Reviewer's report

Title: Apoptosis of Keratocytes in Corneal Infection: Could it be a protective phenomenon?

Version: 1 Date: 14 June 2004

Reviewer: Haydee E.P. Bazan

Reviewer's report:

General
The authors of this study attempt to correlate keratocyte apoptosis with several infectious processes induced by fungal, bacterial, viral, or Acanthamoeba keratitis in tissues from patients who underwent keratoplasty. They report keratocyte apoptosis independent of the infectious agent. There are several reports that have shown that damage of the epithelium releases inflammatory cytokines that induce rapid apoptosis in the stroma. In this study, apoptosis was found mainly in the posterior stroma, suggesting that there is some mechanism that prevents the keratocytes of this area from entering necrosis, possibly to subdue the inflammatory reaction. It would be interesting to pursue these findings using animal models. The authors are well aware that although their study reviewed a lot of samples from different cases, it is still very limited, in that the samples are taken from patients who underwent keratoplasty, therefore the tissue had already gone the route of irreversible damage.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
There are concerns with respect to the figures.
- Figure 2 shows in high magnification H&E staining from a tissue with Acanthamoeba keratitis. Is the loss of keratocyte nuclei in the picture a result of apoptosis or necrosis? It would be helpful to show a low-magnification picture of the same section with TUNEL staining.

- It is difficult to distinguish nuclear fragmentation in Figures 3 and 4. It would be more convincing to show photographs of different infections at lower magnification along with a control for comparison. In addition, keratocytes close to the pathological foci appear to be normal. How do the authors explain this phenomenon?

- The title is based on a hypothesis, not proof in this MS. What kind of protection occurred in the cases analyzed, if all the tissues had been replaced?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
“Figure 7” must read “Figure 5.”

- The tables are confusing; e.g., Table 1 describes 43 patients. The size of the ulcer is presented for 38 patients, the localization of the ulcer for 42 patients: does this mean that 42 of the 43 patients showed ulceration?

- In Table 2, keratocyte apoptosis, reduced – with respect to what?

- In Table 3 were the sizes of ulcers classified as small, medium, or large? There are similar concerns for the other parameters measured. In “keratocytes,” there are three rows, what is the last
row? In “apoptosis,” what is the difference between the two rows?

Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No

**Declaration of competing interests:**

none