Label-Retaining Cells Are Preferentially Located in Fornical Epithelium: Implications on Conjunctival Epithelial Homeostasis

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Purpose. To determine the cell kinetic properties of epithelial cells from various zones of the conjunctiva.

Methods. The morphology and cell kinetics of bulbar, fornical, and palpebral conjunctival epithelium were studied in neonatal and adult SENCAR mice. To examine the proliferative rate of the conjunctival epithelium, a single administration of tritiated thymidine (3H-TdR) was used to detect cells in "S" phase. Proliferative rates were also assessed by determining mitotic activity after an intraperitoneal injection of colchicine to arrest cells in mitosis. To detect slow-cycling cells, mice received 3H-TdR continuously for 1 week. After a 4-week chase, animals were sacrificed and eyes were surgically removed. All tissues were immediately fixed in formalin and processed for histology and autoradiography.

Results. Slow-cycling cells, detected as label-retaining cells (LRCs), were identified in bulbar, fornical, and palpebral epithelia, as well as in limbal epithelium. The greatest number of LRCs was found in fornical epithelium. In addition, we found a number of label-retaining goblet cells. This cell population was shown to incorporate 3H-TdR after a single pulse administration, and mitotic figures were seen in goblet cells after colchicine treatment, indicating that conjunctival goblet cells have proliferative capabilities.

Conclusions. These findings are consistent with earlier in vitro data that the fornical epithelium may be a zone enriched in conjunctival epithelial stem cells. This has important implications in conjunctival epithelial development and is relevant in wound repair. Furthermore, the concept that goblet cells are slow-cycling cells with proliferative capabilities provides new insights into the area of conjunctival homeostasis. Invest Ophthalmol Vis Sci. 1995;36:236-246.

Conjunctival epithelium can be divided into three morphologically distinct zones: bulbar, fornical, and palpebral.1-4 This epithelium forms a physical protective barrier and, through goblet cell secretions, contributes to the formation and maintenance of a "tear film" on the ocular surface. In performing these functions, conjunctival epithelium, like other stratified epithelia, undergoes a series of changes in which epithelial cell loss due to terminal differentiation is balanced by new cell formation. Conjunctival epithelium can also serve as a source of epithelial cells that migrate rapidly and resurface on the corneal stroma after a loss of corneal epithelium due to wounding.5-12 One of the responses to corneal epithelial depletion is increased proliferation of conjunctival epithelial cells.13-15 For example, Thoft and coworkers observed that one day after the removal of corneal epithelia and a portion of bulbar conjunctival epithelia, there was a 10-fold increase in the tritiated thymidine (3H-TdR) labeling index of the remaining conjunctival epithelium.13 This kind of self-renewing epithelium, by definition, must contain stem cells.11 Such cells are thought to play a major role in normal proliferative homeostasis, as well as in a tissue's responsiveness to population depletion, resulting from wounding and...
other extrinsic perturbations. However, despite the obvious functional importance of conjunctival epithelium in ocular physiology, relatively little is known about conjunctival epithelial stem cells.

Our understanding about the location and biologic properties of stem cells has come primarily from investigations on the hematopoietic system, as well as the palmar epithelium, follicular epithelium, corneal–limbal epithelia, and small intestinal epithelium. From these studies, there has emerged an awareness that stem cells in general, and those of epithelia in particular, share certain features. These cells are relatively undifferentiated, are slow cycling but can be preferentially stimulated to proliferate by wounding, and can give rise to transient amplifying cells that have limited proliferative potential. Furthermore, stem cells have great proliferative potential, are usually located in well-protected and well-vascularized areas, and are in contact with a unique stromal environment that provides a "niche" for the maintenance of these cells. Finally, they may be a target for carcinogens and, as such, they play a central role in tumor development.

