Cytologic Evidence of Corneal Diseases with Limbal Stem Cell Deficiency

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Purpose: To determine which human corneal diseases show similar abnormal corneal surfaces, characterized by conjunctival epithelial ingrowth (conjunctivalization), vascularization, and chronic keratitis (i.e., a constellation of signs termed *limbal stem cell dysfunction* [deficiency], which have been noted in experimental rabbit models).

Methods: A total of 134 impression cytology specimens of the perilimbal region collected from 1984 to 1994 were reviewed. Limbal deficiency was diagnosed if conjunctival goblet cells were found on the corneal surface.

Results: Ninety-four patients were found to have limbal deficiency. Category 1 comprised 53 patients with a clear history showing limbal stem cell destruction by chemical/thermal burns, Stevens-Johnson syndrome, multiple surgeries and cryotherapies, contact lens wear, and severe microbial keratitis. Patients in category 2 (n = 41), did not have such a history, but gradual loss of stem cell functions over time was disclosed and included diverse causes such as aniridia, multiple endocrine deficiencies, neurotrophic keratopathy, peripheral inflammatory keratopathy or limbitis, and idiopathy. The 40 remaining patients with suspicious findings did not have limbal deficiency.

Conclusions: Impression cytology can be used to diagnose and monitor corneal diseases with limbal deficiency, which manifest distinct clinical problems and are generally poor candidates for penetrating keratoplasty. The identification of category 1 diseases allows one to consider limbal (stem cell) transplantation for surface reconstruction. The presence of category 2 diseases indicates that limbal stem cell functions can be modulated by developmental, hormonal, neuronal, vascular, and inflammatory factors in the limbal stroma. *Ophthalmology 1995;102:1476–1485*

Homeostasis of corneal epithelium, a rapidly self-renewing tissue, is achieved by orderly proliferation and differentiation of limbal stem cells to transient amplifying cells and ultimately, terminally differentiated corneal epithelial cell.[1–3] Limbal stem cells differ from corneal transient amplifying cells in location (limbal basal versus corneal basal epithelium), cell cycle time (slow cycling versus rapid cycling), lifespan (long versus short), and differentiation (poor versus full). Studies showing their differences in cell culture proliferative rates,[4,5] expression of certain markers,[6–9] cell cycle time,[2,10] and response to phorbol ester tumor promoter[2,11] between the corneal and limbal epithelium indicate that the control of mitotic kinetics for limbal stem cells is different from that for corneal transient amplifying cells, although the exact mechanism remains unknown.

Experimental support for the limbal location of corneal epithelial stem cells is derived from observations of abnormal corneal epithelial wound healing when the limbal epithelium is partially[12,13] or completely[14,15] removed. These experimental studies produced a spectrum of corneal surface abnormalities characterized by conjunctival epithelial ingrowth (conjunctivalization), vascularization, and chronic inflammation[12–15] which indicate limbal stem cell dysfunction (deficiency). The conjunctival source of the epithelial ingrowth was proved by immunofluorescent

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staining with monoclonal antibodies[12–15] and by the detection of goblet cells with impression cytology.[16] Because vascularization and inflammation also can be seen in other corneal diseases, we consider "conjunctivalization" to be the most reliable diagnostic sign of limbal deficiency.[17,18] In this study, we identified a number of clinical diseases with limbal deficiency and conjunctivalization through the use of impression cytology to detect goblet cells on the corneal surface.

Materials and Methods

We retrospectively reviewed impression cytology specimens collected from 134 patients from 1984 to 1994. All specimens were gathered by applying nitrocellulose filter paper onto the perilimbal area to include both the conjunctival and the corneal surface in four different quadrants.[13] The cytology specimens were processed and stained with periodic acid-Schiff and hematoxylin–eosin in the method previously described.[19] Based on our diagnostic criteria of limbal deficiency that conjunctival goblet cells were present on the corneal surface, we grouped these patients into two major categories: those with and those without limbal deficiency. We compared the clinical data of the patients in these groups.

Results

Conjunctival goblet cells, indicative of limbal deficiency, were detected on the corneal surface of 94 of 134 patients, whereas the cells were not detected in the remaining 40 patients. Figure 1A depicts an example in which few large nonkeratinized squamous cells were removed by the filter paper from the corneal surface (left of the arrow), and magenta-stained goblet cells were found only on the conjunctival surface (right of the arrow). In contrast, in a patient with aniridia and limbal deficiency, no such distinction between conjunctival and corneal epithelium and conjunctival goblet cells was found on both conjunctival and corneal surfaces (Fig 1B). The group of corneal diseases with limbal deficiency was further classified into two subcategories: category 1, aplasia or total loss of limbal stem cells due to destruction; and category 2, gradual loss of limbal stem cell functions due to insufficient stromal support.

Category 1: Aplasia or Total Loss of Limbal Stem Cells due to Destruction

The 53 patients in this category (Table 1) had a clear ocular history of limbal tissue destruction and consequent loss of the limbal stem cell population. The causes for this destruction included chemical burns (n = 32), thermal burns (n = 2), Stevens-Johnson syndrome (n = 7), multiple surgeries or cryotherapies to the limbal region (n = 4), contact lens wear (n = 5), and severe microbial keratitis (n = 3). In addition to conjunctivalization, evidenced by the presence of conjunctival goblet cells on the corneal surface, all patients showed corneal vascularization, decreased vision, and moderate to severe photophobia. Of the patients with chemical and thermal burns, all except two were males, ranging in age from 12 to 84 years (mean ± standard deviation, 43.3 ± 21.4 years; median, 40 years). Eight patients had bilateral involvement, and the remainder had unilateral involvement. The time interval from the injury to the cytologic diagnosis ranged from 2 weeks to 70 years. Twelve patients had limbal deficiency diagnosed by impression cytology within 1 year after injury, suggesting that these patients might have had more severe involvement, which brought them to medical attention sooner. Of these 12 patients, 7 had delayed wound healing or persistent corneal epithelial defects. The diagnosis of limbal deficiency was not made until 3 to 70 years later in the other 22 patients. Most of these patients had received multiple penetrating keratoplasties with repeated graft failures resulting in corneal scarring and vascularization. Of these 22 patients, 9 had a history of recurrent erosions. In some patients, this problem persisted even after corneal transplantation. (See case 1 in the Case Reports section below.)

