and of tears depleted of TL; no protein was seen by gel electrophoresis.

Titration of Phospholipid with TL

The binding of 16AP to TL has been well characterized.[23] To verify specific binding of the fluorescence-labeled phospholipid NBD C_6-HPC to TL, a binding curve was constructed. NBD C_6-HPC characteristically exhibits low fluorescence in the free state (unbound to protein). However, when NBD C_6-HPC is bound to protein in a hydrophobic environment, there is a marked increase in fluorescence intensity, and the peak of fluorescence is shifted to shorter wavelengths (blue shift). The binding affinity of NBD C_6-HPC to TL was determined by the change in fluorescence intensity (Fig. 7). A dissociation constant in the submicromolar range and a single binding site were demonstrated. The fluorescence associated with free lipid is negligible. To ensure that NBD C_6-HPC to TL has binding characteristics similar to those of the native phospholipid, L-α-lysophosphatidylcholine, competitive displacement experiments were performed with TL (Fig. 7B). The IC_{50} parameter

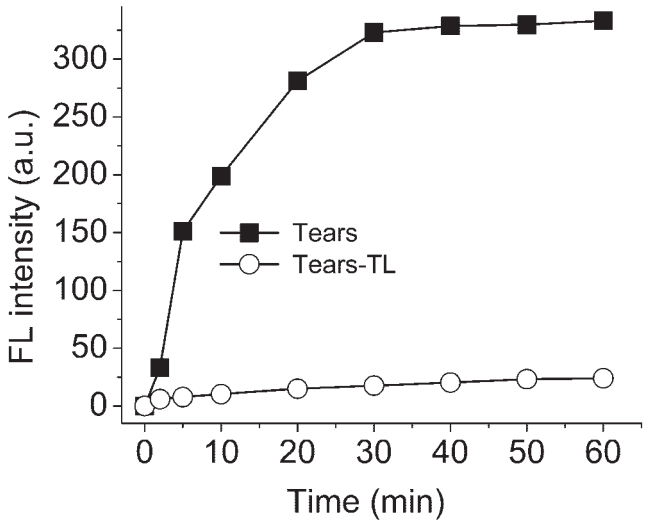

FIGURE 8. Removal of fluorescence-labeled phospholipids from fresh human corneas by tears. Intensity of fluorescence is given as the mean of two measurements.

represents the concentration of L-α-lysophosphatidylcholine that inhibited 50% of the binding of NBD C_6-HPC, as determined by the decrease in fluorescence intensity. In addition, the IC_{50} parameter indicates that the binding constants of the native and fluorescence-labeled phospholipids were close.

Removal of Phospholipid from the Corneal Surface

The removal of phospholipids from the human cornea was monitored by fluorescence spectroscopy. Results are shown in Figure 8 and are similar to those obtained for fatty acids. Fluorescence-labeled phospholipid was readily removed from unfixed corneas by whole tears (Fig. 8). However, tears depleted of TL did not result in a significant increase in fluorescence intensity. The scavenging of phospholipids can be restored to tears depleted of TL by adding purified TL back to the solution (Fig. 9).

The removal of labeled phospholipids from human corneas was followed visually with the use of fluorescence stereomicroscopy. Photographs, all taken at the same exposure, show that fluorescence from phospholipids was barely changed with tears depleted of TL (Fig. 10). Incubation of the cornea with whole tears shows a dramatic reduction of fluorescence after 1 hour. Simultaneous monitoring of the fluorescence spectra revealed results identical to those seen in Figure 8.

Histologic sections of the corneas were obtained after the scavenging experiments. Periodic acid-Schiff (PAS)–and hematoxylin and eosin–stained sections revealed 5 layers of epithelium. PAS staining was observed near the surface of the epithelium, possibly reflecting residual mucins (Fig. 11).

Gel-filtration analysis of the tears overlying the cornea revealed the fraction in which fluorescence-labeled phospholipid bound to TL (Fig. 12). The only peak of fluorescence coeluted precisely with TL, indicating that the phospholipid was removed solely by this protein. Nonspecific binding, seen in Figure 12 as a slight broad increase in fluorescence, was noticeably lower for the fluorescent phospholipid than for the fatty acid (Fig. 6). The SDS tricine gel of the fractions collected in gel filtration are shown (Fig. 12).

To confirm the visual impression that most of the lipid had been removed by TL, the fluorescent lipid in the overlying solutions of whole tears and of tears depleted of TL was extracted with chloroform/methanol. Approximately 98% of total fluorescence was detected in the overlying solution of tears, and 2% remained on the surface of the cornea.