We have recently shown that under identical culture conditions, keratinocytes of rabbit fornix had a much greater in vitro proliferative potential than bulbar and palpebral keratinocytes. Inasmuch as high proliferative potential is an important characteristic of stem cells, it raises the interesting possibility that fornix might be the preferential site of conjunctival epithelial stem cells. Because stem cells are known to be slow cycling, one way to "tag" these cells involves repeated administration of labeled thymidine (³H-TdR) for a prolonged period of time, followed by a long chase period so that only cells that cycle slowly will retain the isotope (the label-retaining cells, or LRCs). Using this approach, we identified LRCs in mouse conjunctival epithelium, and we report here that the fornix contains many more LRCs than other regions of the conjunctival epithelium. This is consistent with our earlier data, indicating that rabbit fornix keratinocytes have a much greater in vitro proliferative potential than bulbar and palpebral epithelial cells. Interestingly, we found that some of the LRCs had a goblet cell phenotype. On further investigation, using mitotic arrest and thymidine autoradiographic techniques, we observed that goblet cells are capable of proliferating. These results have important implications concerning the homeostasis and regenerative properties of mammalian conjunctival epithelium.

MATERIALS AND METHODS

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the University of Pennsylvania Animal Care and Ethics Committee.

Conjunctival Development

To gain further insight into the relationship between bulbar, fornical, and palpebral conjunctival epithelia with respect to proliferation, we analyzed the morphology and cell kinetics of these three zones during development. Late gestational age (15 to 17 days) SENCAR mice obtained from Harlan Sprague provided newborns used in the studies on conjunctival development. Three mice were killed at 1, 2, 4, 6, 8, 10, 12, and 14 days after birth by cervical dislocation, and eyes and eyelids were surgically removed and processed for histology or autoradiography as described below.

To determine the proliferative rate of the developing conjunctival epithelium, one group of neonatal mice with eyelids fused (1, 2, 4, 6, 8, and 10 days old) received a subcutaneous injection of 5 μCi of methyl-³H-TdR (specific activity, 82.7 Ci/mmole) per gram of body weight 1 hour before sacrifice.

Detection of Slow-Cycling Cells

To detect slow-cycling cells, neonatal SENCAR mice were injected subcutaneously in the back with 5 μCi of methyl-³H-TdR (specific activity, 82.7 Ci/mmole; New England Nuclear, Boston, MA) per gram of body weight twice daily (8:00 AM and 4:00 PM) for the first 7 days of life (5 to 15 μCi per animal per day, depending on the weight of the mouse). After a 4-week chase when mice received only food and water ad libitum, animals were killed and eyes were removed with the eyelids still attached. Specimens were fixed with 10% formalin in phosphate-buffered saline and embedded in JB4 plastic embedding medium, and sections were cut at 3 μm as previously described. Sections were processed for autoradiography (see Autoradiography), and those cells with silver grains retained in their nuclei (LRCs) were detected.

Determination of Proliferative Activity of Adult Conjunctiva

To examine the proliferative rate of the adult conjunctival epithelium, ³H-TdR was used to detect cells in "S" phase. One hour before sampling, 4-week-old female SENCAR mice (Harlan Sprague) received intraperitoneal injections of 40 μCi ³H-TdR (specific activity, 82.7 Ci/mmole; New England Nuclear, Boston, MA) in 0.1 ml of sterile phosphate-buffered saline. Eyes were removed and prepared for histology as described.

We also assessed the proliferative activity of the conjunctival epithelium by determining mitotic activity. Because mitosis is a relatively rare event, we used mitotic arrest techniques to enhance the number of mitotic figures present in tissue sections. Mice were...
treated with intraperitoneal colchicine (10 mg in 0.1 ml phosphate-buffered saline per animal; Sigma, St. Louis, MO) that arrests cells in mitosis, and eyes were removed 8 hours later and prepared for histologic examination as described. In an earlier experiment, mice were sacrificed hourly for 12 hours after an intraperitoneal injection of colchicine, and a mitotic index (number of mitotic figures per 1000 basal cells) was determined for each time point. The maximum number of mitotic figures occurred 8 hours after colchicine injection.

