Corneal Epithelial Wound Healing in Partial Limbal Deficiency

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Previous studies have shown that the corneal epithelial stem cells are located at the limbal basal layer. The limbal stem cells are regarded as the ultimate source for corneal epithelial cell proliferation and differentiation. This paper examines epithelial wound healing in rabbit corneas with partial limbal deficiency (PLD), which was created by the surgical removal of two-thirds of the limbal zone (superior and inferior). Four to eight months after PLD creation, all corneas appeared normal, without vascularization. The residual stem cell capacity then was challenged by two sizes of corneal epithelial debridement created with combined n-heptanol and mechanical scraping. In the first group, two consecutive 6-mm defects were created 1 month apart. After the first wounding, three of eight PLD corneas had delayed wound healing and two of the three had vascularization, as compared to controls (n = 7). After the second wounding, both controls (n = 7) and the remaining PLD (n = 5) corneas showed similar rapid healing. In the second group, a large defect of up to 1 mm within the limbus was created. Healing was completed in 25–40 days in PLD (n = 6) corneas, a more marked delay compared to the 10–12 days for controls (n = 6) (P = 0.001). In addition, all PLD corneas showed increased vascularization and had epithelium of the conjunctival phenotype, verified by the immunofluorescent staining positive to AM-3 monoclonal antibody but negative to AE-5 monoclonal antibody. Thus, a deficiency of limbal stem cells contributes to the triad of conjunctival epithelial ingrowth, corneal vascularization, and delayed healing with recurrent erosion. In PLD, corneal epithelium is still compromised, particularly when a large epithelial cell mass is removed. Invest Ophthalmol Vis Sci 31:1301–1314, 1990

The normal ocular surface is covered by corneal, limbal, and conjunctival epithelia, each of which has a distinct cellular phenotype. The cornea is covered by a tightly adherent nonkeratinized stratified squamous epithelium, which ensures optical smoothness. In contrast, conjunctival epithelium contains mucin-secreting goblet cells. The limbal epithelium is the transitional zone between the corneal and conjunctival epithelia. It differs morphologically from the cornea in that it possesses Langerhans cells and melanocyte and from the conjunctiva in that it lacks goblet cells. These three epithelial phenotypes maintain the ocular surface integrity.

In postnatal development, stem cells are needed for all self-renewing tissues that have a high degree of cellular differentiation. Corneal epithelium is well known for its rapid self-renewing capacity, which together with a coordinate cellular differentiation maintains the entire corneal epithelial cell mass. In exploring this self-renewing process, several studies have shown that there is centripetal cellular movement responsible for the replacement of central corneal epithelial cellular attrition, resulting from either normal desquamation or traumatic loss. The exact anatomic location of the stem cells of the corneal epithelium remained unclear until Schermer et al indicated their limbal location based on evidence provided by the expression pattern of a major 64-kD corneal epithelial keratin. Additional evidence supporting this finding was provided by Ebato et al, who showed that limbal epithelial cells grow much better than peripheral or central corneal epithelia in an explant culture, and by Cotsarelis et al, who showed that limbal basal cells can be selectively stimulated by topical ocular application of a tumor promoter, as well as by wounding of the central corneal epithelium.

Based on these findings, we speculated that the limbal stem cells play a crucial role as an ultimate source of corneal epithelial cellular proliferation and differentiation. Our laboratory previously studied corneal epithelial wound healing in a rabbit model by...
surgically removing the entire limbal zone, in a procedure called total limbal deficiency.\textsuperscript{14} In that model, the limbal zone, including 2 mm of the peripheral cornea and 3 mm of the adjacent conjunctiva, was surgically removed. In response to two consecutive central 7.5-mm epithelial debridements, the corneas with total limbal deficiency exhibited abnormal wound healing characterized by delayed healing, recurrent erosion, increased corneal vascularization, and conjunctivalization of the resultant epithelial phenotype.\textsuperscript{14} This finding demonstrated that the corneal epithelium loses its renewal capacity in the absence of the limbus. Clinically, Kenyon and Tseng\textsuperscript{15} recently reported encouraging results in the use of limbal autograft transplantation for various ocular surface disorders that cause destruction of the limbal zone. In a rabbit model, Tsai, Sun, and Tseng\textsuperscript{16} also showed that limbal transplantation is a better source than conjunctival transplantation in restoring the corneal epithelial phenotype for a combined chemically and mechanically damaged corneal surface. These results indicate that the corneal epithelial phenotype can be recovered by the replacement of the limbus.