There were seven patients (5 females and 2 males) with Stevens-Johnson syndrome, ranging in age from 11 to 81 years (35.7 ± 28 years; median, 26 years). Three patients had symmetric bilateral involvement, and four showed asymmetric involvement with one eye more severely damaged. All patients showed corneal vascularization and surface epithelial irregularity. In addition, four patients had recurrent corneal epithelial defects. (See case 2 in the Case Reports section below.)

In four patients, limbal deficiency developed after multiple surgeries and/or cryotherapies to the limbal region. Two patients had complicated cataract surgeries: one later underwent wound repair two times, and the other underwent extensive removal of epithelial downgrowth. The other two patients had conjunctival intraepithelial neoplasia, and underwent multiple tumor resections and cryotherapies. (See case 3 in the Case Reports section below.)

We identified five patients (4 women and 1 man) with limbal deficiency from contact lens-induced keratopathy. The patients ranged in age from 20 to 58 years (33.8 ± 17.2 years; median, 28.5 years). All patients showed bilateral involvement, often with one eye more severely involved. They all had severe photophobia, decreased vision, corneal vascularization, and an irregular surface. (See case 4 in the Case Reports section below.)

The last group in this category consisted of three patients with severe microbial keratitis. The patients included two women (42 and 71 years of age) and one man (55 years of age). In the first patient, chronic bilateral asymmetric ocular surface problems developed with photophobia, dryness, recurrent erosions, and corneal vascularization after measles infection at 3 years of age. In the other two patients, ocular surface problems developed after severe limbus to limbus *Pseudomonas* keratitis, resulting in persistent epithelial defects. All three patients showed goblet cells on impression cytology.

