A Mouse Model of Limbal Stem Cell Deficiency Induced by Topical Medication With the Preservative Benzalkonium Chloride

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Purpose. To develop a mouse model of limbal stem cell deficiency (LSCD) by topical administration of benzalkonium chloride (BAC).

Methods. BAC solutions (0%-0.5%) were applied to the mouse ocular surface for 4 weeks. Corneal neovascularization, inflammation, and epithelial status were observed under slit-lamp microscope. The eyeball and ocular surface tissues were collected at 4 and 12 weeks and labeled with a series of antibodies. Limbal structure was evaluated by light and transmission electron microscopy (TEM). Corneal impression cytology was performed at 12 weeks, and specimens were labeled with periodic acid Schiff (PAS) reagents.

Results. BAC (0.5%) four times per day for 28 days successfully induced the typical manifestations of LSCD, including corneal neovascularization, severe inflammation in the stroma, and diffuse epithelial defect ($P < 0.001$). Conjunctival epithelium markers K19 and K13 were positive on the corneal surface. Expression of the putative limbal stem cell markers Pax6 and ABCG2 was abolished in the limbal epithelium. $β$-catenin was negative in the basal layer. TEM revealed the irregular basement membrane and the loss of stem cell—specific ultrastructure in the limbal basal epithelium. In the 0.5% BAC group, goblet cells could not be observed on day 28 but emerged after the cessation of BAC, and remained over the cornea after 8 weeks. K13-positive cells were still present over the cornea with the loss of K12.

Conclusions. Topical administration of BAC at high concentration and frequency in mouse induces ocular surface changes resembling those of LSCD in humans, representing a novel model of LSCD.

Keywords: limbal stem cell deficiency, benzalkonium chloride, inflammation, squamous metaplasia, goblet cell
endothelium and innervations, and the conjunctiva, as well as other ocular tissues. In many patients, preservatives can be the major cause of or aggravating factor for ocular surface disorders, especially in those with dry eye and glaucoma, for example. Pauly et al. systematically investigated the dose-dependent toxicity of BAC to the ocular surface in Lewis rats after long-term administration, with the observation of corneal neovascularization, inflammation, and epithelium erosion. However, whether BAC was related to corneal conjunctivalization was not mentioned, and the status of limbal epithelium remained unclear. This present study was therefore conducted to evaluate the toxicity of BAC on the ocular surface of the normal BALB/c mouse and to develop a LSCD model with topical medication of BAC, aiming to provide a better platform for future investigations.

**Materials and Methods**

**Animals and Administration of Benzalkonium Chloride**

Forty male BALB/c mice (18–20 g, purchased from Shanghai Laboratory Animal Center [SLAC], Shanghai, China) were used for this study, all of which were free of clinically observable ocular surface diseases. The mice were maintained in a standard environment throughout the study as follows: room temperature 25°C ± 1°C, relative humidity 60% ± 10%, and alternating 12-hour light–dark cycles (8 AM–8 PM). All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The mice were randomly assigned to four groups of 10 mice each. For 28 days, each group received topical administration of PBS as the control and BAC solution (0.1%, 0.25%, 0.5%, respectively) at the right eye four times daily (9 AM, 1 PM, 5 PM, 9 PM). The BAC was dissolved in PBS.

**Experimental Procedure**

At each time point (on days 0, 1, 3, 7, 11, 15, 20, and 28), clinical ocular surface alterations were evaluated. Criteria for the diagnosis of LSCD included persistent corneal new vessels, inflammation in the stroma, and corneal epithelial damage, according to the methods described below.

The best concentration of BAC for the induction of LSCD was determined based on the data (see Results). Six global...
specimens in each group were dissected on day 28 for histological analysis by light microscopy, immunostaining, and transmission electron microscopy (TEM) as described below. After 28 days, the other four mice in each group stopped receiving BAC instillation and were kept alive for corneal impression cytology and periodic acid Schiff (PAS) staining. After 8-week cessation of BAC treatment, the four mice were evaluated by slit lamp and killed for PAS and immunostaining.