The objective of the current study is to examine the reserve stem cell capacity in corneas with partial limbal deficiency (PLD), a problem that can be encountered in some clinical situations as well as in donor eyes for limbal autograft transplantation.

Materials and Methods

The use of animals in these investigations conformed to the ARVO Resolution on the Use of Animals in Research.

Rabbit Model of PLD

A total of 14 New Zealand albino rabbits of both sexes, weighing 2–3 kg, were anesthetized with intramuscular xylazine HCl (50 mg) and ketamine HCl (50 mg). In one eye of each rabbit, two pieces of limbal strips involving the limbal circumference of 10–2 and 4–8 o’clock were removed. To ensure thorough removal of limbal epithelium, a dissection of the limbal zone was performed with a partial-thickness corneal incision 2 mm from the limbus with a no. 69 Beaver blade, followed by lamellar dissection toward the limbus with a no. 66 Beaver blade, and completed by conjunctival peritomy 3 mm beyond the corneolimbal junction with a pair of scissors at the same locations along the limbal circumference. No suture was used to secure the free conjunctival edge. Topical gentamicin sulfate and dexamethasone ointments were applied three or four times per day for 1 week, followed by dexamethasone ointment alone once or twice per day for a second week. All of the injured eyes healed without complication within 1–2 weeks. Four to eight months later, these 14 rabbits were subdivided into two groups, each receiving a different size epithelial debridement.

Challenge of the Reserve Stem Cell Capacity of PLD

The reserve capacity of limbal stem cells in PLD rabbits was challenged by two different sizes of epithelial debridement, as follows.

In the first group, eight PLD corneas and seven normal control corneas received two consecutive 6-mm central corneal epithelial debridements created at a 1-month interval. In brief, after adequate general anesthesia was induced with intramuscular injection of xylazine HCl and ketamine HCl, one eye was proptosed. After a trephine marking, the central 6-mm corneal surface was rubbed with a cotton tip wet with n-heptanol\textsuperscript{17} for a period of 1–2 min. This rubbing was performed in a repetitive circular motion, starting from the central cornea to the wound margin and then moving backward from the wound margin to the central cornea. The ocular surface then was rinsed with sterile saline and the corneal epithelium removed by gentle scraping with a Bard-Parker blade. Topical gentamicin ointment was given twice per day for 3 days. One month later, the eyes were examined with a Zeiss surgical microscope, and those corneas without corneal vascularization received the second debridement using the same procedure.

In the second group, six PLD corneas and six normal controls received one large corneal epithelial debridement up to 1 mm within the limbus. The wound margin was outlined with a Bard-Parker blade. The rest of the wounding procedure was the same as described above. Topical gentamicin ointment was also applied twice per day for 3 days.

Study of the Reserve Proliferative Capacity

To study the reserve proliferative capacity of PLD corneas, the healing process was evaluated by external photographs, with and without 1% fluorescein staining. Photographs were taken under general anesthesia daily in the first week after wounding, and then every 2–3 days until the wound was completely healed. Those corneas healed with vascularization were examined weekly until the rabbits were sacrificed. The longest follow-up was 6 months. The fluorescein-stained external photographs of each wound was projected and traced onto a piece of paper. The wound area was determined by a Zeiss Videoplan 2 image analyzer with the Mop-Videoplan program (Kontron Elektronik GmbH, Eching, West Germany).

The healing curve was plotted by tracing the
mean and standard deviation of the wound area measurement of each given postoperative day. The curves of the experimental PLD group and normal control group were compared.

