IATROGENIC LIMBAL STEM CELL DEFICIENCY*

BY *Edward J. Holland,* MD, AND *Gary S. Schwartz,* MD, (BY INVITATION)

ABSTRACT

Purpose: To describe a group of patients with limbal stem cell (SC) deficiency without prior diagnosis of a specific disease entity known to be causative of SC deficiency.

Methods: We performed a retrospective review of the records of all patients with ocular surface disease presenting to the University of Minnesota between 1987 and 1996. Patients were categorized according to etiology of limbal deficiency. Patients who did not have a specific diagnosis previously described as being causative for limbal deficiency were analyzed. Risk factors, clinical findings and sequelae were evaluated.

Results: Eight eyes of six patients with stem cell deficiency not secondary to a known diagnosis were described. All eyes had prior ocular surgery involving the corneoscleral limbus. Six eyes had been on chronic topical medications and all eyes had concurrent external disease such as pterygium, keratoconjunctivitis sicca, rosacea or herpes simplex virus keratitis. All eyes had superior quadrants affected corresponding to areas of prior limbal surgery. Sequelae of disease included corneal scarring and neo-vascularization, and five eyes had with visual acuity of 20/200 or worse.

Conclusions: Because the epitheliopathy started peripherally and extended centrally in all patients, we feel it represents a stem cell deficiency. The fact that all patients were affected superiorly, at sites of a prior limbal surgical incision, points to surgical trauma to the SC as the likely major etiologic factor for the deficiency. The surgical trauma to the limbal SC probably made these cells more susceptible to damage from other external disease influences and toxicity from chronic topical medications. Because the stem cell deficiency is secondary to prior ocular surgery and chronic topical medications, we propose the term "iatrogenic limbal stem cell deficiency".

*From the Department of Ophthalmology, University of Minnesota School of Medicine, Minneapolis.

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INTRODUCTION

Recent studies have shown that the stem cells responsible for the renewal of the corneal epithelium are located at the corneoscleral limbus.[1-12] When significant limbal stem cell deficiency exists, conjunctival epithelial cells invade and populate the corneal surface. This process of conjunctivalization results in a thickened, irregular, unstable epithelium, often with secondary neovascularization, inflammatory cell infiltration, and disruption of the basement membrane.[13] Punctate corneal epithelial defects and larger confluent defects are common in patients with stem cell deficiency and ultimately lead to corneal scarring, ulceration, and loss of vision.

A limited number of specific disease entities are known to be associated with limbal stem cell deficiency. These entities include aniridia, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, chemical injury, thermal injury, severe contact lens–induced keratopathy, corneal intraepithelial neoplasia, and trachoma. Aniridia is a primary stem cell disorder that may result from a deficiency in the development or maintenance of limbal stem cells.[14-16] Secondary disorders of limbal stem cells are more common. Stevens-Johnson syndrome and ocular cicatricial pemphigoid may result in severe inflammation with conjunctival scarring, goblet cell depletion, aqueous tear deficiency, and the eventual loss of limbal stem cells.[17] In both chemical and thermal injuries, there is direct injury to the limbal stem cells. Chronic severe contact lens–induced keratopathy results from chronic trauma to the corneoscleral limbus from the contact lens and/or upper field.[18] We believe that Hypoxia may also have a roll in stem cell deficiency secondary to contact lens use. Extensive corneal intraepithelial neoplasia almost always originates at the limbus, and likely results in the replacement of healthy stem cells with neoplastic cells.[19]

However, there is another group of patients who present with stem cell deficiency, yet who do not have any of the aforementioned disease entities. These patients appear to have a disease process that is slower to develop, milder, and not associated with a single underlying diagnosis. The purpose of this study is to better characterize this form of stem cell deficiency, identify risk factors, and postulate an etiology.

METHODS

We retrospectively reviewed all charts and operative records of patients presenting to the cornea clinic at the University of Minnesota Hospitals and Clinic with stem cell deficiency between 1987 and 1996. Patients with stem cell deficiency that was due to a single, specific diagnosis were excluded. Therefore, all patients with stem cell deficiency associated with aniridia, chemical injury, thermal injury, Stevens-Johnson syndrome, ocu-

lar cicatricial pemphigoid, corneal intraepithelial neoplasia, severe contact lens-induced keratopathy, and trachoma were excluded from this study.