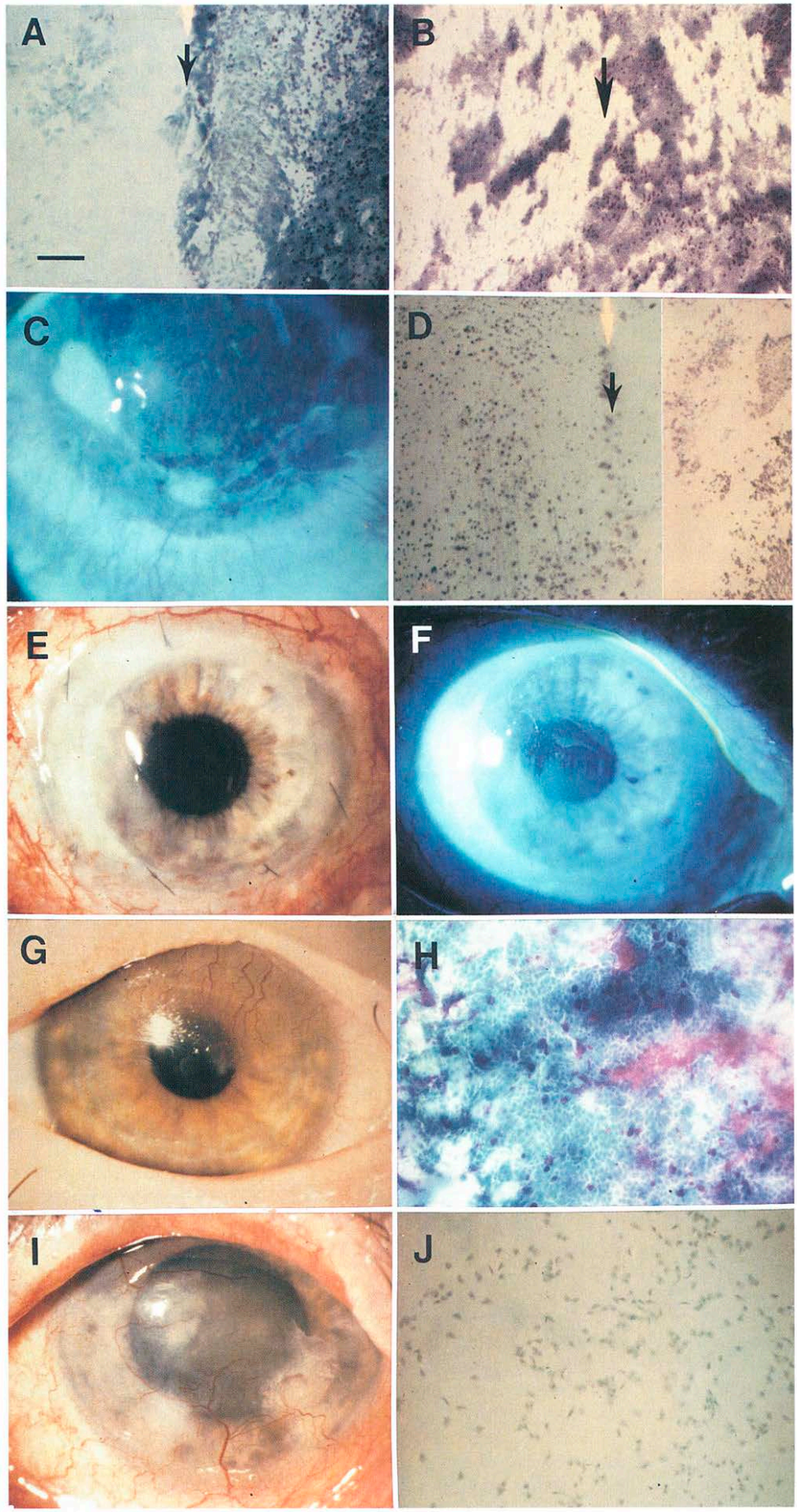

Figure 1. **A,** impression cytology of the normal perilimbal area shows few large squamous superficial cells on the corneal surface (left of the arrow) and the goblet cell-containing conjunctival epithelium (right of the arrow). For this figure and all others shown below, the arrowheads point toward the limbus (bar = 250 μm). **B,** impression cytology of a representative patient with aniridia shows the loss of the normal limbal landmark with goblet cell-containing conjunctival epithelial cells found on the corneal surface (left of the arrow). **C,** case 1. A patient with unilateral alkaline injury occurring 18 years ago in whom progressive vascularization and an irregular fluorescein-stained epithelium developed with defects after recent corneal transplantation combined with cataract extraction and lens implantation. **D,** impression cytology of the inferior perilimbal area shows goblet cells (stained by their mucin granules) on the corneal surface (left of the arrow). **E** and **F,** 2 months after limbal autograft transplantation, the return of a normal stable and smooth corneal surface is shown, without fluorescein staining. **G,** case 2. A patient with bilateral Stevens-Johnson syndrome occurring 6 years ago in whom progressive vascularization developed on the right superior cornea. **H,** impression cytology obtained from the central corneal surface shows goblet cell-containing conjunctival epithelial cells with mucus aggregate (stained pink). **I,** another patient with Stevens-Johnson syndrome and similar corneal scarring and vascularization. **J,** impression cytology of the temporal perilimbal area shows scattered, large squamous epithelial cells on the corneal surface (all impression cytology specimens are stained with periodic-acid-Schiff and modified Papanicolaou as previously described[18]; photographs were taken at the same magnification as A).

Table 1. Corneal Diseases with Limbal Deficiency

	No. of Patients
Category 1 Aplasia: Total Loss of Stem Cells due to Primary Destruction	53
Chemical/thermal injuries	34
Stevens-Johnson syndrome	7
Multiple surgeries or cryotherapies of the limbal region	4
Contact lens-induced keratopathy	5
Severe microbial keratitis	3
Category 2 Hypofunction: Gradual Loss of Stem Cell Function due to Insufficient Stromal Support	41
Aniridia (hereditary)	13
Keratitis associated with multiple endocrine deficiencies (hereditary)	2
Neurotrophic keratopathy (neuronal or ischemic)	13
Peripheral inflammatory disorders and chronic limbitis	5
Idiopathic	8
Total	94

Category 2: Gradual Loss of Limbal Stem Cell Functions due to Insufficient Stromal Support

Category 2 included 41 patients with corneal diseases of diverse causes (Table 1). These patients did not have a history of trauma or external injury, but clinical features of limbal deficiency still developed over time. This group included the genetic disease of aniridia (n = 13), keratitis associated with multiple endocrine deficiencies (n = 2), neurotrophic keratopathy (n = 13), peripheral inflammatory keratopathy or limbitis (n = 5), and idiopathy (n = 8). All 13 patients with aniridia showed bilateral limbal deficiency. These included six women and seven men, ranging in age from 30 to 73 years (47.8 ± 15.6 years; median, 42.5 years). These patients typically had marked photophobia and decreased vision. The corneal changes, which began early in life and were progressive in nature, included superficial pannus and an irregular fluorescein-stained abnormal epithelium, similar to previous reports.[20] Results of impression cytologic examination in all patients showed conjunctivalization with conjunctival epithelium and goblet cells appearing on the corneal surface. A typical example is illustrated in Figure 1B.

There were two patients with keratitis associated with multiple endocrine deficiencies. Shortly after birth, both patients manifested severe photophobia and an irregular corneal epithelial surface with progressive pannus. (See case 5 in the Case Reports section below.)

There were 13 patients with neurotrophic keratopathy in whom limbal deficiency developed. Limbal deficiency was related to neuronal components in 11 patients (8 patients with diabetes mellitus and 3 patients with previous herpetic simplex infection) and ischemic components in 2. All patients (8 women and 5 men) were typically elderly, ranging in age from 53 to 97 years (74.1 ± 10.5 years; median, 73 years). The condition in 5 of the 13 patients might have been aggravated by additional surgeries for cataract, glaucoma, and cornea. The remaining patients did not have any surgery nor photocoagulation for diabetic retinopathy. (See case 6 in the Case Reports section below.)

The next five patients belonged to the peripheral inflammatory keratopathy or limbitis group. These included three patients with chronic limbitis and two with pseudopemphigoid. All the patients were female and most were elderly, ranging in age from 15 to 79 years (58 ± 25.7 years; median, 71 years). (See cases 7 and 8 in the Case Reports section below for review of their clinical features.)

We classified the last group of eight patients as idiopathic because their ocular and systemic illnesses were insignificant. (See case 9 in the Case Reports section below.)