Measurement of New Vessels and Inflammation

Length of new vessels (NV) was examined and scored by a single masked observer, who was an ophthalmologist, under slit lamp (SLM-3; Kanghua Science & Technology Co., Ltd., Chongqing, China) at each time point as previously described. The cornea was divided into four quadrants, which were scored individually. The measurement of vessels was quantified by the ratio of the largest length of vessels to the corneal radius, briefly as follows: no vessels grown into clear cornea, 0; one-quarter or less but not zero, 1; between one-quarter and one-half, 2; between one-half and three-quarters, 3; and more than three-quarters, 4. The final scores for NV were calculated by summing the scores of the four quadrants (total, 16 points).

The severity of inflammatory response was analyzed under slit lamp as previously described. The inflammatory index was analyzed based on the following parameters: ciliary hyperemia (absent, 0; present but less than 1 mm, 1; present between 1 and 2 mm, 2; present and more than 2 mm, 3); central corneal edema (absent, 0; present with visible iris details, 1; present without visible iris details, 2; present

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**Figure 2.** Representative images showing the inflammatory responses with BAC treatment on day 28. In BAC-treated eyes (B, D), fewer layers of corneal epithelium were observed in the central cornea but more layers of epithelium in the limbal area when compared with the control (A, C). Infiltration of inflammatory cells, as well as new vessels, was intensive in the stroma of BAC-treated eyes. CD4-positive cells also emerged in the stroma of both the limbal and central cornea in 0.5% BAC-treated eyes (F, H). No CD4-positive cells were observed in the control eyes (E, G). Black triangles, cavity of new vessels; black arrows, CD4-positive cells. Scale bar: 100 μm.
FIGURE 3. Representative images showing corneal conjunctivalization and partial loss of corneal phenotype on day 28. No differences in K19 or K13 expression in the conjunctiva between control (A, G) and BAC-treated eyes (D, J). The expression of K19 and K13 was weaker in the basal layers of the ocular surface of both groups. K19 and K13 staining was weak in the limbal epithelium of the control eyes (B, H), but much stronger with 0.5% BAC treatment (E, K). Invasion of K19- and K13-positive epithelium could be observed in the central cornea of BAC-treated eyes (F, L) but not in the control eyes (C, I). White arrows indicate the terminal ends of K19 (F) or K13 (L) invasion in the cornea. K10-positive cells were detected.
without visible pupil, 3), and peripheral corneal edema (absent, 0; present with visible iris details, 1; present without visible iris details, 2; present with no visible iris, 3). The final inflammatory index result was obtained by summing the scores of the different parameters divided by a factor of 9.

**Evaluation of Fluorescein Staining**

One microliter 1% liquid fluorescein sodium was dropped into the conjunctival sac. Ninety seconds later, corneal epithelial damage was graded with a cobalt blue filter under a slit-lamp microscope. The cornea was divided into four quadrants, which were scored respectively as previously described with essential modification, briefly as follows: absent, 0; slightly punctate staining less than 30 spots, 1; punctate staining more than 30 spots, but not diffuse, 2; severe diffuse staining but no positive plaque, 3; positive fluorescein plaque, 4. The scores of each quadrant were added to arrive at a final grade (total, 16 points).

**Corneal Impression Cytology**

Corneal impression cytology (IC) was used to dynamically monitor the emergence of goblet cells over the cornea. Corneal IC specimens were collected every 2 days in the first week and every 7 days in the following period until day 56 (8 weeks) after the cessation of BAC instillation. Topical anesthesia by 0.4% oxybuprocaine was given to help immobilize the mice. One 3-mm circular disc of cellulose acetate membrane filter paper (Advantec Toyo Roshi Kaisha, Tokyo, Japan) was placed on the central corneal surface. After the filter paper was pressed for 6 seconds with constant pressure, it was gently lifted and fixed with 4% paraformaldehyde. The specimens were stained with PAS reagents and hematoxylin.

**Immunostaining**

On day 28, the global tissues were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles, Inc., Elkhart, IN) and frozen at −80°C. Two global tissues of the mice after cessation of BAC treatment for 8 weeks were also embedded in OCT. OCT-embedded frozen sections (6 μm thick) were cut with a cryotome (CM 1850UV; Leica Microsystems AG, Wetzlar, Germany) and stored at −80°C. Global sections were fixed in acetone at 4°C for 10 minutes and permeated with 0.2% Triton X-100 in PBS for 20 minutes.