We then evaluated risk factors for the development of stem cell deficiency. Specific factors that were studied included the number and types of prior ocular surgery, the coexistence of ocular disease that may be associated with ocular surface disease, and the use of chronic topical medications.

We determined the location and extent of the epitheliopathy and the degree of stromal involvement for each case. Visual acuity at the most recent examination was also recorded.

RESULTS

Eleven eyes of 9 patients who had epitheliopathy consistent with limbal stem cell deficiency and who met the aforementioned requirements were evaluated (Table I). Age of patients at presentation ranged from 42 to 90 years; mean age was 73.4 years and median age 80 years. Six eyes of 5 male patients and 5 eyes of 4 female patients were included in the study.

All eyes had had previous ocular surgery. The number of procedures per eye ranged from 1 to 4, with a mean of 2.3 and a median of 2. The most common procedure seen among these patients was intracapsular cataract extraction, which had been done in 7 of the 11 eyes. Six of the 11 eyes had undergone penetrating keratoplasty, 3 of which had had the procedure repeated at least once. All 11 eyes had had surgery involving the superior limbus. One patient had had multiple conjunctival autografts during repeated pterygium excision, 2 had undergone extracapsular cataract extraction, and 1 had had transsclerally sutured posterior chamber intraocular lens.

In addition, 8 eyes had been exposed to long-term treatment with topical medications, including pilocarpine, levobunolol, betaxolol, trifluridine, and sulfacetamide-dexamethasone. One eye had also received a 4-week course of topical mitomycin C four times daily following pterygium excision with conjunctival autograft.

Three eyes of 2 patients had a long history of diabetes mellitus. Five eyes of 4 patients had keratoconjunctivitis sicca diagnosed by a Schirmer test performed without topical anesthesia with less than 2 mm of wetting. Other possible contributing factors included chronic aphakic or pseudophakic corneal edema (4 eyes), rosacea (patient 3, both eyes), and herpes simplex virus keratitis (patient 5).

All patients were affected in the superior quadrant (Figure 1). Two eyes of 1 patient had epitheliopathy extending over the entire cornea. The epitheliopathy started peripherally in all 11 eyes. It extended to the visual axis, resulting in marked decreased visual acuity in 8 eyes of 6 patients.

TABLE I: SUMMARY OF CLINICAL DATA

Patient No., Age (yr), Gender	Eye	Visual acuity	Prior surgeries	Contributing ocular conditions
1,42,F	L	20/70	Pterygium excisions with conjunctival transplant x 3, superficial keratectomy	Topical mitomycin QID x 30 days
2,81,F	R	20/400	ICCE	Keratoconjunctivitis sicca, diabetes mellitus, pilocarpine and beta-blocker therapy for many yr
2,81,F	L	20/400	ICCE	Keratoconjunctivitis sicca, diabetes mellitus, pilocarpine and beta-blocker therapy for many yr
3,76,M	R	20/200	ECCE, PK With sulcus PCL, tube-shunt procedure	Rosacea, keratoconjunctivitis sicca, topical beta-blocker therapy for 5 yr, antibiotic/steroid therapy for several yr
3,76,M	L	HM	ICCE, PK with TS-PCL	Rosacea, keratoconjunctivitis sicca, antibiotic/steroid therapy for several yr
4,82,F	R	20/400	ICCE, PK, repeat PK with TS-PCL	Rubeola keratitis
5,62,M	L	20/30	PK x 3, ECCE with PCL	HSV keratitis, chronic viroptic, nasolacrimal duct obstruction
6,66,F	L	20/70	Cataract needling, PK with TS-PCL, repeat PK	Aphakic corneal edema, keratoconjunctivitis sicca
7,82,M	R	20/60	ICCE, PK with TS-PCL	Glaucoma, diabetes mellitus, aphakic corneal edema, topical beta-blocker and pilocarpine therapy for 11 yr
8,90,M	R	20/150	ICCE	Aphakic corneal edema, glaucoma, topical beta-blocker and pilocarpine therapy for many yr
9,80,M	R	20/40	ICCE with ACL	Pseudophakic corneal edema

ACL, anterior chamber lens; ECCE, extracapsular cataract extraction; HM, hand motions; HSV, herpes simplex virus; ICCE, Intracapsular cataract extraction; PCL, posterior chamber lens; PK, penetrating keratoplasty; TS-PCL, transsclerally sutured posterior chamber lens;