**Autoradiography**

Tissue sections 2-3 μm thick were picked up on glass slides and air dried; the slides were then dipped in a nuclear track emulsion (Ilford, London, UK) and diluted 1:1 with distilled water at 40°C. These coated slides were air dried and exposed for 10 to 14 days at 4°C in light-tight boxes. The autoradiographs were developed in Kodak D-19 developer (Eastman Kodak, Rochester, NY) for 5 minutes at 20°C, rinsed in distilled water, and fixed for 10 minutes. After a 30-minute wash, the slides were lightly stained with hematoxylin and cosin.

A labeling index was determined for the different conjunctival zones by counting at least 1000 nuclei from three different sections for each determination, and the results were expressed as the percentage of labeled nuclei.

**RESULTS**

**Morphology of Murine Conjunctival Epithelium**

**Neonatal.** Although the organization and development of rabbit and rat conjunctival epithelium have been described in detail, relatively little attention has been directed to the murine conjunctival epithelium. At birth, mouse conjunctival epithelium consists of one to two layers of relatively flattened and undifferentiated cells. As development proceeds (2 to 4 days after birth), the bulbar and palpebral basal keratinocytes maintain a flattened appearance, whereas the fornical basal keratinocytes become cuboidal (Fig. 1A). During the next several days, the fornical keratinocytes assume a columnar shape typical of adult tissue. Palpebral keratinocytes become cuboidal, whereas the bulbar conjunctival cells remain relatively flattened. Overall, cell density is greatest in the fornical region. This increase in epithelial cell density in the fornix is paralleled in the underlying stroma, which is also more cellular in the fornix compared with either the bulbar or palpebral regions. In addition, the fornical stroma is the most heavily vascularized (Figs. 1B, 1D). Finally, before eyelid opening, goblet cells were first seen in the fornix approximately 10 days after birth (Fig. 1D).

Goblet cell density remained highest in the fornix throughout subsequent stages of development.

**Adult.** Based on its anatomic location, degree of stratification, and density of goblet cells, adult conjunctival epithelium can be divided into three zones (Fig. 2A): bulbar (Fig. 2D), fornix (Fig. 2E), and palpebral (Fig. 2F). The bulbar zone is covered by a stratified squamous epithelium and is contiguous with the limbal zone (Fig. 2C) of the cornea (Fig. 2B). The bulbar epithelium consists of a single basal cell layer, two to three cuboidal wing cell layers, and one slightly flattened superficial cell layer. Bulbar epithelium contains goblet cells and is, therefore, easily distinguished from the adjacent limbal epithelium. The fornical epithelium (Fig. 2E) contains one to two cell layers and appears much less stratified than either the bulbar or the palpebral region. Two types of cells are present within the fornical zone: small, round epithelial cells with a relatively large nuclear:cytoplasmic ratio, and numerous goblet cells. Goblet cells are columnar and have small, foot-like structures at the base in contact with the basement membrane. The nucleus is most often positioned beneath a prominent mass of mucous droplets, which bulges the cell into its characteristic "wine goblet" appearance. The apical portion of the goblet cell is often exposed to the surface. Goblet cells usually appear in clusters, each of which can contain as many as five goblet cells. The palpebral epithelium (Fig. 2F) is the most highly stratified of the three zones, consists of four to seven cell layers, and is contiguous with the epidermis of the eyelid. In contrast to the fornical epithelium, palpebral goblet cells appear singly and increase in number toward the fornix.

**Proliferative Rates of the Conjunctival Epithelium**

**Neonatal.** To study the proliferative status of the developing conjunctival epithelium, we used tritiated thymidine-labeling to detect cells in the "S" phase of the cell cycle. When [3H]-Tdr was administered as a single pulse immediately after birth, labeled nuclei were found to distribute randomly in the basal cell layer of the neonatal conjunctival epithelium. During the ensuing developmental period (2 to 10 days after birth), labeled nuclei were preferentially observed in the fornical zone and in the eyelid region (Figs. 1A, 1C).