Case Reports

Case 1. A 62-year-old man sustained a plaster and cement injury to his right eye 19 years earlier, resulting in progressive discomfort, corneal scarring with vascularization, and conjunctival cicatrization. He underwent penetrating keratoplasty combined with cataract extraction and lens implantation and recovered satisfactory visual acuity of 20/25. Three months later, recurrent erosions appeared on the graft near the host cornea with encroaching blood vessels refractory to photocoagulation (Fig 1C). Results of impression cytologic examination showed conjunctival goblet cells on the vascularized host corneal surface (Fig 1D), confirming limbal deficiency. To secure the visual result and prevent potential allograft rejection, limbal transplantation was performed using healthy tissue from the left eye, which resulted in a smooth and stable corneal epithelial surface and regression of the corneal vascularization (Figs 1E and 1F).

Case 2. In an 11-year-old girl, Stevens-Johnson syndrome developed after taking penicillin at 6 years of age. This condition was complicated by dry eyes. Results of initial cytologic exam-

ination of the bulbar conjunctiva showed severe squamous metaplasia without goblet cells (for examples, see reference 19). Topical retinoic acid ointment alleviated her irritating symptoms. One year ago, her right eye became more irritated, red, and photophobic, and there was development of the progressive pannus and an irregular surface approaching the visual axis superior cornea (Fig 1G). Results of repeated impression cytologic examination on the pannus showed epithelial cells and numerous goblet cells (Fig 1H), confirming limbal deficiency.

Case 3. An 83-year-old man had conjunctival intra-epithelial neoplasia removed from the right superior limbal region 18 years earlier. Recurrence was noted 2 years ago, and conjunctival scraping was performed. Postoperatively, he had persistent ocular redness and photophobia. In addition, visual acuity decreased to 20/70 due to superior superficial vascularization and an irregular "whorl-like" fluorescein-stained surface (Figs 2A and 2B). Results of impression cytologic examination showed that this corneal surface was covered by epithelial cells and goblet cells which were continuous with the surrounding conjunctiva (Fig 2C). Limbal transplantation was performed by taking healthy limbal tissue from the left eye, resulting in the total resolution of his irritant symptoms, regression of vascularization, a stable surface (Fig 2D), and visual acuity of 20/50 for more than 2 years.

Case 4. A 33-year-old woman had progressive irritation, photophobia, and decreased visual acuity to 20/400 in the right eye and 20/300 in the left for 11 years after 10 years of soft and hard contact lens wear for correction of myopia. During this period, her right eye had received penetrating keratoplasty, which was repeated once 3 years later due to graft rejection. Both corneas showed superficial 360° vascularization and an irregular epithelial surface (Fig 2E). Results of impression cytologic examination disclosed that this abnormal corneal surface was covered by epithelial cells and goblet cells (Figs 2F and 2G).

Case 5. A 10-year-old boy had multiple endocrine deficiencies: Addison disease, hypoparathyroidism, autoimmune disease, and systemic candidiasis syndrome. In the last year, decreased visual acuity to 20/30 in the right eye and 20/40 in the left, progressive photophobia, and protective ptosis developed (Fig 2K). Both superior corneal surfaces showed irregular "whorl-like" changes with fluorescein staining (Figs 2L and 2M, respectively). Fine superficial vascularization also was noted on the 360° peripheral cornea (indicated by an arrow in Fig 2L). The impression cytologic examination performed on the superior corneal surface showed epithelial cells with goblet cells (Fig 2N). The patient remained symptomatic with intermittent improvement after topical preservative-free methylprednisolone treatment. The other patient, seen at 4 months of age, was suspected to have an endocrine abnormality because of subnormal cortisone and androstenediol levels and one episode of candidiasis. He had skin changes compatible with keratosis pilaris. Results of his impression cytologic examination also showed clear evidence of conjunctivalization (Fig 2O).

Case 6. An 80-year-old woman had a 15-year history of diabetes mellitus, leading to bilateral background diabetic retinopathy and ischemic maculopathy. She had not received any surgeries or photocoagulation. Both eyes had itching and burning, and the left superior cornea showed a fluorescein-stained, "whorl-like," irregular epithelium (Fig 3A). Three months later, her left eye had extreme photophobia and superficial peripheral corneal vascularization after routine dilated eye examination. Results of impression cytologic examination showed that conjunctival epithelial cells with goblet cells were present on the corneal surface (Fig 3B). Her symptoms eventually resolved after a course of topical preservative-free methylprednisolone.

Case 7. A 54-year-old woman had chronic itching and redness of both eyes since 19 years of age. Later, her allergic symptoms subsided but her eyes remained red, and 360° peripheral corneal pannus was noted in each eye with epithelial irregularity and stromal haze, leading to decreased vision (Fig 3C). Results of impression cytologic examination showed that epithelial cells together with goblet cells were present on the corneal surface (Fig 3D).

Case 8. A 69-year-old woman had an unknown chronic conjunctivitis and limbitis of the right eye worse than the left eye for 4 months. Inflammation progressed, despite various treatments for possible medicamentosa and presumed chlamydial and herpetic infections. Diagnostic scrapings and conjunctival biopsies were negative for chlamydial infection and ocular cicatricial pemphigoid. Results of serologic tests were negative for Epstein-Barr virus, tuberculosis, Lyme, and syphilis. The right cornea showed vascularization, irregular surface, and erosion (Figs 3E and 3F), the inferior fornix showed symblepharon and subepithelial fibrosis (Fig 3G), and the 360° limbus showed severe limbitis (Fig 3H). Because the conjunctival biopsy showed pronounced lymphocytic infiltration, we gave her topical cyclosporine treatment, which slowly suppressed the inflammation. Results of impression cytologic examination performed 11 months after the onset of inflammation showed that the peripheral corneal surface was covered by conjunctival epithelial cells with goblet cells (Fig 3I).

