Anterior Corneal Pathology in Chronic Corneal Edema

We read with interest the recent article by Alomar et al.¹ and would like to congratulate the authors for their detailed description of confocal microscopy observations in corneas with chronic edema and their excellent description of the correlation of these observations to histopathologic findings. We certainly appreciate the vast amount of time that was spent acquiring images of such excellent quality.

In their article, the authors suggested that subepithelial fibroblasts located between the epithelium and Bowman’s layer were “keratocyte-derived cells.” We have also observed by confocal microscopy this reticular network of cells located in the same region, as have other investigators,² contrary to the authors’ claim of first publication. This subepithelial fibrosis is the primary reason for increased light scatter in corneas with chronic edema.³,⁴ The authors attempted a morphologic comparison of keratocyte and subepithelial fibroblast nuclei, but they did not convincingly demonstrate that these cells were derived from keratocytes. First, the morphologic size of the nuclei determined from light (or confocal) microscopy cannot conclusively determine the origin or similarity of cell types. Second, the authors attempted to show similarity between the cell types by measuring the average length of the nuclei from a few sagittal histologic sections. This is not an accurate quantitative method, as the authors pointed out in the Discussion, because the histologic sections may not have traversed the maximum length of all nuclei, and the shape of the nuclei was not considered. A more accurate quantitative approach would have been to examine multiple, serial sagittal histologic sections, or better, en face (coronal) histologic sections, to determine the maximum length of individual nuclei. An ideal approach, considering that these corneas were examined by using confocal microscopy, would have been to measure the dimensions and shape of the nuclei in the en face confocal images. This information was available to the authors, but surprisingly, they did not report the data. Confocal microscopy is a valuable quantitative tool that enables examination of the cornea in vivo,⁵ while avoiding artifacts created by tissue shrinkage during fixation for histologic examination. With this approach, the authors would have found that the maximum diameter of the nuclei was greater than that estimated by histology, which was predisposed to underestimating the diameter. The authors also did not discuss alternative origins of subepithelial fibroblasts, such as from epithelial cells.

Despite the lack of conclusive evidence of the origin of the subepithelial fibroblasts, it is certainly possible that these cells are derived from keratocytes. The authors alluded to the hypothesis that keratocytes may migrate through Bowman’s layer to transform into subepithelial fibroblasts, and indeed, Iwamoto and DeVoe⁶ examined multiple serial sections by electron microscopy and found one stromal cell traversing Bowman’s layer and concluded that this was a rare finding. As the authors found, and our experience supports, subepithelial fibroblasts are present in most corneas with chronic edema, although cells traversing Bowman’s layer are rarely found by light microscopy. In support of keratocytes as the origin of subepithelial fibroblasts is that anterior keratocytes are depleted in corneas with chronic edema, a finding in a previous histologic validation of confocal microscopy observations⁷ (also contrary to the authors’ claim of first publication). Did Alomar et al. examine multiple serial sections by light microscopy to determine whether cells traversed Bowman’s layer and whether the presence of subepithelial fibroblasts was associated with anterior keratocyte depletion? If so, their observations would support this hypothesis.

As the authors discussed, understanding anterior corneal pathology in corneas with chronic edema will be helpful for understanding the long-term clinical outcomes of these eyes after endothelial keratoplasty. The detailed analysis provided by Alomar et al. is an important contribution to this topic.

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References


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Author Response: Anterior Corneal Pathology in Chronic Corneal Edema

We are pleased to respond to the letter written by Drs. Patel and McLaren in relation to our paper correlating in vivo confocal microscopy (IVCM) findings with histologic findings in corneas with chronic edema.¹ We thank them for their generous commendation of our work in their opening sentences. They have essentially raised two issues.

First, they contended that we have made “first claim” to the IVCM demonstration of subepithelial fibrosis. They have challenged this by quoting their own work, which is unpublished, and providing another reference.² The latter paper was designed to establish the normative database for corneal backscatter analysis by IVCM. They have included one patient, which was not normal, of Fuchs endothelial dystrophy where a reticular network of subepithelial fibrosis was demonstrated. This was a passing reference as it was not the main thrust of the paper. Moreover, the paper was published in August 2011, and our paper was published in September 2011. Clearly, there was
no means by which we could have known of the content of this article before we submitted ours.

Patel and McLaren go on to quote one of their papers wherein they refer to the IVCM demonstration of subepithelial fibrosis in chronic edema as “S.V.P, unpublished data 2008.” We feel that a reference to unpublished data is not a bona fide claim to priority. We would like to add here that we first presented our preliminary data at the European Association for Vision and Eye Research (EVER) congress held in Portoroz, Slovenia, on October 4, 2008. The published abstract includes a statement on the presence of subepithelial fibroblasts in Fuchs endothelial dystrophy. Dr. McLaren and another eminent ophthalmologist attended the presentation and discussed the findings with Dr. Alomar. It thus appears that our “first claim” to the IVCM demonstration of subepithelial fibrosis in chronic corneal edema is justified, although others may have made the same observation contemporaneously.

Their second contention is that we did not convincingly demonstrate that the subepithelial fibroblasts were derived from keratocytes, although they go on to discuss why this is most likely to be the case. We do not think that examination of keratocytes in serial sections, either transverse or coronal, would give an accurate measure of keratocyte nuclei as the orientation, of the nuclei within the cells can be in any direction. We presented similarities in shape, orientation, and size as circumstantial evidence in support of the keratocyte origin of the subepithelial cells. Moreover, we have provided evidence of keratocyte migration through breaks in Bowman’s zone in a previously published paper, which was quoted in our manuscript. We do not believe that keratocytes can migrate through an intact Bowman’s zone; only in the presence of breaks does this occur. Bowman’s zone breaks are known to occur in chronic edema, as we demonstrated in Figure 5 of our paper. Patel and McLaren hypothesize in their elegant paper, that keratocytes migrate into the subepithelial region. Interestingly, however, in their paper, they argued that keratocyte depletion from the anterior corneal stroma is due to apoptosis of keratocytes. They made no comment on the possible anterior migration of keratocytes into the subepithelial region. It is surprising and intriguing that they made no mention of the subepithelial location of fibroblasts by histology or IVCM, despite their unpublished observations of 2008. These corneal samples would have provided them with an ideal opportunity to study this phenomenon and support their recent hypothesis that depletion of keratocytes from the anterior stroma is related to their anterior migration and accumulation in the subepithelial space. Careful scrutiny of their paper made it clear that, unlike our study, they did not perform histology and IVCM on the same corneal samples. Histology can be properly correlated with IVCM only if the same tissue samples (and preferably the same sites) are compared. Hence our “first claim” of histologic correlation with IVCM features is also sustained.

References

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