For immunofluorescent labeling, after preincubation with 2% BSA in PBS for 60 minutes to block the nonspecific sites, sections were incubated at 4°C overnight with antibodies of K19, MUC5AC, β-catenin (1:50; Santa Cruz Biotechnology, Santa Cruz, CA), and K10 (1:300; Abcam, Cambridge, UK). After further incubation in Alexa Fluor 488- or Alexa Fluor 555-conjugated secondary antibodies (1:1000; Invitrogen, Carlsbad, CA), sections were rinsed, counterstained with 4',6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI), mounted, and photographed using a confocal laser.
scanning microscope (Fluoview FV1000; Olympus, Tokyo, Japan) mentioned above.

For immunohistochemical staining, the activity of endogenous peroxidases was quenched with 0.6% hydrogen peroxide for 30 minutes. After incubation with 2% BSA, the antibodies of P63 (1:250; Abcam) and ABCG2 (1:25; Abcam) were applied and incubated at 4°C for 14 to 18 hours. After rinsing with PBS, the sections were further incubated with biotinylated anti-rabbit IgG (1:50) using Vectastain Elite ABC kits (Vector Laboratories, Burlingame, CA) according to the manufacturer’s protocol. The reaction product was then developed with diaminobenzidine (DAB). Sections were mounted and examined with a light microscope (Eclipse 50i; Nikon, Tokyo, Japan). P63 and ABCG2 were previously reported to be predominantly expressed in the nucleus and cytoplasm, respectively, of normal progenitor cells. As there is only one layer of epithelial cells in the limbal zone of normal mice, counterstaining with hematoxylin was not performed to avoid possible interference during observation.

**Figure 5.** Representative images showing the loss of putative stem cell markers with BAC treatment on day 28. In the control eyes, P63 was strongly positive in the nucleus of limbal epithelium (A) and basal epithelium of the central cornea (B), while ABCG2 was expressed in the cytoplasm of limbal epithelium (E) but weakly in the central cornea (F). With 0.5% BAC treatment for 28 days, both P63 (C, D) and ABCG2 (E–H) were found to be absolutely negative in the limbal and central cornea. The dashed line (F–H) indicates the assumed basement membrane of epithelium. Scale bar: 100 μm (A–H).

**TEM and Light Microscopy**

On day 28, two eyes randomly selected from each group were enucleated and fixed for 2 hours in a mixture (pH 7.4) of 2.5% glutaraldehyde and 4% paraformaldehyde in PBS. The limbal tissues were cut to a size of 4 mm in length and 2 mm in width
without touching the epithelium and further embedded, sliced, and stained. The ultrastructure of limbal epithelium was examined and photographed with TEM (JEM2100HC; JEOL, Tokyo, Japan).

Hematoxylin and eosin (HE) staining was performed in cryosections fixed in acetone. Two specimens from mice after cessation of BAC for 8 weeks were embedded in paraffin, cross sectioned, and stained with PAS reagents and hematoxylin. These sections were examined using the light microscope (Nikon).

**Statistical Analysis**

Analysis of the significance of differences between groups was performed using one-way ANOVA (SPSS 16.0 statistical software; SPSS, Chicago, IL). \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Macroscopic and Slit-Lamp Examinations**

The effects of BAC of different concentrations (0%, 0.1%, 0.25%, and 0.5%) were evaluated by neovascularization scoring, inflammatory index, and fluorescein sodium staining in the cornea. Based on the clinical diagnostic criteria, topical administration of 0.5% BAC four times daily for 28 days was considered the optimal procedure to induce putative LSCD in BALB/c mice \( (P < 0.001, \text{Fig. 1}) \). Corneal new vessels induced by BAC at 0.5% concentration emerged progressively after the treatment (Fig. 1A), and corneal inflammatory index (Fig. 1D) and fluorescein staining scores (Fig. 1G) indicated rapid and persistent inflammatory response and epithelium damage. Lower concentrations of BAC (0.1% and 0.25%) caused mild corneal new vessels, moderate inflammation, and epithelium...
damage. Representative images of the eyes receiving PBS and 0.5% BAC are also shown in Figure 1.

**BAC-Induced Inflammation in the Cornea**

Histological analysis of sections revealed that the 0.5% BAC-treated group had much more inflammatory cell infiltration in the cornea and limbus than the PBS-treated group, which was consistent with the results of corneal inflammatory index. Stratified and irregular limbal epithelium was observed in the BAC-treated eyes (Figs. 2A–D). Interestingly, CD4-positive cells were intensively marked in the stroma of the limbal and central cornea of 0.5% BAC-treated eyes on day 28 while negatively labeled in the PBS-treated eyes, validating the immune response with BAC administration (Figs. 2E–H).