DISCUSSION

The experiments provide several lines of evidence that TL scavenges fatty acids and phospholipids from the human corneal surface. First, tears remove lipid from the cornea, whereas tears depleted of TL do not. Second, when purified TL is added back to tears depleted of TL, scavenging is restored. Third, recombinant TL also removes fluorescent lipid from the cornea. Fourth, gel filtration of the tears used to scavenge the fluorescence-labeled lipid reveals that the lipid coelutes with TL. The data also indicate that TL is the only component in tears that has any appreciable ability to remove lipids. The gel-filtration profiles of tears used in the fatty acid and the phospholipid experiments reveal that the peak absorbance fractions of TL coincide with a sharp simultaneous peak of high-intensity fluorescence attributed to the labeled lipid. The profile of tears depleted of TL in the fatty acid scavenging experiments (Fig. 6) shows only 2 minor broad peaks that may represent nonspecific binding and free lipid unassociated with proteins (Fig. 6). Free lipid might be expected because the ratio of bound to free lipid is determined by the equilibrium established between protein and ligand. Solubilized free lipids would be driven toward the tear–air interface to occupy a more energetically favorable hydrophobic environment. These data are compelling evidence that TL acts as a potent scavenger to extract otherwise insoluble lipids from the corneal surface and to solubilize the lipids in the aqueous layer. In gel-filtration profiles, no fluorescence-labeled phospholipid was detected that was unbound to TL. This finding may reflect avid lipid binding to TL, less ligand after rinsing, or both, leading to the reduced ligand/TL ratio in the scavenging experiment.

The experiments shown also provide a sense of the rate at which TL scavenges lipid. The time frame for lipid extraction by TL was rapid when one considers that the distribution volume was 200 μL in the cuvettes and mixing chamber, in contrast to the much smaller volume in tears on the eye (7–8 μL tear volume).[24] Data shown in Figure 3 suggest that extraction is enhanced with more aggressive mixing. The action of the eyelids over the tear film is more efficient than the action

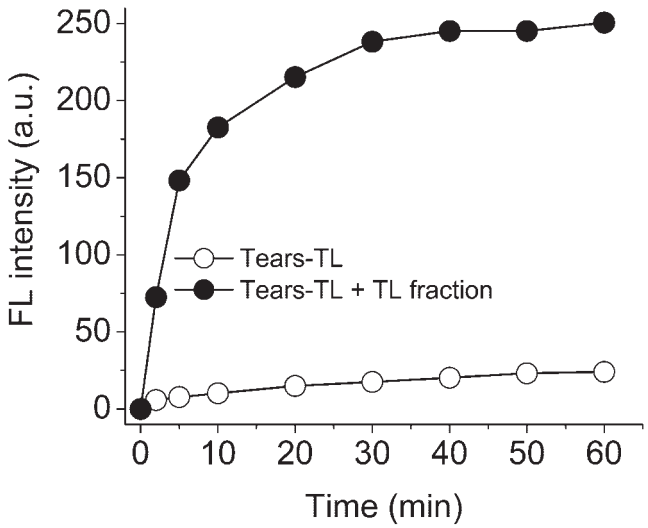

FIGURE 9. Removal of fluorescent phospholipids from fresh human corneas by TL (n = 2).