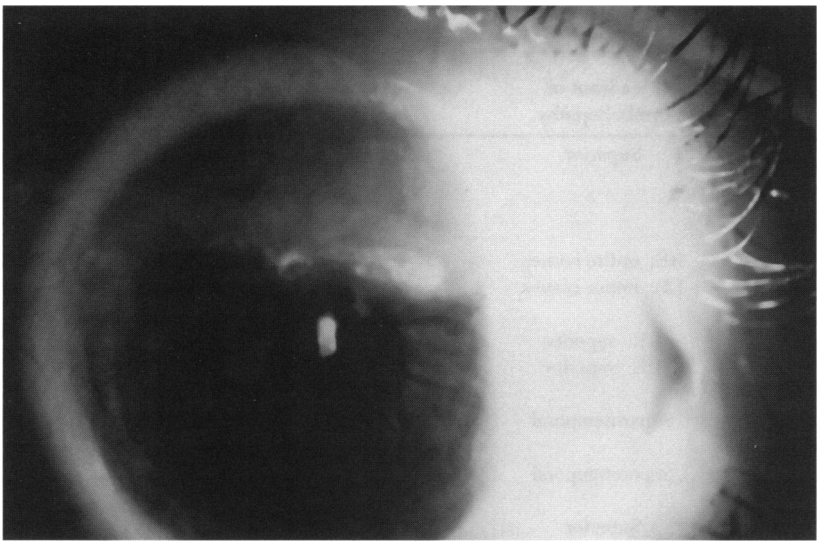

FIGURE 1
Patient 1. Slit-lamp photograph demonstrating superior sectoral area of thickened, irregular epithelium with neovascularization and lipid keratopathy.

Two patients with only peripheral disease maintained visual acuity of 20/40 or better. In 3 cases, the visual acuity was reduced to 20/60 or 20/70, and in the remaining 6 cases it was reduced to 20/150 or worse. In 4 cases (patients 1, 2 [both eyes], and 4), anterior stromal scarring developed as a result of the chronic epitheliopathy (Table II).

DISCUSSION

A stable ocular surface depends on the proper functioning of the limbal stem cells. A limited number of disorders have been described that lead to ocular surface instability from abnormal stem cell function. Aniridia is a primary disorder where improper development of the anterior segment results in a decreased number of stem cells. In all likelihood, the remaining cells, in addition to being decreased in number, are also dysfunctional. Aniridic patients are not born with abnormal ocular surfaces. As they get older, epitheliopathy develops in the peripheral cornea and slowly extends centrally. This advancement of surface disease indicates a progression of limbal stem cell dysfunction or loss.

Another cause of stem cell dysfunction is severe conjunctival deficiency. Examples of this category include patients with Stevens-Johnson syndrome and ocular cicatricial pemphigoid, diseases resulting in inflamma-

TABLE II: SEQUELAE OF LIMBAL STEM CELL DEFICIENCY

Patient No.	Quadrant of epitheliopathy	Central cornea affected?	Stromal sequelae of epitheliopathy
1	Superior	Yes	Stromal scarring, neovascularization, lipid keratopathy
2	RE: entire cornea	Yes	Stromal scarring
	LE: entire cornea	Yes	Stromal scarring
3	RE: superior	Yes	None
	LE: superior	Yes	None
4	Superotemporal	Yes	Stromal scarring
5	Superotemporal	No	None
6	Superior	No	None
7	Superior	Yes	None
8	Superior	No	None
9	Superotemporal	Yes	None

LE, left eye; RE, right eye.

tion of the conjunctiva and limbus. In these patients, the primary disease affects the conjunctiva, and the stem cells are involved secondarily. The corneal surface is almost always normal throughout the early stages of these disease processes. Only later, when the stem cell population is diminished from the constant conjunctival inflammation, does the corneal epithelium become abnormal. Eventually, if stem cell loss continues, conjunctivalization of the cornea will occur and the patient will show clinical signs of stem cell deficiency.

In other patients, stem cell deficiency develops because of loss of stem cells from chemical or thermal injury. Within this category are those patients with ocular surface disease secondary to alkali, acid, or thermal injury. These patients sustain loss of the majority of their stem cell populations at the time of the injury. In all likelihood, they sustain further, gradual stem cell loss in the period following injury owing to inflammation. The injury to the conjunctival tissue is another important factor that leads to the worsening of the stem cell function seen in these patients.