Case 9. A 52-year-old man had a history of chronic open-angle glaucoma for 16 years with timolol treatment for 8 years. His ocular history was pertinent for 1 year of contact lens wear and a minor welding burn 25 years ago. He had progressively blurred vision for 3 years and was noted to have peripheral keratitis in the right eye. No resolution was noted, despite different topical treatments. One year later, his left cornea showed a similar change with superficial epithelial irregularity covered by a fluorescein-stained "whorl-like" epithelium (Figs 3J and 3L) and peripheral superficial vascularization (Fig 3K). Results of impression cytologic examination showed that the peripheral corneal surface was covered by epithelial cells with goblet cells (Fig 3M).

Corneal Diseases without Limbal Deficiency

There were 40 patients suspected to have limbal deficiency based on clinical signs of corneal vascularization, delayed healing, and recurrent erosion, but they did not have cytologic evidence of limbal deficiency. Their clinical diagnoses are summarized in Table 2. Two patients had acid burns and photophobia, corneal vascularization, and pseudopterygium. Results of impression cytologic examination showed conjunctival squamous metaplasia and lid margin keratinization but no goblet cells on the corneal surface. Similarly, there were five patients with Stevens-Johnson syndrome whose cytologic findings also showed squamous metaplasia and keratinization. A representative case is illustrated in Figure 1I. The corneal findings resembled case 2 (Fig 1G). Nevertheless, the cytologic finding of moderate squamous metaplasia was entirely different (Fig 1J). There were nine patients in whom persistent epithelial defects, recurrent erosions and corneal vascularization developed after ocular surgeries. Again, results of impression cytologic examination showed squa-