**Adult.** Labeled nuclei were observed in the basal cell layer of the three conjunctival zones, as well as in limbal and corneal epithelia (Figs. 2B to 2F). As in previous studies, the labeling index for limbal epithelium (2.58%) was equivalent to, if not slightly lower than, that of the corneal epithelium (2.77%; Table 1). Bulbar and palpebral epithelia had similar labeling indices (1.02% and 0.90%, respectively), though they were somewhat lower than limbal and corneal epithelia. Interestingly, fornical epithelium had a lower proliferative rate (0.23%) than the other conjunctival zones.
Enrichment of Conjunctival Epithelial Stem Cells in Fornix

Figure 1. Morphology and cell kinetics of developing SENCAR mouse ocular epithelium. (A) Autoradiogram showing the distribution of 3H-thymidine-labeled nuclei in a transverse tissue section of ocular epithelium from a 4-day-old SENCAR mouse, showing the corneal (C), limbal (L), bulbar (B), fornical (F), and palpebral (P) zones. (B) High magnification histologic section from a 4-day-old SENCAR mouse showing a portion of the fornical (F) and limbal (L) epithelia. Fornical epithelium consists of a single layer of round to columnar cells resting on a highly vascularized stroma (arrows). Limbal epithelium is more stratified, consisting of one- to two-cell flattened layers. Similar to the fornical epithelium, limbal epithelium rests on a highly vascularized stroma (arrows). (C) High magnification autoradiogram from a 4-day-old SENCAR mouse showing a greater distribution of 3H-thymidine-labeled nuclei in the fornical epithelium (F, arrows) compared to bulbar epithelium (B, single arrow). Fornical epithelium consists of a single layer of round to oval keratinocytes, whereas keratinocytes of bulbar epithelium are primarily flattened. (D) High magnification histologic section of a portion of the fornical epithelium from a 10-day-old SENCAR mouse. Single and paired goblet cells (G, arrows) are first seen in fornical epithelium at this time. Note prominent vascular profiles in fornical stroma (large arrows).

Label-Retaining Cells Are Preferentially Located in Fornix

Pulse-labeling techniques detect those cells that are regularly cycling and yield information on the proliferative rate of a tissue. However, this methodology provides little insight into the slow-cycling cells. To identify the slow-cycling cells, we provided a continuous supply of 3H-TdR and then detected the LRCs.15,25 As in previous studies, no LRCs were observed in corneal epithelium (Fig. 3A, Table 1), whereas a subpopulation of LRCs was routinely detected in the basal layer of limbal epithelium (Fig. 3B, Table 1). In conjunctival epithelium, LRCs were observed in the basal layers and the bulbar (Fig. 3C), fornical (Fig. 3D), and palpebral epithelium (Fig. 3E). However, LRCs were not evenly distributed within the conjunctival epithelium (Table 1). The greatest density of LRCs was found in the fornical zone (14% of epithelial cells). Within bulbar epithelium, 5% of epithelial cells were detected as LRCs, and slightly fewer than 1% of palpebral keratinocytes were LRCs.

Goblet Cells Have Proliferative Capabilities

In previous investigations, we detected relatively undifferentiated LRCs in the basal layer of murine epithelium, hair follicle, and limbal epithelium. Consistent results were obtained in the present study with
FIGURE 2. Morphology and cell kinetics of adult SENCAR ocular epithelium. (A) Histologic section of ocular epithelium from adult SENCAR mouse showing the relationship of the corneal (C) and limbal (L) epithelium with bulbar (B), fornical (F), and palpebral (P) conjunctival epithelia. Scale bar = 50 μm. (B-F) Autoradiograms showing distribution of \(^{3}H\)-thymidine-labeled nuclei in corneal (B, arrowheads), limbal (C, arrowheads), bulbar (D, arrowheads), and palpebral (F, arrowheads) epithelia after a pulse administration of nucleotide. \(^{3}H\)-thymidine-labeled nuclei were rarely observed in fornical (E) epithelium. G = goblet cells. Scale bar = 10 μm.