Another cause of ocular surface disease due to stem cell deficiency is contact lens–induced keratopathy, which can lead to severe conjunctivalization of the cornea.[18] The ocular surface disease here is most likely due to chronic injury to the limbal stem cells. The injury to the stem cells is probably due to chronic ischemia in these patients, as it is worse in the superior quadrants than in all other quadrants.

Corneal intraepithelial neoplasia is a less commonly described cause of ocular surface disease from limbal stem cell deficiency. Corneal intraepithelial neoplasia is felt to cause stem cell deficiency from replacement of normal stem cells with neoplastic ones. Typically, the ocular surface disease starts sectorally at the location of the limbal corneal intraepithelial neoplasia. This abnormal corneal tissue then spreads circumferentially, and if enough of the limbus is eventually involved, total ocular surface failure may result.

The aforementioned categories account for the vast majority of patients with stem cell deficiency reported in the literature. However, there is another group of patients who have stem cell deficiency yet do not fit into any of these categories. It is likely that in many of these patients, stem cell deficiency goes undiagnosed because the disorder does not fit into a classic etiology. In the present study, we describe 11 eyes of 9 patients with stem cell deficiency not attributable to any previously described etiology.

Tseng[20] categorized causes of stem cell deficiency according to whether they represented hypofunction or aplasia of stem cells. Those causes listed as resulting from stem cell hypofunction included aniridia, multiple endocrine deficiency, neurotrophic keratopathy, chronic limbitis, and pterygium and pseudoterygium. Causes listed as resulting from stem cell aplasia included chemical or thermal injury, Stevens-Johnson syndrome, and multiple surgeries or cryotherapies to the limbal region. The last category is of interest to the current study. Although this entity was named in Tseng's study, details of patients with this condition were not described. We feel this category corresponds to the cases that we are presently describing, and we have performed our current study to further describe patients within this category.

We describe 11 eyes of 9 patients with clinical findings consistent with limbal stem cell deficiency. All eyes had a chronic, progressive epitheliopathy that began in the peripheral cornea and progressed centrally. In some cases, the epitheliopathy was accompanied by fine neovascularization. The clinical findings were not consistent with other causes of epitheliopathy, such as keratoconjunctivitis sicca, blepharokeratoconjunctivitis, or toxic epitheliopathy. In addition, the epitheliopathy neither responded to standard therapies for dry eye management nor resolved after reduction or cessation of topical medications.

Each of the eyes described had had prior surgery involving the corneoscleral limbus, and the median number of prior surgeries per eye was two. We hypothesize that direct trauma to the limbus at the time of surgery results in loss of stem cells. Ocular surgery involving the corneoscleral limbus is, of course, quite common, yet limbal stem cell deficiency from prior surgery is quite rare. We feel that surgery to the limbus does not typically cause loss of enough of the stem cell population to result in ocular surface disease. However, surgical manipulation of the limbus does initiate a localized loss of stem cells that predisposes patients to development of the clinical findings of limbal deficiency when exposed to further stem cell trauma.

In support of this argument, all of our patients were affected in the superior quadrant corresponding to the site of prior limbal surgery. It is likely that both the length and location of these incisions are influential in the development of stem cell deficiency. Two eyes of 2 patients had had extracapsular cataract extraction utilizing an 11-mm limbal wound. Seven eyes of 6 patients had had intracapsular cataract extraction with wounds typically involving 180° of the corneal perimeter (Figure 2). The anterior nature of intracapsular cataract extraction wounds and older extracapsular cataract extraction wounds likely caused direct trauma to the limbal stem cells at the time of the surgery. It is interesting to note that none of the patients reported in this series had had cataract surgery by phacoemulsification. It is very likely that phacoemulsification incisions, by nature of their being both shorter and more posterior than the extracapsular and intracapsular cataract extraction ones, result in significantly less trauma to the limbal stem cell population.

Another possible factor for the superior location of the stem cell damage is the contribution of the upper eyelids. Although difficult to evaluate, it is possible that mechanical forces elicited by the upper eyelids may cause localized ischemia and further stem cell damage.