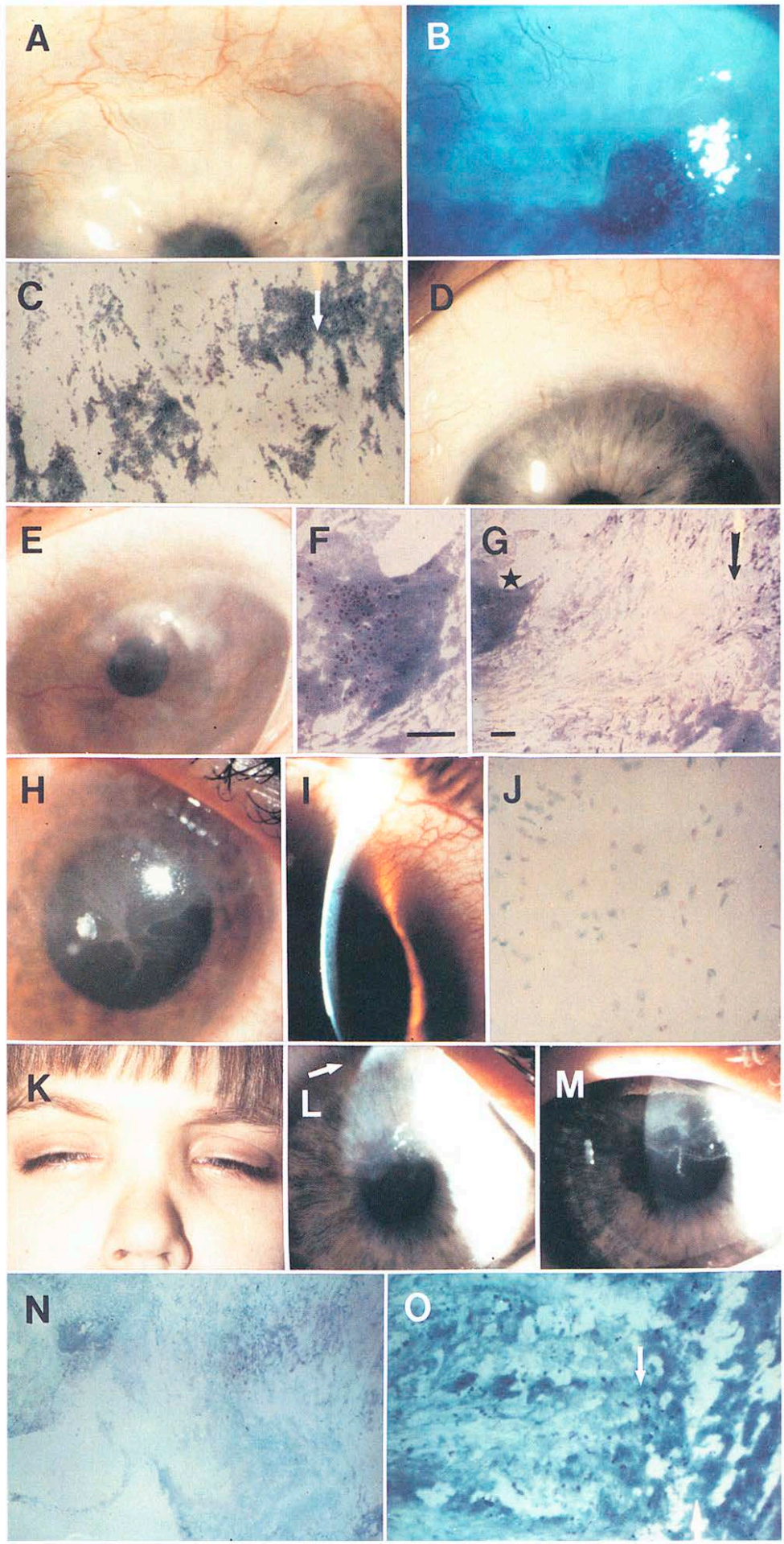

Figure 2. **A** and **B**, case 3. A patient with corneal vascularization and a migratory, fluorescein-stained, irregular corneal surface epithelium after multiple ocular surgeries to remove superior limbal conjunctival intraepithelial neoplasia. **C**, impression cytology of the superior perilimbal area shows goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow). For this figure and all others shown below, the arrowheads point toward the limbus. **D**, 5 weeks after limbal autograft transplantation. The return of a normal stable and smooth corneal surface is shown without vascularization. **E**, case 4. A patient with bilateral contact lens-induced keratopathy shows superficial 360° corneal vascularization, scarring, and surface irregularity. **F** and **G**, impression cytology of the left temporal perilimbal area shows migratory, goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow, G) (bars = 250 μm). **F**, the higher magnification of the area marked by an asterisk in Figure 2G shows many goblet cells. **H** and **I**, another contact lens wearer with bilateral thimerosal-induced keratopathy, an irregular, "whorl-like" superior corneal surface, and peripheral diffuse vascularization. **J**, impression cytology of the superior corneal surface shows many large squamous epithelial cells. **K** through **M**, case 5. A patient with bilateral keratitis associated with multiple endocrine deficiencies. Severe photophobia is shown. Both superior corneal surfaces show superficial vascularization (white arrow) and an irregular whorl-like epithelium. **N**, impression cytology of the left superior corneal surface (M) shows migratory goblet cell-containing conjunctival epithelial cells. **O**, impression cytology of another similar case shows goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow) (all impression cytology specimens are stained with periodic acid-Schiff and modified Papanicolaou as previously described[18]; photographs of C, J, and O are of the same magnification as F; photograph of N is of the same magnification as G).