Study of Reserve Differentiative Capacity

During the entire follow-up period of 6 months, one rabbit of each group was sacrificed with an intravenous overdose of pentobarbital. For normal controls, the small defect group was sacrificed at day 6 and 14, and the large defect group at day 1, 6, 18, and 60 after healing. For PLD, the small defect group was sacrificed at day 3 and 21 after healing, and the large defect group at 4, 7, 8, 12, and 24 weeks after wounding. The resulting epithelial phenotype, determined by immunofluorescent study, was used to study the reserve differentiative capacity of the PLD corneas.

The corneoscleral button was removed, sectioned into two halves, and embedded for frozen sections. The resulting epithelial phenotype over the corneal surface healed from the two different sizes of epithelial debridement was evaluated by immunofluorescent staining with two monoclonal antibodies, AM-3 and AE-5, which recognize specifically the nonglycosylated portion of rabbit ocular mucin core protein and a 64-kD keratin specific to corneal epithelium, respectively. AM-3 was developed and characterized in our laboratory and used to verify conjunctival epithelial phenotype. AE-5 (a kind gift of Tung-Tien Sun), has been characterized previously by his laboratory and used as a marker for corneal epithelial phenotype.

The conditioned supernatant of the hybridoma of both of the above mentioned antibodies was used as a primary antibody. The supernatant of the parental secretor myeloma cells was used as primary antibody control. A dilution of 1:200 fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody was used as a secondary antibody. After immunofluorescent staining, the slide was examined with a Zeiss Axiophot equipped with epifluorescence capacity and photographed. A composite of serial photographs taken under a 10X objective lens was made for each specimen and included the epithelia covering corneal, limbal, and adjacent conjunctival surfaces and is reported here for purposes of comparison.

Statistical Analysis

The paired and pooled data of both experimental and control groups were analyzed by the student t-test and the Wilcoxon-Mann-Whitney test, with the assistance of the Department of Biostatistics, Bascom Palmer Eye Institute.

Results

In all 14 PLD corneas, the limbal wounds healed rapidly within 1–2 weeks, even though two thirds of the limbal circumference had been surgically removed. The corneal epithelium of all of these corneas remained intact and the corneal surface was smooth and clear during the entire follow-up period of 4–8 months. To study the reserve proliferative capacity of PLD corneas, we compared the healing curve of PLD corneas to that of normal controls in response to two different sizes of corneal epithelial debridement.

In the group of small epithelial defects, the first 6-mm corneal epithelial debridement was created in eight PLD and seven normal control corneas. When the healing curve was compared as a group, PLD corneas appeared to heal slightly more slowly, but the results were not statistically significant when compared to the normal controls (P > 0.5; Fig. 1A). Extensive variation indicated by a large standard deviation was noted in the PLD group and possibly was due to the fact that three PLD corneas showed a marked delay in wound healing (Fig. 1A). The second 6-mm epithelial debridement was performed 1 month after the first. Three animals in the PLD group were excluded: two developed corneal vascularization and one died. The remaining five PLD and seven control corneas showed rapid healing in 3–4 days and 4–9 days, respectively (Fig. 1B). The difference in healing rate between these two groups was not statistically significant (P > 0.5; Fig. 1B). No additional corneal vascularization was noted in either group after the second wounding. Despite the lack of statistical significance, the healing rate in the second wounding tended to be faster than the first for both the control and PLD groups (Fig. 1A, B).

When individual eyes were examined after the first wounding, all seven controls and five of eight PLD corneas healed smoothly within 4–9 days without corneal vascularization. An example (case 6) of each group is shown in the left and central columns of Figure 2. However, the remaining three PLD corneas showed delayed wound healing, especially notable on the superior and inferior corneal aspects, adjacent to the site of limbus removal. One such example (case 8) can be seen in the Figure 2 (right column). Indeed, the extension of the wound to the peripheral cornea also was noted in these three corneas on the first postoperative day (Fig. 2, second row, right column). Two of these three PLD corneas healed with corneal vascularization coming from the limbal deficient areas (data not shown).

In the group of animals with large epithelial defect, both PLD (n = 6) and normal control (n = 6) corneas received one large epithelial debridement that ex-
Fig. 1. Comparison of the healing curves of the control and PLD corneas in response to (A) the first central 6-mm epithelial debridement and (B) the second debridement. The healing curve was derived by plotting the mean of the residual defect area with its standard deviation. For (A), control n = 7 and PLD n = 8; for (B), control n = 7, and PLD n = 5. There is no statistical significance (P > 0.5) for either (A) or (B).