Long-term use of topical medications including pilocarpine, beta blockers, antibiotics, and corticosteroids appeared to play a significant role in 6 eyes. Topical medications such as these are known to be toxic to the corneal epithelium. It is possible that chronic use of these medications is also toxic to the limbal stem cells. One eye was exposed to topical mitomycin C for 30 days. Mitomycin C is an antimetabolite that specifically targets dividing cells. The entire ocular surface was exposed to mitomycin C, but the only site where the patient's stem cells decompensated was the site of the previous conjunctival autograft. It is probable that the surgical trauma in combination with the antimetabolic effect of the mitomycin C led to irreversible stem cell damage and ocular surface disease.

Other external disease diagnoses that may have contributed to stem cell failure and were present in some of these patients included kerato-

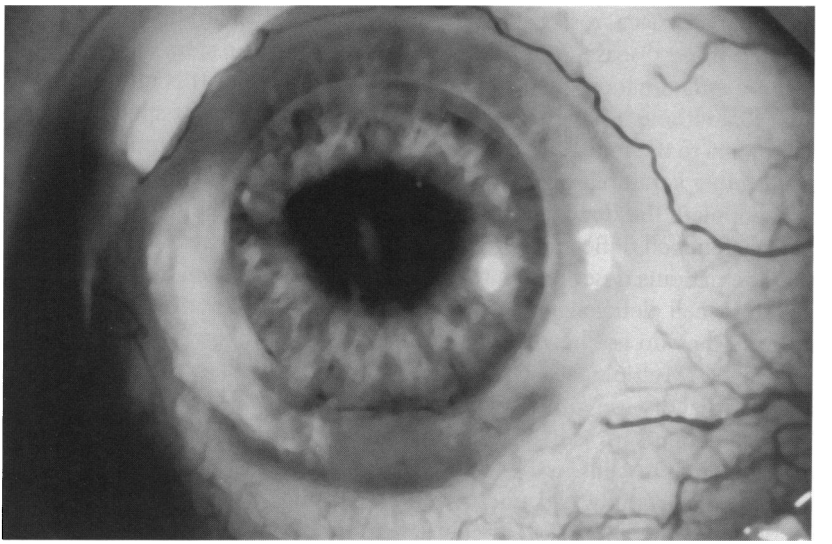

FIGURE 2A

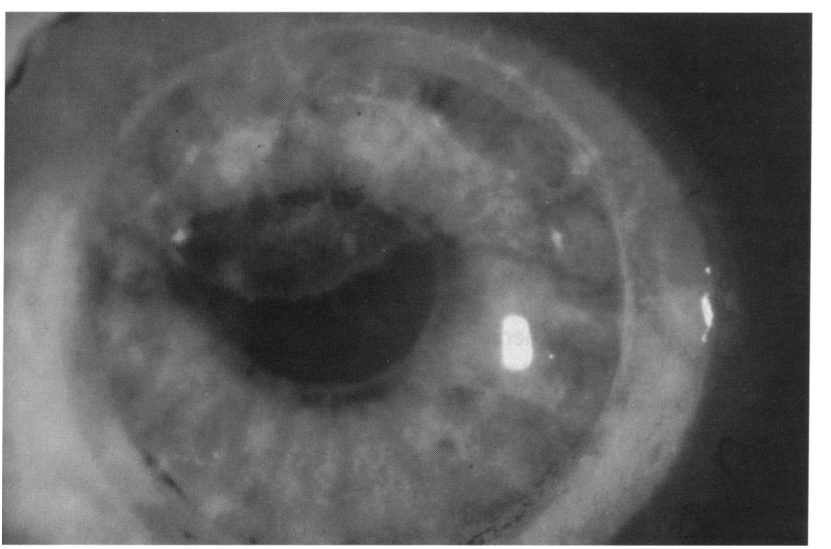

FIGURE 2B

Patient 3, right eye. A. Slit-lamp photograph demonstrating abnormal limbal vascular pattern superiorly in patient with previous intracapsular cataract extraction, and subsequent penetrating keratoplasty with transsclerally sutured posterior chamber lens. Patient also had had a tube-shunt procedure. B. Fluorescein dye demonstrates abnormal epithelium of superior aspect of graft.

conjunctivitis sicca, rosacea, and herpes simplex virus keratitis. It is possible that these disease entities led to exhaustion of the stem cells through chronic inflammation or increased epithelial turnover. It is interesting to note that although penetrating keratoplasty does not cause specific surgical trauma to the limbal stem cells, 6 eyes in this study had a total of 9 prior penetrating keratoplasties. The increased demand on the host epithelium to repopulate the donor graft is probably a factor for the development of limbal stem cell deficiency or exhaustion in these patients.