respect to the morphology of LRCs from limbal, palpebral, and bulbar epithelia (Fig. 3D). Unexpectedly, however, we found a number of label-retaining goblet cells within the fornical epithelium. To see whether these label-retaining goblet cells were capable of replicating, we arrested mitosis during an 8-hour period using colchicine. Indeed, some mitotically arrested goblet cells were observed, proving that these cells were capable of proliferating (Figs. 4A to 4D). In addition, occasional goblet cell nuclei were found to be labeled after a single pulse administration of \(^{3}H\)-Tdr (Figs. 4E to 4F).

DISCUSSION

Fornix Is the Stem Cell-Rich Zone of the Conjunctival Epithelium

In an in vitro investigation of the growth characteristics of rabbit bulbar, fornical, and palpebral keratino-
Table 1. Comparison of the Distribution of Pulse-Labeled Keratinocytes (LI) With Label-Retaining Keratinocytes (LRC) in Various Ocular Zones

<table>
<thead>
<tr>
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<th>LI % (n = 6)</th>
<th>LRC % (n = 10)</th>
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<tbody>
<tr>
<td>Corneal</td>
<td>2.77 ± 1.2</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Limbal</td>
<td>2.58 ± 1.2</td>
<td>5.38 ± 1.76</td>
</tr>
<tr>
<td>Bulbar</td>
<td>1.02 ± 0.32</td>
<td>4.96 ± 1.76</td>
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<tr>
<td>Fornical</td>
<td>0.23 ± 0.2</td>
<td>13.63 ± 3.0</td>
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<tr>
<td>Palpebral</td>
<td>0.90 ± 0.62</td>
<td>0.90 ± 0.45</td>
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All values are means ± SD.
LI = Pulse-labeled keratinocytes; LRC = label-retaining keratinocytes.

Because stem cells are slow cycling, they rarely incorporate tritiated thymidine after single pulse administration. This explains why, as we have shown earlier, stem cell-rich regions (e.g., limbal epithelium, bulge region of the hair follicle, tips of deep rete ridges of palmar epithelium) usually do not incorporate large amounts of pulse-administered tritiated thymidine.15,17,21,25 In the adult mouse, the fornical epithelium had a lower labeling index than either palpebral or bulbar epithelia. This labeling pattern is

Figure 3. Detection of label-retaining cells in SENCAR mouse ocular epithelium. Label-retaining cells (LRC) are rarely, if ever, detected in corneal epithelium (A) but are routinely present in limbal epithelium (B). Occasional LRCs are detected in bulbar (C) and palpebral (E) epithelia, whereas numerous LRCs are observed in fornical epithelium both in basal cells (arrowheads) and goblet cells (D, LRG). Label-retaining fibroblasts (LRF) are present in the stroma of all zones. G = goblet cell. Scale bar = 10 μm.
entirely consistent with previously described findings for other epithelial stem cell systems.

Fornical Epithelium Is the Major Proliferative Zone During Conjunctival Growth and Development

After pulse administration of tritiated thymidine, numerous labeled nuclei were routinely detected in the basal cells of fornical epithelium during the first 10 days of life. If the fornix were enriched in conjunctival epithelial stem cells, proliferation of these cells would be expected during development, because theoretically this would result in the generation of transient amplifying cells needed for continued conjunctival growth and expansion. Proliferation of the normally quiescent fornical epithelial cells during a specific stage of conjunctival growth and development is analogous to what occurs in the bulge region of mouse hair follicle (site of follicular epithelial stem cells) during the early growing phase of the hair.