**Corneal Conjunctivalization**

Fluorescent staining was performed to reveal alteration of conjunctival epithelium markers K19 (Figs. 3A–F) and K13 (Figs. 3G–L) on the ocular surface. On day 28, no differences in K19 or K13 expression in the conjunctiva were observed between the control and the BAC-treated eyes. The K19 and K13 staining in the limbal epithelium was weak in the control but increased after 0.5% BAC treatment. The invasion of K19-positive and K13-positive epithelium could be observed in the...
central cornea of BAC-treated eyes but not in the control eyes. In the 0.5% BAC group, K12 expression was lost in the peripheral cornea but still positive around the central area (Figs. 3S, 3T). These results revealed the presence of conjunctival phenotype and the loss of corneal phenotype over the corneal surface after 4 weeks of treatment with 0.5% BAC.

Topical administration of 0.5% BAC for 28 days also induced apparent cytoplasmic K10 expression in the superficial layer of corneal, limbal, and conjunctival epithelium, indicating that nonkeratinized epithelium of the ocular surface was partially replaced by keratinized squamous epithelium (Figs. 3M–R). However, MUC5AC-positive goblet cells were not detected accordingly on the corneal surface of the 0.5% BAC-treated eyes on day 28. The presence of goblet cells was confined to the conjunctival fornix of the control eyes, but almost undetectable in the BAC-treated eyes (Fig. 4).

Loss of Limbal Stem Cell Markers by BAC

The expression of putative LSC markers, P63 and ABCG2, was analyzed by immunohistochemical staining (Fig. 5). In the absence of BAC treatment, P63 was strongly positive in the nucleus of limbal epithelium and basal epithelium of central cornea; ABCG2 was positively expressed in the cytoplasm of limbal epithelium and negatively in the central cornea. With the 0.5% BAC treatment for 28 days, both P63 and ABCG2 were found to be absolutely negative in the limbal and central cornea.

A LSC-associated marker, β-catenin, was also applied for labeling (Fig. 6). In the control group, β-catenin was positive in all layers of limbal and central epithelium. In contrast, its expression was lost in the limbal basal epithelium after 0.5% BAC treatment, but still positive in the superficial layer around the limbus. In central cornea, β-catenin was apparently weaker and even partly abolished in the basal and suprabasal epithelium after BAC treatment. Interestingly, 8 weeks after cessation of BAC, β-catenin expression was found in all layers of limbal and central epithelium.

Ultrastructural Analysis

In normal mice, there was only one layer of epithelium at the limbus. A group of small, round-shaped cells could be observed in the limbal basal epithelium. These putative stem cells resided on a regular and intact basement membrane without cytoplasmic processes, and presented with rich heterochromatin and a high nuclear to cytoplasmic ratio, while the nucleolus might be absent or not (Fig. 7A). In contrast, a cluster of disordered, stratified, and larger epithelial cells was observed in the limbal basal epithelium of the 0.5% BAC-treated eyes, without obvious heterochromatin or nucleolus of high density, resting on an irregular and wrinkled basement membrane (Fig. 7B). The altered morphology of limbal epithelial cells suggested that the characteristics of LSCs, such as high proliferative potential, slow cell cycle, and low level of differentiation, were probably abolished by long-term BAC treatment.
Recovery of Goblet Cells and Persistent Corneal Conjunctivalization After Cessation of BAC

After BAC treatment for 28 days, four mice in each group stopped receiving BAC instillation and were kept alive. Corneal impression cytology specimens were collected every 2 days in the first week and every 7 days in the following period, and the PAS assays were performed. Goblet cells were first observed in the specimens collected on day 4 after the cessation of BAC (data not shown). Goblet cells consistently existed on the corneal surface even in the specimens collected 8 weeks after the cessation of BAC (Fig. 8F). PAS staining for the paraffin sections also showed the recovery of goblet cells in the conjunctival fornix and corneal surface after 8 weeks of cessation of BAC (Figs. 8G, 8H).