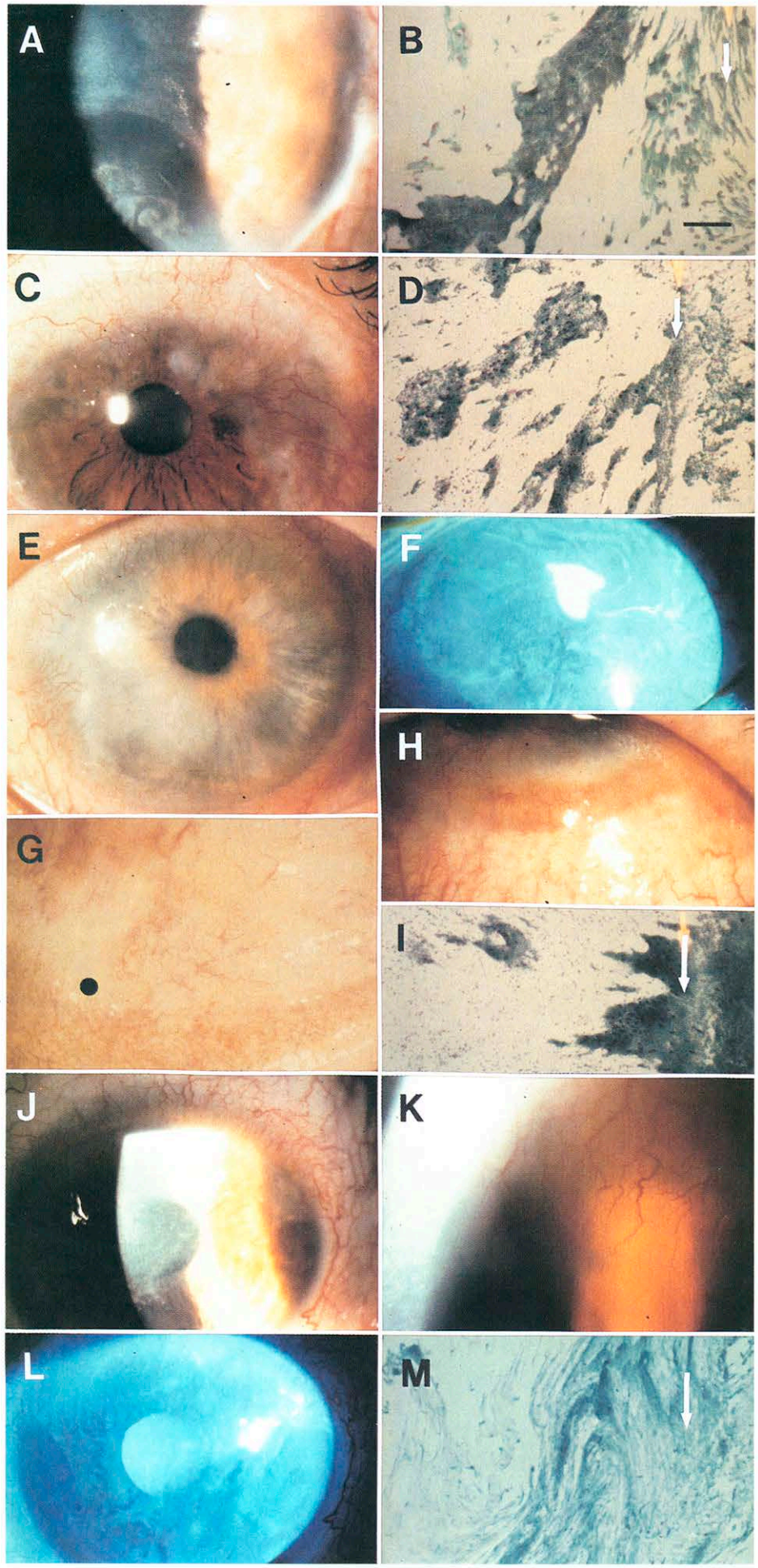

Figure 3. **A,** case 6. A patient with bilateral neurotrophic keratopathy from diabetes mellitus. A migratory, pigmented, and irregular epithelium is seen with fine peripheral vascularization. **B,** impression cytology of the superior perilimbal area shows goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow). For this figure and all others shown below, the arrowheads point toward the limbus (bar = 250 μm). **C,** case 7. A patient with bilateral allergic limbitis. Perilimbal scarring, an irregular corneal epithelium, and corneal vascularization are shown. **D,** impression cytology of the superior perilimbal area shows goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow). **E** through **H,** case 8. A patient with chronic limbitis of an unknown etiology. Diffuse superficial vascularization (E), a migratory and fluorescein-stained epithelium on the corneal surface are seen with a persistent epithelial defect (F), conjunctival symblepharon (G, indicated by a dot), and pronounced limbitis (H). **I,** impression cytology of the nasal perilimbal area shows goblet cell-containing conjunctival epithelial cells. **J** through **L,** case 9. A patient with bilateral idiopathic keratopathy. An irregular corneal surface (J), peripheral superficial vascularization (K), and a fluorescein-stained migratory epithelium (L) are shown. **M,** impression cytology of the temporal perilimbal area shows migratory, goblet cell-containing conjunctival epithelial cells on the corneal surface (left of the arrow) (all impression cytology specimens were stained with periodic acid-Schiff and modified Papanicolaou as previously described[18]; all micrographs were taken at the same magnification as B).

Table 2. Corneal Diseases without Limbal Deficiency

Group	Clinical Signs Suspected of Limbal Deficiency	No. of Patients
1, Acid burn	Corneal vascularization Pseudopterygium	2
2, Stevens-Johnson syndrome	Corneal vascularization	5
3, Abnormal ocular surface after ocular surgery	Persistent epithelial defect Corneal vascularization	9
4, Contact lens-related keratopathy	Irregular epithelial surface Recurrent erosions Corneal vascularization	5
5, Neurotrophic keratopathy	Irregular epithelial surface Corneal vascularization	12
6, Peripheral inflammatory disorders Ocular cicatricial pemphigoid Terrien marginal degeneration Atopic keratoconjunctivitis Rosacea keratitis	Corneal vascularization	7
Total		40

mous metaplasia and keratinization, mucous aggregates, and some migratory epithelial cells without goblet cells on their corneal surfaces.

In the group of patients with contact lens-related keratopathy, we also identified five patients who had a history of wearing soft contact lenses for 2 to 7 years. These patients had recurrent corneal erosions (1 patient), corneal vascularization (superiorly, 3 patients; 360°, 2 patients), and irregular corneal surfaces (all patients). Two of these patients had been identified to have thimerosal toxicity. One such patient is shown in Figures 2H and 2I. Results of impression cytologic examination showed conjunctival squamous metaplasia which was limited to the perilimbal area (Fig 2J), mucous aggregates, and papillary response of the tarsal conjunctiva. In the group with neurotrophic keratopathy, 12 patients were suspected to have limbal deficiency because they also had persistent corneal epithelial defects, an irregular epithelial surface, and corneal vascularization. Nevertheless, results of impression cytologic examination did not show limbal deficiency. Similar findings also were noted in seven patients with peripheral inflammatory keratopathy and chronic limbitis. Three patients had ocular cicatricial pemphigoid/pseudopemphigoid, two had Terrien degeneration, one had atopic keratoconjunctivitis, and one had ocular rosacea.

Discussion

This study establishes that the features of limbal stem cell deficiency, observed in experimental rabbit models created by limbal tissue removal,[12–15] also can be found in a number of human corneal diseases. These features include conjunctivalization (i.e., conjunctival epithelial ingrowth onto the corneal surface), corneal vascularization, chronic keratitis, and recurrent or persistent epithelial defects. Because the conjunctival epithelium is known to be more permeable than the corneal epithelium,[21] conjunctivalized corneal surfaces frequently are stained abnormally by fluorescein (Figs 1–3, see also reference 20). This view is supported by reports comparing the staining property of fluorescein with those of rose Bengal,[22,23] lissamine green B, and sulforhodamine B.[24] Unlike fluorescein, which detects cell–cell junctional changes as described above, rose-Bengal staining is most ideal for detecting deficiencies in the pre-ocular mucus layer in such pathologic situations as squamous metaplasia. Patients with limbal deficiency often have severe photophobia and decreased vision. Clinical detection of limbal deficiency is important because patients with these diseases generally are poor candidates for conventional corneal transplantation. This is because corneal vascularization and inflammation associated with conjunctivalization of the corneal surface increase the risk of allograft rejection (reviewed in references 17 and 18). Clinically, Kinoshita et al[25] have recognized that some of these diseases are characterized by the disappearance of the limbal palisades of Vogt, a sign suggestive of destruction of the stem cell-containing limbal tissue. Among all the above signs, we have proposed earlier[17,18] that the most reliable diagnostic criterion is conjunctivalization. As shown in this report, this phenomenon can be best detected clinically through the use of impression cytology, a relatively noninvasive conjunctival biopsy technique originally reported by Egbert et al.[26] In addition to its well-characterized applications for detecting and staging bulbar or tarsal conjunctival squamous metaplasia in various ocular surface disorders,[19,27–29] impression cytology is useful for detecting goblet cell-containing conjunctival epithelium on the corneal surface. We propose that this technique be used to diagnose and monitor the state of limbal deficiency.

