Limbal Lacuna: A Novel Limbal Structure Detected by In Vivo Laser Scanning Confocal Microscopy

Siamak Zarei-Ghanavati, MD
Arturo Ramirez-Miranda, MD
Sophie X. Deng, MD, PhD

ABSTRACT
Limbal lacuna is a novel limbal structure detected by in vivo laser scanning confocal microscopy in normal subjects and the unaffected limbus in sectoral limbal stem cell deficiency. This unique structure harbors normal limbal epithelial cells deep inside the limbal stroma and the palisades of the Vogt are absent in these subjects. This well-protected location could better shield limbal stem/progenitor cells from damage and might serve as an alternative niche for limbal stem cells.

INTRODUCTION
Limbal stem cells are presumed to reside at the limbus, and the palisades of Vogt has been suggested as the niche of limbal stem cells. Therefore, the palisades of Vogt is usually used as a hallmark for the normal limbus, and all studies to date have focused on this structure. In vivo laser scanning confocal microscopy acquires live images of microstructures at the cellular level, and some studies have used this method to evaluate human limbal structures, including the palisades of Vogt.

Using laser scanning confocal microscopy, we detected and characterized the limbal lacuna, a novel structure in the human limbus that may serve as an alternative niche for limbal stem cells.

DESIGN AND METHODS
We found limbal lacunae-like structures in four eyes of four cases, two healthy subjects and two patients with sectoral limbal stem cell deficiency, using the Heidelberg Retina Tomograph III Rostock Corneal Module laser scanning confocal microscope (Heidelberg Engineering GmbH, Dossenheim, Germany). The ages of the healthy subjects were 71 and 79 years, and the ages of the patients with limbal stem cell deficiency were 49 and 80 years. Confocal imaging was performed in all four quadrants: superior, nasal, temporal, and inferior. The causative etiology of sectoral limbal stem cell deficiency was Stevens–Johnson syndrome in one eye and multiple ocular surgeries (cataract surgery with superior main wound and two trabeculectomies) in the other. In these two eyes, the area of vortex keratopathy associated with late fluorescein staining and epithelial thinning was limited to the superior peripheral cornea and the central and inferior cornea epithelia appeared normal.

From Jules Stein Eye Institute, University of California, Los Angeles, California.

Originally submitted February 27, 2011. Accepted for publication October 28, 2011. Posted online December 8, 2011.

Supported by Prevent Blindness of America (SXD), International Council of Ophthalmology, International Helmerich Fellowship (SZ-G), and Conde de Valenciana Foundation and Pan-American Ophthalmological Foundation (AR-M).

The authors have no financial or proprietary interest in the materials presented herein.

The authors thank Mr. Jose Castellano for his technical help in acquiring the confocal images and Dr. Alberto Haber-Olguin for his help with the three-dimensional reconstruction of the images.

Address correspondence to Sophie X. Deng, MD, PhD, Cornea and Uveitis Division, Jules Stein Eye Institute-UCLA, 100 Stein Plaza, Los Angeles, CA 90095. E-mail: deng@jsei.ucla.edu
do: 10.3928/15428877-20111201-07
FINDINGS

Well-demarcated, lacuna-like structures that contained clusters of highly packed limbal epithelial cells that extended into the deep underlying limbal stroma were seen (Figs. 1A and 1B). The general characteristics of lacunae were the same in all four cases. The lacunae were filled with normal limbal epithelial cells that had distinct borders, hyporeflective cytoplasm, and invisible nuclei. The lacuna structures were located in the posterior limbus. In contrast, the palisades of Vogt consists of highly reflective linear cords within limbal epithelial cells. These cords are lined with epithelial basal cells (Fig. 1C).

In the eyes without limbal stem cell deficiency, the
diameter of the lacuna at the longest meridian was 250 µm in one eye and 260 µm in the other. The lacuna was approximately 77 µm below the surface limbal epithelium, and the depth of the valley was more than 130 µm from the superficial epithelium. Stromal vessels could be seen adjacent to the lacuna (Fig. 1A, arrow). In three cases, we did not find any alternating cords or long striated structures that had been previously described as the typical palisades of Vogt on confocal imaging. In the fourth case, one of the healthy subjects, a few thin and relatively short cords were seen (Fig. 1B). These cords may be the atrophic remnant of the palisades of Vogt.

We found the structure in the superior quadrant in all cases except one. In the cases of superior sectoral limbal stem cell deficiency, the lacunar structure was found only in the unaffected inferior limbal area (Fig. 2A). The diameter at the longest meridian of the lacunae in these two cases was 325 and 375 µm. The lacunae were approximately 17 and 57 µm below the surface limbal epithelium and extended more than 64 and 137 µm, respectively, from the superficial epithelium into the stroma (Figs. 2B and 2C). The actual depth of the lacunae could not be determined in both cases. Three-dimensional reconstruction of the images confirmed the extension of the epithelial cells into the limbal stroma (Figs. 2D, 2E, and 2F). The mean epithelial cell density within the lacunae was 4,192 ± 91 cells/mm². This cell density was comparable to that between stromal projections within the palisades of Vogt in normal subjects, which was 4,211 ± 108 cells/mm² (unpublished data from our data base).

DISCUSSION

Normal limbal structures imaged using laser scanning confocal microscopy have been described previously. The palisades of Vogt and small round stromal projections into the limbal epithelium are the only two limbal structures noted. It has been proposed that limbal stem cells located beside these cord structures and the palisades of Vogt serve as a niche for limbal stem cells. Although the palisades of Vogt is an important and commonly seen limbal structure, it is not always present in normal individuals. Townsend found that the palisades of Vogt was absent in 20% of the normal population. It has also been documented in healthy individuals that presence of palisades of Vogt declines with age. This finding suggests that the palisades of Vogt may not be the only anatomic location where limbal stem cells reside and that the palisades of Vogt is not a sine qua non structure for healthy limbal stem cells.

Limbal crypts described as solid cords of epithelial cells that extended from the peripheral limbus into the underlying stroma have been observed by Dua et al. only in histopathologic specimens. The limbal lacuna shares a similar feature with the limbal crypt in that the structure invades the limbal stroma. However, limbal lacunae described in our series are different because they are observed in older normal subjects and in partial limbal stem cell deficiency in the absence of the typical palisades of Vogt.

It has been proposed that the limbal epithelial crypt may serve as a limbal stem cell niche. Our confocal images of limbal lacunae show that they are separated from the stroma with relatively acellular material that is similar to the Bowman’s layer. The lacunae are located more than 100 µm deep into the stroma from the surface epithelium, and this location could better protect limbal stem cells from environmental damage. The prevalence of the palisades of Vogt declines with age and the typical palisades of Vogt becomes absent or largely atrophic. We found this structure in older subjects. We think that the well-organized and highly refractive structure of the palisades of Vogt in younger subjects might preclude detection of the limbal lacuna. The presence of limbal lacunae containing normally appearing limbal epithelial cells suggests that this structure serves as an alternative niche for limbal stem cells. In addition, finding this limbal lacuna structure in two patients with partial superior sectoral limbal stem cell deficiency with fairly preserved cornea epithelium supports our hypothesis that the limbal lacuna provides a niche for the remaining surviving limbal stem cells in the limbus to maintain the corneal epithelium.

REFERENCES