After 8 weeks of cessation of BAC, K13 was uniformly expressed in the corneal and conjunctival epithelium. No K12 expression could be observed on the corneal surface. K10-positive cells could still be observed in the conjunctiva and the cornea. These data indicated that the corneal conjunctivalization and the loss of the corneal phenotype were persistent at least within 8 weeks after the cessation of BAC (Fig. 9).

Discussion

Developing animal models that simulate LSCD would be important for the clarification of mechanisms and evaluations of therapeutic treatments. In this study, we introduced a mouse model of LSCD induced by topical administration of a commonly used preservative, BAC, with high frequency and concentration. The observations by slit lamp revealed that the manifestations of ocular disorders induced by topical treatment with 0.5% BAC were consistent in principle with LSCD in humans, notably including persistent corneal new vessels, chronic inflammation in the stroma, epithelial defects, and corneal conjunctivalization.

For decades, detection of goblet cells on the corneal surface has been considered the gold standard for the diagnosis of LSCD. However, the pathological condition of the corneal surface in patients with LSCD is always complicated, especially in those who suffer from severe dry eye or ocular surface inflammation, such that the proliferation and differentiation of vulnerable goblet cells is overwhelmingly interrupted. The number of goblet cells was previously reported to be apparently reduced or even abolished in animals and patients with dry eye. Impression cytology can not always remove all layers of epithelial cells, thus leading to difficulties in assessing LSCD, especially in early stages. Immunolabeling of histological sections was therefore performed to detect clues on day 28. Cumulative evidence indicated that the identification of conjunctiva-specific K19- or K13-positive epithelial cells on the corneal surface would be a simple and practical method to identify LSCD. As the invasion of conjunctiva remained constant under various ocular surface stresses. Previous studies revealed that K19-positive cells were present on the corneal surface in a higher proportion (82%) than goblet cells (59%) in patients with clinical definite LSCD, and that K13 might be a specific marker for corneal epithelial stem cells.

Our results supported these studies with the observation of K19- and K13-positive epithelial invasion into the corneal surface after BAC treatment in BALB/c mice on day 28, in the absence of MUC5AC-positive goblet cells. Based on these issues, we considered that although the presence of goblet cells is needed to confirm the diagnosis of LSCD, their absence does not exclude the diagnosis. The depletion of goblet cells was not permanent and could represent a temporary stage before the alleviation of consistent BAC insults. This is supported by our data showing that the goblet cells would recover over the corneal surface after the cessation of BAC treatment (Fig. 8), with the persistent presence of conjunctival K13 (Fig. 9B).

Although Majo et al. reported that the limbus was not the only niche for corneal epithelial stem cells and that the murine corneal epithelium contained potential stem-cell-like cells, our findings supported the pre-existing data indicating that the corneal stem cells of the mouse reside in the limbal region, with high expression of putative SC markers such as P63 and ABCG2. As a P53 homologue, P63 is known to be essential for epithelial development and stratification, and is consistently expressed in the corneal basal epithelium with maximal levels at the limbus in the mouse. ABCG2, a member of the adenosine triphosphate–binding cassette (ABC) superfamily, was correlated with the proliferative capacity of SCs and appears to be the most reliable marker for the identification of corneal epithelial SCs. The BAC-induced loss of P63 and ABCG2 might indicate insufficiency for the proliferation and differentiation of the LSCs, thus leading to poor re-epithelialization. β-catenin is not a specific marker for putative stem cells, as there were no significant differences of expression density between the cornea and limbus. However, β-catenin is a pivotal component of the cadherin cell adhesion complex and an essential molecule in the Wnt signaling pathway, a key regulator of epithelial proliferation and differentiation. The presence of Wnt signals allows LSCs to maintain their capacity to undergo proliferation upon wounding. Based on our findings, downregulation of β-catenin in the basal layer of cornea and limbus could be considered a sign of impairment of the capacity of epithelial stem cells by BAC. Interestingly, β-catenin was abundantly expressed again in all layers of epithelium over the limbal and central cornea at 8 weeks after the cessation of BAC; but this result did not imply the recovery of LSC function, since the limbal epithelium had converted to conjunctiva.