Tended up to 1 mm within the limbus. All wounds in control corneas without limbal removal healed rapidly, in 10–12 days, but none of the PLD wounds healed until 25–40 days after wounding (Fig. 3). The healing rate seemed to be comparable for both control and PLD corneas in the first 5 days after wounding, but the healing was significantly delayed from the 8th day onward after the debridement (P = 0.001; Fig. 3). All control corneas healed smoothly; one such example (case 2) is shown in Figure 4A and 4B. No corneal vascularization was noted in any of the controls (Fig. 4A) except in one, which showed mild peripheral vascularization on the upper cornea during the early healing course, which regressed immediately after complete epithelialization (data not shown). In contrast, all PLD corneas healed with irregular wound edges, and most importantly, with progressive corneal vascularization and inflammation. One example (case 3) is shown in Figure 4C–H. All six PLD corneas became heavily vascularized, except case 5, which had only mild vascularization during the follow-up period of 6 months (Fig. 5).

To study the potential reserve differentiative capacity of PLD corneas, we compared their resulting epithelial phenotypes to those of normal controls using cell-specific monoclonal antibodies in the immunofluorescent test. AM-3 monoclonal antibody reacts specifically with ocular mucin,18,19 and recognizes conjunctival goblet cells of normal rabbits (Fig. 6A). AE-5, which recognizes a major 64-kD corneal keratin,10 decorates the full-thickness corneal epithelium and suprabasal cell layers of limbal epithelium (Fig. 7A). After two consecutive 6-mm epithelial debridements, all control corneas retained the corneal epithelial phenotype, as indicated by staining negative to AM-3 (Fig. 6B) but positive to AE-5 (Fig. 7B). Five of eight PLD corneas that healed without vascularization also retained a corneal epithelial phenotype (Figs. 6C, 7C). However, the remaining two vascularized PLD corneas exhibited AM-3-positive goblet cells extending to the midperiphery (Fig. 6D) as well as absence of normal full-thickness corneal epithelial AE-5 staining, leaving only punctate pattern (Fig. 7D). This result suggests the coexistence of isolated AE-5 positive cells intermixed with AM-3 positive goblet cells. The central cornea still retained the corneal epithelial phenotype, which was negative to AM-3 but positive to AE-5 staining (Figs. 6D and 7D). This distribution was consistent with the presence of peripheral corneal vascularization.

In the study of one large epithelial debridement, all normal control corneas healed without vascularization and maintained the corneal epithelial phenotype as indicated by positive AE-5 staining and negative AM-3 staining. One specimen obtained 18 days after
Fig. 2. Serial external photographs of normal control (case 6, left) and PLD corneas (case 6, center; case 8, right) after the first 6-mm central epithelial debridement. To illustrate the defect areas, 1% fluorescein staining was used. Photographs were taken immediately after debridement (OP, top row), and day 1, 5, and 9 thereafter (D1, 5, 9).
healing is shown in Figures 6E and 7E. The positive expression of AE-5-specific antigen extended to the perilimbal conjunctiva with concomitant loss of AM-3 specific antigen expression at that region (Figs. 6E, 7E).

In contrast, all PLD corneas showed conjunctivalization of the resultant epithelial phenotype, which stained positive with AM-3 but weak or negative with AE-5 antibody. For example, 4 weeks after debridement, the cross section through the limbal deficient area showed AM-3-stained goblet cells on most parts of the cornea except the central area, which still retained the corneal epithelial phenotype (Figs. 6F, 7F). However, a section adjacent to the residual limbus showed extension of the goblet cells only to the perilimbal area, and the AE-5-positive epithelium on the rest of the cornea (Figs. 6G, 7G). Twelve weeks after debridement, one section of the corneal surface was covered by conjunctival epithelium containing more AM-3-positive goblet cells (Fig. 6H), and there was a nearly total loss of AE-5 expression (Fig. 7H). Six months after debridement, the corneal surface still had an epithelium mixed with both AM-3-positive and AE-5-positive characteristics (data not shown).