The patients described in this study are different from those with limbal stem cell deficiency that fits one of the well-described categories, because they do not have a single disease entity that leads to their limbal deficiency. Multiple factors, including surgery, use of topical medications, and external disease, probably all contribute to limbal stem cell deficiency in these patients. It is likely that prior surgery, with its traumatic insult to the limbal stem cells, is the most important etiologic factor, since each patient in this study had undergone surgery involving the corneoscleral limbus, and the area of stem cell deficiency always corresponded to the area of prior limbal surgery. It must be borne in mind that the overwhelming majority of patients undergoing surgery to the corneoscleral limbus will not go on to have clinically significant limbal stem cell deficiency. Concomitant ocular diseases such as keratoconjunctivitis sicca and herpes simplex virus keratitis, together with long-term use of topical medications, must be recognized as important factors contributing to the slow, chronic insult to the limbal stem cell population.

The clinical course of this disorder is a slowly progressive epitheliopathy beginning at the peripheral cornea and progressing centrally. It may be sectoral in an area corresponding to an area of previous limbal surgery. This sectoral nature can lead to the appearance of a wedge-shaped area of abnormal epithelium immediately adjacent to normal epithelium (Figure 3). By and large, the sectoral nature separates this form of limbal stem deficiency from those forms described previously, which tend to involve the entire limbus.

Although these patients have not been previously described, they are probably more common than the literature might suggest. It is important to recognize this disorder as a limbal deficiency, because standard medical therapies will not address its cause. The sequelae of this condition include stromal scarring and significant loss of vision, as was seen in 6 eyes in this study. Although not related to a single disease process, as is the case for the other causes of limbal deficiency, we feel these patients make up an additional category of limbal deficiency because of the many similarities within the group. Each of these patients had undergone surgery involving the corneoscleral limbus, and many patients had also received extended courses of topical medications. We believe that these medical and surgi-