Clustering of Conjunctival Stem Cells Is Consistent With Stem Cell Distribution in Other Stratified Squamous Epithelia

That conjunctival epithelial stem cells are not randomly distributed throughout the tissue, but show a preferential fornical location, is consistent with the distribution patterns of putative stem cells in other stratified squamous epithelia. For example, corneal epithelial stem cells are clustered in the limbal region; interscalene interfollicular stem cells have been postulated to reside at the bottom of the deep rete ridges; stem cells of follicular structures, such as hair, eyelash, and vibrissae, have been demonstrated recently to concentrate in the upper portion of the outer root sheath known as the bulge; in murine epidermis, stem cells are thought to reside in the center of a column of keratinocytes known as the epidermal proliferative unit (EPU); and pluripotent stem cells, which give rise to epithelial goblet cells, are thought to reside at the bottom of the small intestinal crypts.

The apparent nonrandom and clustered location of epithelial stem cells supports the concept of a stem cell "niche"—perhaps maintained by the underlying stroma—which provides a microenvironment for the maintenance of the "stemness." Careful analysis of the microanatomic compartments of stem cell systems reveals many common features; With respect to the external environment, epithelial stem cells are positioned deep in the tissue, presumably to provide maximal protection from environmental insults; the stroma underlying these regions is organized into a network of randomly arranged collagen and elastin fibers; a high degree of vascularity and a high density of fibroblasts and other mesenchymal cells (e.g., mast cells) are characteristically interspersed within the collagen–elastin network. Consistent with these attributes, fornical epithelium is located further from the external environment than either the bulbar or palpebral epithelia. In addition, fornical stroma is the most heavily vascularized and most cellular of the three conjunctival zones. Thus, the preferential distribution of a subpopulation of cells with stem cell characteristics in the fornix is consistent with the anatomic distribution of stem cells in other epithelia.

The Fornix Plays a Major Role in Conjunctival Development

Fornical epithelial proliferation during the early stages of neonatal life is indicative that this region plays an important role during conjunctival epithelial maturation. Consistent with this idea is the observation that goblet cells are first seen in the fornical region of the 8-day-old mouse; with time, they appear in bulbar and palpebral zones. In a recent study on the development of human conjunctival goblet cells, alcian blue and PAS-positive staining cells (histochemical markers for goblet cells) first appeared in the fornix region at 8 weeks of gestational age. In 9-week specimens, goblet cells were most numerous in the fornix. Some goblet cells were also present in the palpebral conjunctiva but were rarely seen in the bulbar region. Because one of the hallmarks of conjunctival maturation is the presence of goblet cells, these findings are further evidence of the central role that the fornix plays in conjunctival epithelial development.

Goblet Cells Have Proliferative Capabilities

Conjunctival epithelium constantly renews itself and has a remarkable regenerative capacity, as evidenced...
by the conjunctiva’s ability to recapithelialize a denuded cornea and limbus. Furthermore, conjunctival epithelial proliferation has been shown to increase in response to wounds that involve only the central corneal epithelium. Traditionally, the (non-goblet) basal epithelial cell has been considered the major proliferative cell responsible for both conjunctival epithelial maintenance and corneal epithelial replacement. However, our finding that a subpopulation of morphologically mature goblet cells can be labeled after exposure to continuous and pulse administrations of tritiated thymidine, as well as the detection of mitotically arrested goblet cells, are indicative that this cell type also has a significant proliferative capability. The ability of goblet cells to proliferate has been observed in several other epithelial tissues. For example, 0.8% of the “pit” cells (surface mucous cells with a goblet cell phenotype) located in the glandular epithelium of mouse stomach can be labeled after a single exposure to TdR. A similar situation exists in nonstimulated mammalian airway epithelium where basal, Clara, serous, and mucous (goblet) cells have all been observed to divide. In hamster tracheal epithelium, small numbers of basal (0.3%) and mucous (0.1%) cells were observed to be either resting or proliferating more slowly than other labeled cells. However, after perturbation (e.g., cigarette smoke or mechanical trauma), an increased percentage of tracheal epithelial goblet cells was observed to proliferate, suggesting that goblet cells could play a major role in tracheal epithelial repair.