When we compared the relative risk of developing corneal vascularization and conjunctivalization of the resultant epithelial phenotype in PLD and normal control groups, there was no statistically significant difference, as a result of the two consecutive small epithelial debridements ($P > 0.4$), even though two of eight PLD corneas did develop corneal conjunctivalization. However, the difference between the two groups was statistically significant in response to one large epithelial debridement ($P = 0.002$). None of the control corneas, but all six PLD corneas, exhibited corneal vascularization and conjunctivalization.

**Discussion**

By definition, stem cells are present in all self-renewing tissues that have a high degree of cellular differentiation. These cells are long-lived and relatively quiescent with respect to cell mitosis under the state of steady growth, but have a great potential for clonogenic cell division, and ultimately are responsible for cellular proliferation and differentiation. Most of our knowledge about stem cells comes from studies of blood cells and such epithelial tissues as intestinal epithelium, seminiferous epithelium, and epidermal keratinocytes. According to the information from these studies, stem cells, after a round of mitosis, generate transient amplifying cells that are short-lived and function primarily to amplify cell numbers through active mitosis. The transient amplifying cells finally differentiate into postmitotic cells that are committed to cellular differentiation. The ultimate expression of the functional aspect of the tissue is achieved by the terminal differentiation of the postmitotic cells. Therefore, stem cells and transient amplifying cells compose the proliferative compartment of the tissue and differ from each other in life span as well as in mitotic activity.

Evidence has now been gathered to demonstrate that stem cells of the corneal epithelium are located at the limbal region. Previously, several investigations had suggested that peripheral cornea and limbus play a significant role in corneal epithelial renewal, but Schermer et al pointed out the limbal location from their study of the expression pattern of a major 64-kD corneal keratin. They further suggested that transient amplifying cells are located at the corneal basal layer and postmitotic cells at the suprabasal cell layers. Recently, other studies have indicated that the mitotic activity in the peripheral cornea and limbus is higher than that in the central cornea, suggesting that more transient amplifying cells are located in the peripheral cornea. The current study together with...
Fig. 5. External photographs of all six PLD corneas (cases 1–6) after one large epithelial debridement. In each case, the photograph was chosen from the last available photographic examination, of which the duration of follow-up is provided in the upper right. All six corneas except case 5 showed heavy vascularization. In addition, stromal opacity and granuloma pyogenicum developed on all of the control corneas.

several of our previous reports\textsuperscript{14–16} further substantiates this important concept. Other properties of limbal stem cells are described in detail in a recent review by Tseng.\textsuperscript{30} However, it remains unknown whether stem cells and transient amplifying cells assume a different role in the healing of a central cor-
neal epithelial wound. In this regard, the results of the current study provide some useful information, which deserves more discussion.

In this study, the limbal wounds of all PLD corneas healed rapidly even when two thirds of the limbal circumference was removed. In contrast to the characteristic AE-5 suprabasal staining of the normal limbal zone (Fig. 7A), the limbal deficient area showed full-thickness AE-5 positivity, immediately contiguous to conjunctival epithelium containing AM-3 positive goblet cells (data not shown). This result indicates that the limbal epithelium had been surgically removed thoroughly at the wound area and that the limbal wound was healed by the surrounding transient amplifying cells, with possible contribution by the adjacent conjunctival epithelium. Because the resultant corneal epithelium did not show any epithelial breakdown during 4–8 months of follow-up, the remaining stem cells and transient amplifying cells in PLD corneas were still sufficient to maintain the ordinary corneal epithelial cell renewal. It is unknown whether the corneal epithelial integrity can be maintained by these residual proliferative compartments for an indefinite period of time. To challenge the residual proliferative capacity, two consecutive central 6 mm epithelial debridements were created. The defects of PLD corneas were healed without difficulty, as compared to the normal controls with intact limbus (Fig. 1A and B). This result indicates that the reserved proliferative capacity of the remaining stem cells and transient amplifying cells in the peripheral PLD cornea could still handle the challenge of at least two small central epithelial defects. We do not know, however, how many challenges these cells can tolerate. One can speculate that if stem cells can manage to generate sufficient numbers of transient amplifying cells, then there should not be a problem in toleration.