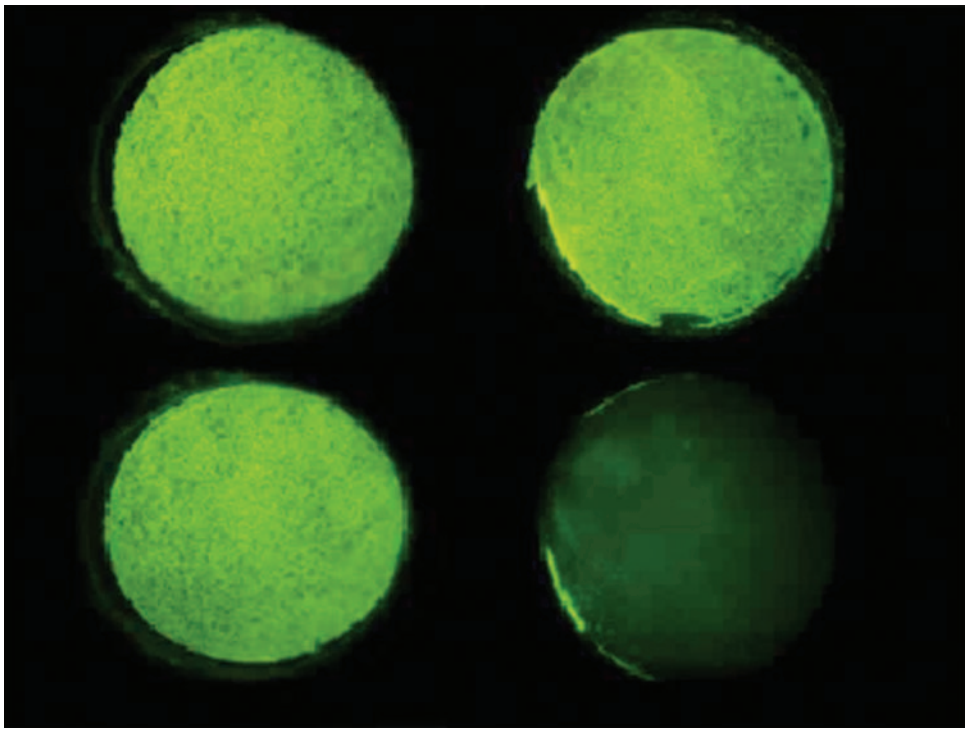

FIGURE 10. Removal of fluorescent phospholipid from the unfixed cornea by tears, as monitored by fluorescence stereomicroscopy. NBD C_6-HPC was applied to cornea and rinsed in buffer (*upper images*), treated with tears depleted of TL (*lower left image*), and tears (*lower right image*). Fluorescence intensity of the overlying solutions is compiled in the data points in Figure 9 for time 0 (*upper images*) and 60 minutes (*lower images*).

of our mixing chamber and should facilitate extraction speed in the normal eye.

Binding experiments and competitive displacement experiments for NBD C_6-HPC verify that the fluorescence-labeled phospholipid has binding characteristics similar to those of the native phospholipid and justifies its use in these experiments.

The extensive fluorescence in Figure 10 clearly documents that substantial lipid binds to the corneal surface despite the thorough rinsing after application in buffer. The fluorescence implies a relatively hydrophobic environment for the lipid on the cornea because NBD C_6-HPC does not fluoresce in aqueous solution. The persistence of lipid after irrigation supports the hypothesis that lipid left in contact with the corneal surface makes it unwettable.[3,25] The figure demonstrates the efficiency of TL to remove a great deal of lipid from the corneal surface and to confirm our rough estimates of fluorescence intensity of 98% in the tears and 2% in the cornea.

The exact nature of lipid binding to the cornea is not completely clear. The corneal epithelium is intact, but the status of the transmembrane mucins is unknown. Every effort has been made to obtain corneas that were not perturbed, with the inherent limitations in the collection process and experimental protocol. It is expected that soluble mucins are removed during surgery or during the gentle rinsing phase of the experiment, which removes excess lipids. Furthermore, loss of some apical epithelial cells from washing is inevitable.[26] The loss of transmembrane domain mucins that reside in apical epithelium might enhance lipid binding to the cornea as the mucin protective layer is reduced. These factors might have influenced the relative ease of binding fluorescence-labeled lipids to the cornea. However, loss of mucins from some apical epithelium is a normal process through apical cell shedding and ectodomain shedding.[1,2,27] Minor ocular trauma encountered during daily activities, such as eye rubbing, irrigation during showers, and swimming, would also result in loss of some soluble mucins and apical epithelium. Fluorescein staining, indicative of epithelial disruption, is an objective clinical

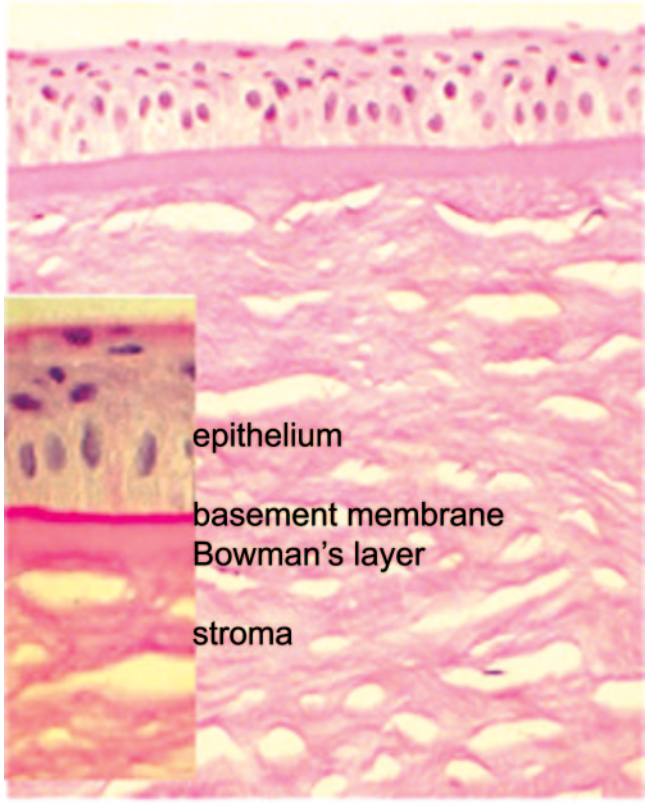

FIGURE 11. Photomicrograph of a corneal section stained with periodic acid-Schiff after lipid removal by tears (original magnification, ×100). *Inset* (original magnification, ×250) shows details of five layers of intact corneal epithelium, epithelial basement membrane Bowman layer, and stroma.