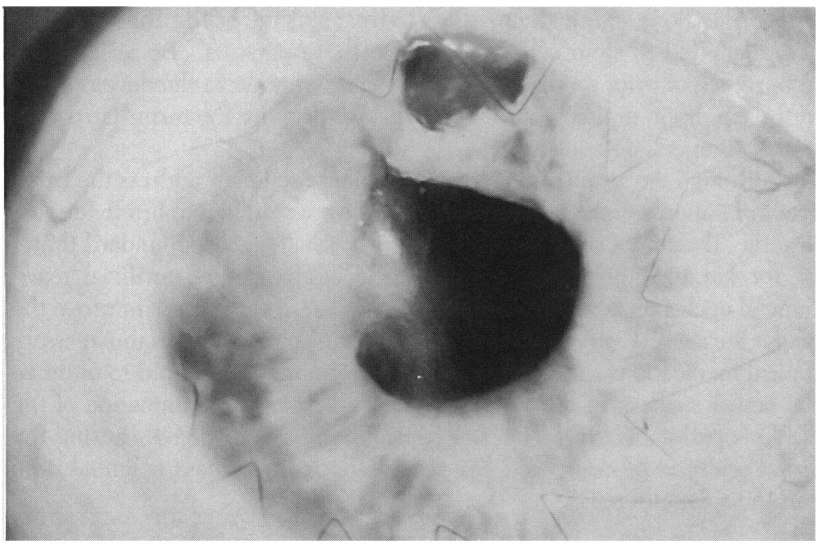

FIGURE 3A

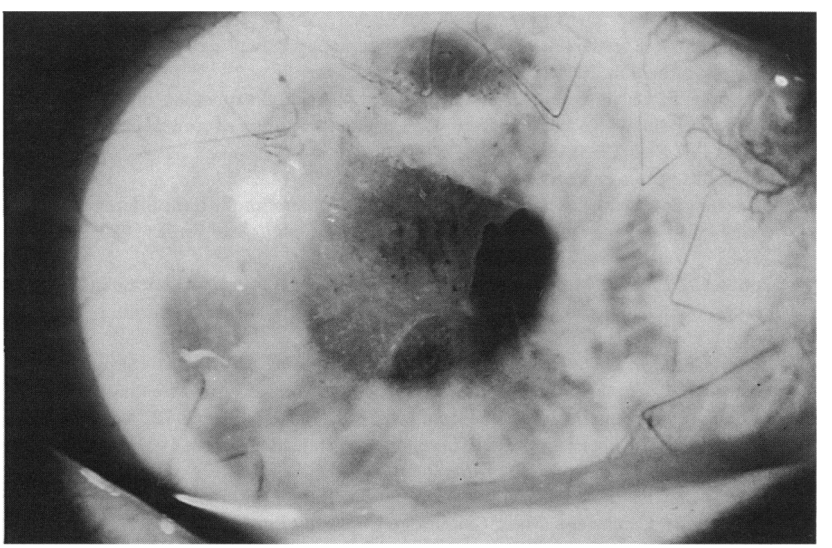

FIGURE 3B

Patient 4. A. Slit-lamp photograph demonstrating wedge-shaped area of stromal scarring secondary to chronic epitheliopathy in patient with prior intracapsular cataract extraction, penetrating keratoplasty, and repeated penetrating keratoplasty with transsclerally sutured posterior chamber lens. B. Fluorescein dye demonstrates that abnormal epithelium originates from superotemporal limbus and extends into pupillary axis.

cal interventions, although necessary to treat specific ocular abnormalities, eventually led to stem cell deficiency in these patients. Because of the importance of prior medical and surgical intervention to the development of the condition in this group of patients, we propose the term "iatrogenic limbal stem cell deficiency."

Although the scope of this study does not specifically address the treatment of patients with stem cell deficiency, we would like to briefly discuss it here. Treatment of these patients can be challenging. Standard therapy for keratoconjunctivitis sicca, such as nonpreserved artificial tears, punctal occlusion, and lateral tarsorrhaphy, can in some cases improve the ocular surface. Every effort should be made to discontinue unnecessary topical medications. Although these interventions were found to improve the ocular surface, in none of our patients did we find elimination of the epitheliopathy. Therefore, if the central cornea is involved, causing the visual acuity to be decreased, the only definitive treatment is limbal stem cell transplantation.

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DISCUSSION

DENIS M. O'DAY, MD. Dr Edward Holland has just reviewed for us an interesting group of patients. In this retrospective study dating to 1986, he has presented evidence for the existence of another etiologic category in the emerging syndrome of limbal stem cell deficiency. These cases add support to the four reported by Drs Puangsricharern and Tseng in 1995.[1] However, in Dr Holland's cases, chronic use of topical medications and preexisting corneal disease are implicated as precipitating factors in addition to the limbal surgery. The study has the inherent limitations of a retrospective design. Because of this, caution is necessary in ascribing the etiology to a particular set of circumstances or in identifying potential risk factors for the disease.

As is rather typical of a relatively newly described disorder, disease definition can be problematic, particularly when the pathogenetic mechanisms are still incompletely understood. Therefore, establishing criteria for the diagnosis of limbal stem cell deficiency is a priority. The clinical features are well described and form the basis for the diagnosis in these cases. Unfortunately, they are not specific for this entity. Similar changes can be seen from other causes.[1] Puangsricharern and Tseng's study of 134 patients included 40 with corneal disease suggestive of limbal stem cell deficiency in whom impression cytology failed to manifest cytologic evidence of the disease. Some of these patients had diagnoses similar to those in the present series, and all qualified by clinical criteria for the diagnosis of limbal stem cell deficiency. Cytologic findings were either normal or showed squamous metaplasia. There is some argument about the diagnostic sensitivity of impression cytology. Since this disease evolves over time, the cytopathologic changes that are seen by this technique may possibly lag behind the clinical manifestations. Nevertheless, it would seem prudent to be as rigorous as possible in defining the syndrome. Diagnostic cytologic examination seems to be one way to do this.

This issue aside, the study raises some interesting questions. If the role of limbal surgery and, in particular, cataract surgery in the pathogenesis of

the disease is accepted, why are they such uncommon causes, given the prevalence of cataract surgery and the need, until recently, for a large incision? Also, why do topical medications, known to preferentially alter the inferior cornea, exert their effect primarily on the superior limbal stem cells?

If further investigation does confirm the findings of the study, the impact on ophthalmic practice could be profound. I hope Dr Holland continues his studies prospectively. I appreciate the opportunity to comment on his paper.

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TOM O. WOOD, M.D. One of the procedures that Ed Holland has made popular is using living related donors for stem cell deficient patients. If we remove half of the limbus from a donor, are we threatening their vision later in life?