It should be noted that three of eight PLD corneas did show delayed healing, although not statistically significant when PLD as a group was compared to the control. This can be explained by the further loss of the remaining transient amplifying cells that occurred during the extension of the wound area to the peripheral cornea, noted on the first postoperative day (Fig. 2, right column). We cannot explain why this wound extension happened only in these 3 PLD corneas. Because transient amplifying cells on the peripheral cornea were damaged, the healing rate was delayed to a similar extent to those of the PLD corneas receiving one large epithelial debridement.

When the remaining transient amplifying cells were nearly totally removed by one large epithelial debridement, up to 1 mm within the limbus, the healing rate in PLD corneas was still comparable to the controls during the first 5 days after wounding (Fig. 3). This early rapid healing in PLD corneas presumably was effected by the remaining transient amplifying cells in the suprabasal portion of the limbus or in the peripheral 1 mm of cornea; however, because of their limited life span, from the 8th day onward, the healing in PLD corneas was significantly slower than that of the controls (P = 0.001; Fig. 3). These results indicate that transient amplifying cells on the peripheral cornea and limbus seem to be responsible for immediate wound healing. We speculate that upon tissue demand for cellular regeneration, the limbal stem cells may need more time to be activated to generate new transient amplifying cells. Without sufficient stem cells, there would be no sustained supply of transient amplifying cells, and thus the wound healing would eventually be retarded. This concept can be supported further by the observation of markedly delayed healing when the limbus was totally removed, as described in a recent experiment.14

If the limbus is intact, a normal corneal epithelial phenotype could be maintained even after two consecutive small epithelial debridements (Figs. 6B, 7B) or one large debridement (Figs. 6E, 7E). This result supports the notion that the limbus indeed contains the stem cells for corneal epithelium. The corneal epithelial phenotype could also be maintained in the PLD corneas when sufficient transient amplifying cells were preserved in the peripheral cornea (Figs. 6C, 7C). Nevertheless, when the populations of these transient amplifying cells were further depleted either by one large epithelial debridement (Figs. 6G, H and 7G, H) or by subsequent wound extension after a small epithelial debridement (Figs. 6D, 7D), the PLD corneas manifested a mixed corneal and conjunctival phenotype. These results suggest that the stem cell population in the remaining one third of the limbus cannot sustain the corneal epithelial phenotype when most of the transient amplifying cells are removed. But with additional surrounding transient amplifying cells, the corneal epithelial phenotype could be maintained. The mixed expression of corneal and conjunctival epithelial phenotype indicates that the residual limbal stem cells either are nonfunctional or are insufficient to generate enough transient amplifying cells for reepithelialization and prevention of conjunctival epithelial ingrowth. This observation suggests that there might be dysfunction of stem cell renewal or generation of transient amplifying cells under this posttraumatic circumstance. Further investigations in the aspects of stem cell regulation will help us clarify this question.