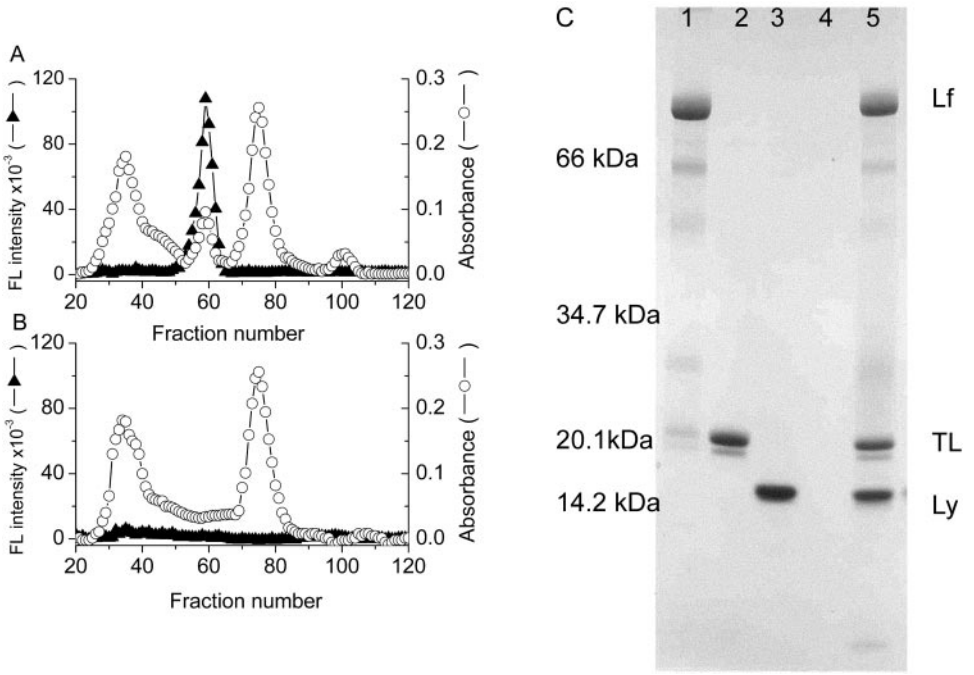

FIGURE 12. Gel-filtration profile of tears (**A**) and tears depleted of TL (**B**) that were used in experiments to test the removal of fluorescence-labeled phospholipid from the cornea. Absorbance peaks of lactoferrin, TL, and lysozyme are shown from left to right. Peak of fluorescence coincides with the absorbance peak of TL. (**C**) Coomassie-stained SDS tricine 10% acrylamide gel of pooled fractions shown in chromatogram (**A**). *Lane 1*, 22 to 48; *lane 2*, 52 to 63; *lane 3*, 70 to 82; *lane 4*, 97 to 105; *lane 5*, tears 5 μL. Lf, lactoferrin; Ly, lysozyme. Masses shown on *left* for standards: bovine albumin (66 kDa), pepsin (34.7 kDa), bovine β-lactoglobulin (20.1 kDa), and egg white lysozyme (14.2 kDa).

sign for dry eye disease but has been documented at various times even in healthy persons.[28] Of course, during normal daily activities, it is unlikely that human cornea would be challenged with the excess lipid placement that was applied here. Whether the fluorescent lipids contaminate mucin or epithelium depleted of mucin, it is likely that both conditions would be present in normal and dry eye states to varying degrees. The fact that formalin-fixed corneas responded almost identically to the fresh, unfixed corneas in these experiments suggests that the corneal surfaces were similar.

The importance of lipid scavenging is likely to have clinical significance for patients with dry eye disease. In dry eye disease, reduced mucin production has been clearly demonstrated in conjunctiva (i.e., MUC5AC and MUC16).[27] TL is reduced in seborrheic blepharitis and meibomian gland dysfunction,[29] and concentrations of TL and other protein components are decreased.[30] Furthermore, the concentration of TL correlates with tear film stability.[31] In dry eye disease the tear film is thin, and the cornea is readily exposed to lipid contamination.[5,6] The precise nature of the interaction of lipids with molecules at the corneal surface remains to be explored.

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