The biological characteristics of BAC are mainly based on its interaction with lipids, proteins (including G proteins) in the biological membranes, and even genes. BAC has a wide variety of cellular toxicity to the ocular tissues. In addition, BAC is able to cause evaporation of the aqueous tear film as a detergent for the lipid layer, thus leading to a dry eye condition. Preservatives could also induce apoptosis at low concentrations and necrosis at high concentrations in cell cultures and animals, as well as in humans. These alterations may stimulate production of a series of biological mediators in the ocular surface and lead to subsequent proinflammatory and inflammatory response and the creation of a vicious circle. Our results indicated a BAC-induced severe inflammatory condition in the ocular surface, including the upregulation of inflammatory factors such as TNF-α (data not shown) and the infiltration of CD4-positive T cells, reflecting chronic and immune responses under longstanding BAC administration. One possibility is that the LSCs were damaged directly by BAC instillation in the BALB/c mouse. Given that the niche regulation for SCs can be altered under severe chronic inflammation, leading to insufficiency of SC functions, we speculated that BAC might induce SC failure in different ways simultaneously, including direct cytotoxicity and indirect damage to the niche microenvironment surrounding the SCs through inflammatory infiltration of CD4-positive T cells, for example. Nonetheless, the detailed mechanisms need further investigation.

Squamous metaplasia is a hallmark of a variety of severe ocular surface disorders including severe dry eye and LSCD, which is commonly seen under long-term deficiency of tear film and chronic inflammatory infiltration in the ocular surface, including the limbal epithelium. The loss of goblet cells could be associated with the ocular surface squamous metaplasia and long duration of BAC insult. After the cessation
of BAC, goblet cells recovered in the conjunctival fornix as well as the corneal surface. This indicated that LSCD and squamous metaplasia could simultaneously exist (Fig. 8G), which is consistent with previous studies regarding LSCD in chemical burns,\textsuperscript{8} for example. Some researchers have pointed out that the grade of squamous metaplasia correlates with the severity of LSCD.\textsuperscript{38} In fact, the squamous metaplasia after BAC treatment was well recorded in our previous study showing that K10 could be observed on day 7 with twice-daily instillation of BAC,\textsuperscript{40} when conjunctival K19 or K13 were still negative over the cornea (data not shown). This indicated that epithelial squamous metaplasia emerged much earlier than corneal conjunctivalization in this model. In other words, LSCD was not the initiating factor for squamous metaplasia, although it might aggravate the condition of squamous metaplasia. In addition, most of the K10 expression emerged in the superficial layer of epithelium but was absent in the basal layer more adjacent to the inflammatory cells in the stroma, suggesting that the squamous metaplasia was probably induced by long-term tear insufficiency. On the other hand, the basal epithelium might also have certain mechanisms to escape from inflammatory damage. These results were consistent with the findings of Li et al.\textsuperscript{39} in an ex vivo model of squamous metaplasia induced by air exposure.

In our study, the pathology induced by BAC is not specific to the limbus and the cornea, as BAC impacts the entire ocular surface, leading to inflammation and epithelial squamous metaplasia. Prolonged chronic inflammation may change the cell fate and lead to the emergence of keratins such as K10, one of the markers of epithelial squamous metaplasia. However, based on our results, we supposed that the emergence of conjunctival K19 and K13 over the cornea probably resulted from the dysfunction of LSC not but from a cell fate change with chronic inflammation in the stroma of the cornea. Our data showed that the K19- and K13-positive epithelium invaded contiguously into the paracentral cornea with sharp tips (Figs. 3E, 3L, white arrows). Accordingly, K12 expression was diminished in the peripheral cornea (Fig. 3T) at 4 weeks and completely lost in the whole cornea 8 weeks after BAC termination. These findings indicated that LSCD developed gradually and that the conjunctivalization progressed from the limbus toward the central cornea until it fully covered the cornea (Fig. 8B). If the K19 and K13 were induced by chronic corneal inflammation through cell fate determination, the supposition was that they would be first recorded over the central or paracentral epithelium in a discontinuous pattern similar to that of K10 (Figs. 3R, 8F).

In conclusion, our findings demonstrated that the ophthalmic preservative BAC could induce LSCD in the BALB/c mouse, with the manifestations including corneal conjunctivalization, neovascularization, severe inflammation in the corneal stroma and limbus, and epithelial defect. The phenotypic alterations included corneal invasion by conjunctiva, emergence of goblet cells over the cornea, and loss of putative LSC markers. This novel mouse model of LSCD would be appropriate for detailed investigation of the mechanisms and evaluation of certain therapeutic methods in acquired LSCD.

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