Of 134 patients surveyed, 94 with limbal deficiency have been identified and can be divided into two subca-

tegories (Table 1). It is easy to understand that limbal deficiency can develop in the patients in category 1 because they, like the experimental models,[12–15] have an identifiable history of pathogenic destruction of limbal tissues. As shown in Table 1, such destruction can come from chemical or thermal injuries, Stevens-Johnson syndrome, multiple surgeries or cryotherapies, contact lens wear, or extensive microbial infection. We predict that this list may be expanded to include other diseases in which the destructive processes involve the limbal region.

Although all these patients had some clinical signs suggestive of limbal deficiency, not all showed cytologic evidence of limbal deficiency as defined by our criteria. As shown in Table 2, 21 patients had conditions similar to those in category 1 but did not show cytologic evidence of conjunctivalization. There are several possible explanations for this finding. First, the destruction might not be severe or diffuse enough to cause total loss of the stem cell population. Second, some of these patients may have subclinical limbal deficiency that may progress eventually to an overt state of limbal deficiency as their stem cell population depletes further. This hypothesis is supported by the 22 patients in category 1 with chemical/thermal injury in whom limbal deficiency did not develop until 3 to 70 years later. Third, it is conceivable that impression cytology may not be sensitive enough to detect all patients with subtle or early changes of conjunctivalization. To amend this potential problem, we advise to repeat impression cytology in suspicious cases. Last, some patients may not manifest limbal deficiency. This may explain cytologic findings of squamous metaplasia or keratinization, another pathologic state common in chemical injuries and Stevens-Johnson syndrome (Figs 1I and 1J). Even without limbal deficiency, diseases listed in Table 2 still carry vascularization, inflammation, and inadequate healing capacity, factors known to be a risk for conventional corneal transplantation. Cytologic demonstration of the absence of limbal deficiency will allow us to search for other causes and treatments. For contact lens-induced keratopathy, this concept is especially important because impression cytology can help differentiate limbal deficiency from a similar-appearing entity, thimerosal toxicity, which shows squamous metaplasia (Figs 2H–2J).

Diagnosis of limbal deficiency in category 1 diseases is crucial because it allows us to use limbal (stem cell) transplantation as a surgical procedure for corneal surface reconstruction.[30–33] In patients with unilateral destruction, we advise the use of limbal autograft transplantation. As shown previously[30,32] and in cases 1 and 3 (Figs 1 and 2), limbal transplantation can achieve rapid surface healing, stable ocular surface without recurrent erosions or persistent epithelial defects, regression of corneal vascularization, and restoration of a smooth and optically improved ocular surface, resulting in improved visual acuity, and probably increased success for subsequent keratoplasty. In patients with bilateral, especially asymmetric, involvement, impression cytology is useful in determining the severity in limbal destruction in the less-involved eye with respect to the extent of limbal circumference. This information is helpful to distinguish which patients require limbal autograft transplantation versus keratoepithelioplasty[34] or limbal allograft transplantation.[33]

We were astonished by the discovery of the category 2 diseases, which include such diverse diseases as aniridia, keratitis associated with multiple endocrine deficiencies, neurotrophic keratopathy, peripheral inflammatory keratitis or chronic limbitis, and idiopathic keratopathy (Table 1). These diseases do not present with the previously mentioned history but have gradual loss of limbal stem cell functions over time. All 13 patients with aniridia showed corneal changes similar to those previously described[20] (i.e., superficial pannus and irregular fluorescein-stained epithelium occurring early in life and progressive in nature). One other histopathologic study[35] also has shown ectopic conjunctival goblet cells on the corneal surface, pannus, and attenuation of the Bowman membrane—features supporting the diagnosis of limbal deficiency. Aniridia may represent a model of genetic disease for studying limbal deficiency caused by developmental factors. For keratitis associated with multiple endocrine deficiencies, a rare disease, the clinical features of our two patients resemble those described by others.[36,37] Both of our patients had keratitis, which is a common finding in 25% to 52% of patients, depending on the series.[36,37] The identification of limbal deficiency in this disease suggests that limbal stem cell functions are under hormonal modulation. The presence of limbal deficiency in 13 of the 25 patients with neurotrophic (neuronal and ischemic) keratopathy is an important finding. Although the pathogenesis remains unclear, we speculate that limbal stem cell functions also can be modulated by neuronal and vascular factors in the limbal stromal microenvironment. In addition to commonly well-recognized causes such as exposure and dryness, we surmise that limbal deficiency can be another cause for recurrent and persistent corneal epithelial defects. Our study also shows that advanced age and ocular surgery can aggravate compromised limbal function. Because the diseases in category 2 did not lose limbal stem cell population as a result of destruction, we believe that there may be a common underlying pathogenesis, namely that the stromal microenvironment is insufficient to support stem cell functions. Future studies should be directed to explore the mechanisms by which limbal stem cells are regulated by their stroma with respect to developmental, hormonal, vascular, and neuronal factors. These studies may uncover pathogenic mechanisms that underlie the diverse diseases associated with limbal deficiency, including those that are idiopathic. They also will help explain how inflammation can disturb stem cell functions in peripheral inflammatory keratopathy and chronic limbitis (Table 1) and in transplanted limbal tissue.[38] Finally, a better understanding of pathogenic mechanisms will enhance our therapeutic strategies in these difficult conditions.

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