RICHARD FORSTER, MD. Ed, I really enjoyed your paper, but I have a great deal of trouble with the term stem cell deficiency. In the first slide Ed showed, indicating the location of stem cells, we note that they are apparently in the deep layers of the limbus; but most of the diseases that we are talking about that produce what we call stem cell deficiency are superficial diseases. I have trouble imagining how a simple keratoplasty, where we don't go near the limbus, can produce limbal stem cell deficiency. Therefore I think we need better criteria for making the diagnosis and better methods for quantitating limbal stem cells. I think that perhaps they need to be biopsied. Somehow we need to be sure that it is the stem cells that are causing the problem that we so easily call stem cell deficiency.

GEORGE WARING, MD. I want to echo the question as to whether or not we should keep doing allografts or using living related donors. If you have one good eye and take half the limbus from this eye to put in the affected eye, are we compromising the good eye? I have done many limbal allogralts from one good eye to a bad eye and have not noticed this problem. My second question is why you did not biopsy some of these patients to demonstrate what you are postulating is indeed a limbal stem cell deficiency?

GEORGE L. SPAETH, M.D. This is a follow up on Don Minckler's comment on the last paper. Patients who have had mitomycin C or some other

antimetabolite during a guarded filtration procedure are frequently helped by the use of artificial tears. The problem appears to be excessive tearing in response to something wrong on the surface. Have you selectively looked at patients who have had mitomycin filtration procedures in comparison with those who have had standard guarded filtration procedures without an antimetabolite to see if maybe some stem cell problem is responsible for the very severe symptomatology that some of these patients have.

EDWARD J. HOLLAND, M.D. I would like to thank you all for those excellent comments. First to address Dr. O'Day. I think it may be important to try to obtain a biopsy-proven diagnosis to outline this condition. We did not report the results of any histological studies in this paper. However, a couple of patients have gone on to stem cell transplantation and at that time, we did take a specimen and sent it to pathology which confirmed conjunctivalization of the cornea with invasion of goblet cells to the ocular surface. This finding has been used by ocular surface specialists as definitive criteria for stem cell deficiency. I do agree with the question, "Why don't we see this more often?" Millions of patients have undergone intracapsular cataract surgery with large limbal incision of 180 degrees. I think it points out the fact that this disease has to have more than one insult or one hit. If we simply look at cataract surgery alone, overall this is probably not enough of an insult to the stem cells but our patients had cataract surgery, trabeculectomy, topical medications, and penetrating keratoplasty. Therefore, it probably takes more than a single insult to the area of the limbus to produce severe limbal stem cell deficiency.

With respect to Dr. Wood, his concern for donors undergoing living related conjunctival limbal allograft (a term we use in which we take the conjunctiva and the limbus from a relative to treat limbal stem cell deficiency); I think you have some concerns as to what is the risk to the donor. When we recommend that if the donor has any history of ocular surface disease, any previous surgery, or any use of chronic topical medications, we do not use them as donors.

Secondly, we try not to take excessively large pieces of donor cells. We try to restrict it to 2-3 mm of the superior and inferior limbus, thus leaving 60-75% of their stem cells without surgery. In our series, as well as studies reported by Doctors Kenyon and Tseng, we have not seen donor patients develop complications. However, I would certainly carefully screen donors for stem cell transplant procedures and would not recommend the use of any patient with any ocular history of surgery or chronic medication.

Dr. Forster commented on what is the role of penetrating keratoplasty and how can that tip this patient over to stem cell deficiency. We do not

think that penetrating keratoplasty alone is a risk factor. But our patients had numerous other risk factors and maybe the PK just pushed the cornea over the edge and is just another type of insult to the ocular surface. Your comment whether we need to biopsy these patients to confirm the diagnosis, I think is a good one. We made the diagnosis in most of our patients based on their clinical appearance, but it certainly would not be hard to remove this layer, including a small area of the limbus, and send it to pathology to confirm goblet cells invading the comea epithelium.

Dr. Waring's question about biopsy I think has been answered.

Finally, Dr. Spaeth's comments on the risk of topical mitomycin. I am concerned about that. I do not know if topical mitomycin alone can cause stem cell decompensation but our patients decompensated late after many, many years of their injury to the stem cells. They had their cataract surgery 10-15 years ago. They had their chronic medications for a long time and then they developed stem cell deficiency. So I do have a concern that we may see stem cell insult and stem cell deficiency years after glaucoma surgery with topical mitomycin. Thank you.