That conjunctival goblet cells can actually divide, as our data clearly show, raises the question as to where these cells are positioned in the scheme of “stem cell → transient amplifying cell → postmitotic cell.” Their ability to proliferate, at least in mice, does not support the concept that goblet cells are terminally differentiated. What remains uncertain, though, is whether goblet cells are transient amplifying cells or stem cells. Evidence favoring a stem cell role comes from our finding that some goblet cells can be identified as LRCs and, hence are, slow cycling, an important criterion for stem cells. An alternative interpretation of the label-retaining data would be that a goblet cell precursor incorporated TdR before a final round of division, entered a postmitotic state (still having a labeled nucleus), and subsequently differentiated into a goblet cell. In this regard, our observation that at least some of the label-retaining goblet cells could undergo mitosis (labeled mitotic figures after long-term labeling; Fig. 4A) is of major importance, because this proves that these label-retaining goblet cells still have proliferative capabilities. Additional indirect evidence for the slow-cycling nature of goblet cells comes from pulse labeling data, which revealed that only a small number of goblet cells incorporate TdR after a single exposure. As previously mentioned, regions enriched with slow-cycling cells (e.g., limbal epithelium, bulge region of the hair follicle, tips of deep rete ridges of palmar epithelium) usually do not incorporate large amounts of pulse-administered tritiated thymidine. Thus, the combination of slow-cycling kinetics (LRCs), proliferative capability, and infrequent thymidine incorporation after pulse labeling protocols are features consistent with stem cells. In normal situations, goblet cells are relatively quiescent, although it is well established that they can rapidly proliferate in response to wounding. Indeed, the suggestion has been made that numerous goblet cells play the major regenerative role in lung epithelium. Thus, the ability of conjunctival goblet cells to enter the proliferative pool in response to tissue depletion, a typical feature of stem cells, is further evidence of their stem cell nature.

The one inconsistent feature of goblet cells with respect to stem cells concerns their state of differentiation. In all other systems studied so far, the slow-cycling cells (stem cells) are relatively undifferentiated or primitive (e.g., limbal basal cells, nonkeratinized keratinocytes). However, goblet cells have the phenotype of a highly differentiated or specialized cell. A possible explanation for this paradoxical observation centers around the secretory physiology of these cells. Under normal situations, goblet cells function to synthesize and secrete mucin, which serves as a protective barrier for the underlying epithelium. As has been shown in intestinal, tracheal, and lung epithelia, as well as in conjunctival epithelium, secretion occurs either by slow, periodic exocytosis of the contents of single mucin granules (baseline secretion) or through the fusion of many adjacent granules followed by expulsion of the entire stored mass of granules at one time (accelerated or stimulated secretion). After accelerated secretion, the goblet cell resynthesizes new granules and continues its secretory function. Because these secretory events are independent of proliferation and can apparently occur multiple times during the life of a goblet cell, it explains how a goblet cell can be proliferatively slow cycling and detected as an LRC.

**CONCLUSIONS**

We have provided evidence to support our earlier suggestion that the fornical epithelium may be a zone enriched in conjunctival epithelial stem cells. This notion has important implications in conjunctival epithelial development and can be extremely relevant in wound repair. The finding that goblet cells are relatively quiescent, slow-cycling cells with certain stem cell attributes provides new insights into the area of conjunctival homeostasis. Although these studies es-
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establish the proliferative nature of the various conjunctival zones and cell types within these zones, they do not provide information on the lineage of the heterogeneous proliferative cell types. Are there two separate stem cell populations that give rise to goblet cells and stratified squamous epithelial cells, or is there a single pluripotent conjunctival epithelial stem cell capable of giving rise to both cell types? Ongoing studies comparing the growth and differentiation of isolated goblet and epithelial cell fractions should provide insight into some of these issues.

Key Words
conjunctival epithelium, stem cells, development, goblet cells, proliferation

References
1. Kessing SV. Mucous gland system of the conjunctiva. AGPA Ophthalmal Surph. 1968;95.
31. Lavker RM, Margolis-Fryer E, Ostad M, Cotsarelis G, Sun T-T. Cells in the bulge region of mouse hair follicle undergo transient proliferation during onset of...