The ingrowth of conjunctival epithelium signifies the loss of limbal epithelium as a barrier between
corneal and conjunctival epithelia. The reason that the normal limbus has such an epithelial barrier can be explained by the following observations. Activation of mitotic activity in perilimbal conjunctival epithelium has been observed by Danjo et al\textsuperscript{31} when a small central corneal epithelial defect was created in rabbits. As a response to a central wound, the stem cells are activated, giving rise to transient amplifying cells which spread centripetally as one can expect in the classical "centripetal cell movement."\textsuperscript{29-39} During the healing process of the normal controls, we observed the positive expression of \textit{AE-5} with concomitant negative expression of \textit{AM-3} at the perilimbal conjunctiva (Fig. 6C, E, and 7C, E). Recently, Thoft et al\textsuperscript{32} reported positive \textit{AE-5} expression on the perilimbal conjunctival area next to the superior limbus in human corneas. We interpreted this result to be caused by the activation of stem cells on the superior limbus to generate transient amplifying cells from the frequent mechanical stimulation of the upper lid blinking. Based on these findings, we speculate that through this kind of centripetal and centrifugal growth pressure, the limbal epithelium can serve as a barrier between corneal and conjunctival epithelium. It is worth reiterating that the conjunctival epithelial ingrowth is accompanied by corneal vascularization. Such a constellation, conjunctivalization with vascularization, is a hallmark of limbal stem cell deficiency, or limbal deficiency. This is confirmed by the current report, and by our recent experiments with total limbal deficiency in rabbits,\textsuperscript{14,33} in which increasing vascularization always was associated with conjunctival epithelial ingrowth. In earlier studies, we\textsuperscript{34-36} and others\textsuperscript{37-38} also have observed an incidence ranging between 14 and 68% of conjunctival epithelial ingrowth and vascularization when total corneal epithelial defects are extended beyond the limbus in rabbit corneas debrided with n-heptanol (n = 287, combining various reports). Recently, we noted this abnormal healing pattern also resulting from the total removal of limbal basal epithelium.\textsuperscript{33} We cannot explain why corneal vascularization is closely associated with the development of conjunctival epithelial ingrowth. We speculate that corneal epithelium derived from limbal epithelium is intrinsically different from that of conjunctival origin in producing antiangiogenic or anti-inflammatory factors (cytokines). The conjunctival epithelium may produce factors that induce stromal inflammation and vascularization, which may explain why conjunctival stroma is normally vascularized.

Clinically, by the use of impression cytology, conjunctival epithelial ingrowth has been recognized in several ocular surface disorders, including chemical/thermal injuries, Stevens-Johnson syndrome, aniridia, and some contact lens-induced keratopathies.\textsuperscript{39} These disorders also are associated with corneal vascularization. Based on the above experimental data, we can speculate that these disorders may have the common pathogenic basis of limbal stem cell deficiency. Such tissue alterations are the basis of compromised corneal functions and are consistent with the experimental data reported earlier.\textsuperscript{40-42} Patients usually suffer from frequent erosions and decreased vision as a result of irregular optical surface, weak tensile strength,\textsuperscript{40} decreased glycogen content,\textsuperscript{41} and incompetent barrier function.\textsuperscript{42} The fact that such abnormal corneal surfaces can be treated successfully with limbal autograft transplantations\textsuperscript{15,16} further supports the pathogenic theory.

In summary, stem cells and transient amplifying cells may have a different role in corneal epithelial wound healing. During the healing process, the cellular migration and mitosis are engaged primarily by the transient amplifying cells. But because of their limited life span, the increasing demand for new transient amplifying cells triggers limbal stem cell activation and thus generates more transient amplifying cells. The growth pressure generated by stem cell activation and increasing transient amplifying cells constitutes the epithelial barrier between corneal and conjunctival epithelia.

Therefore, we can conclude that limbal stem cells serve as an ultimate source for corneal cellular regeneration and differentiation. Deficiency of limbal stem cells, or limbal deficiency, gives rise to the triad of conjunctival epithelial ingrowth (or conjunctivaliza-
Fig. 7. Composite immunofluorescent staining by the monoclonal antibody AE-5. Arrows are placed underneath the limbal region with the arrow pointing to the cornea and the arrowhead placed directly beneath the anatomic corneolimbal junction. AE-5 recognizes the cornea-specific 64-kD keratin and stains the full thickness of the entire corneal epithelium and suprabasal cell layers of the limbal epithelium (A). In the group receiving two small epithelial debridements, the AE-5 positive staining was noted in the entire epithelium of the corneal surface in both normal control (B) and nonvascularized PLD corneas (C). In the vascularized PLD corneas, the paracentral corneal surface was largely negative to AE-5 except for a few scattered superficial cells, and the central corneal surface was still positive to AE-5 (D). In the control cornea receiving one large epithelial debridement, AE-5-positive staining was noted in the entire corneal epithelium (E) extending to the perileminal conjunctiva (also seen in C). In PLD corneas, 4 weeks after wounding, the AE-5 was positive on the remaining part of the corneal surface (F, G) where AM-3 was negative (see also Fig. 6F, G). Twelve weeks after wounding, the entire corneal surface was negative to AE-5 (H).

References


33. Kruse FE, Chen JYJ, Tsai RJF, and Tseng SCG: Conjunctival