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Review

Apoptosis in the initiation, modulation and termination of the corneal wound healing response

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Abstract

Stromal keratocyte apoptosis has been well-characterized as an early initiating event of the corneal wound healing response, triggering subsequent cellular processes that include bone marrow-derived cell infiltration, proliferation and migration of residual keratocyte cells, and, in some circumstances, generation of myofibroblast cells. Recent studies, however, have suggested a more general role for apoptosis in the overall stromal wound healing response that includes modulation and termination functions. This review article highlights, and ties together, recent studies that have demonstrated the important role apoptosis likely plays in weeks to months following an initial insult to the cornea—depending on the type and extent of corneal injury.

Keywords: apoptosis; corneal wound healing; keratocytes; stroma; bone marrow-derived cells; myofibroblasts

1. Introduction

Immediately after epithelial insult, keratocyte cells underlying the area of injury undergo apoptosis or programmed cell death (Wilson et al., 1996a,b; Dupps and Wilson, 2006), an involutional and controlled form of death in which there is limited release of intracellular contents such as enzymes, chemokines and other components that could directly damage the surrounding structures and cells and promote infiltration of excessive numbers of inflammatory cells with potential to further damage the tissue. The type of epithelial injury dictates the location and extent of this early keratocyte apoptosis response (Helena et al., 1998). Thus, extensive debridement of the epithelium over the central cornea triggers widespread keratocyte apoptosis within the anterior stroma underlying the epithelial injury. Conversely, incisional injuries from a blade or microkeratome stimulate apoptosis at the site of the epithelial and stromal penetration. Even epithelial pressure from a poorly fit contact lens may trigger limited superficial stromal keratocyte apoptosis (Wilson, 1998).

Keratocyte apoptosis is an exceedingly rare event in the normal uninjured cornea. Thus, in studies that included unwounded control corneas in rabbits (Helena et al., 1998; Mohan et al., 2003; Szentmary et al., 2005), mice (Wilson et al., 1997), or humans (Kim et al., 1999), almost no apoptotic keratocytes or other stromal cells were noted in control corneas, even when dozens of tissue sections were examined. Apoptotic keratocytes may, however, be noted away from sites of epithelial injury in keratoconus corneas (Kim et al., 1999), leading to the hypothesis that abnormally high levels of ongoing keratocyte apoptosis could play a role in the pathophysiology of this ectatic corneal disease (Kim et al., 1999; Chwa et al., 2006). Abnormally high levels of keratocyte apoptosis have also been associated with the pathophysiology of aniridia (Ramaesh et al., 2006). Thus, it appears that stromal apoptosis is tightly controlled during homeostasis in the absence of corneal injury or disease. However, once corneal injury occurs—whether mechanical, infectious, or chemical—stromal

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apoptosis becomes an important component of the wound healing response. Stromal apoptosis occurs immediately following corneal injury and, depending on the type and extent of injury, may persist in the tissue for months or even years.

Stromal cells that undergo apoptosis following injury vary depending on the type of injury, extent of injury and the time following injury. This review focuses on the identity of stromal cells undergoing apoptosis and the function of apoptosis response at different time points after corneal injury. To facilitate discussion, apoptosis responses will be divided into: the early phase (detected minutes to a few hours after injury), intermediate phase (hours to weeks after injury) and the late phase (occurring weeks to months, or even years, after injury).

2. Early phase apoptosis

Immediately following any sort of epithelial injury, stromal keratocytes underlying the epithelial injury undergo rapid keratocyte apoptosis (Wilson et al., 1996a,b). It is possible that other stromal cells such as Langerhans' cells, nerves and a few resident and circulating inflammatory cells could also be caught up in the wave of apoptosis, but there is no conclusive evidence one way or the other. In species with thin corneas, such as the mouse, one occasionally notes corneal endothelial cells that also appear to undergo apoptosis in response to extensive corneal epithelial injury, such as scrape (Wilson et al., 1996a,b).

The injury precipitating programmed keratocyte death can be produced by epithelial scrape (Fig. 1), incisional injuries from scalpel blades or microkeratomes, or even significant pressure of a contact lens on the epithelial surface (Wilson et al., 1996a,b; Wilson, 1998; Helena et al., 1998; Mohan et al., 2003). Most commonly, apoptosis is detected using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and with this assay the labeling peaks at approximately 4 h after epithelial injury (Wilson et al., 1996a,b). However, using transmission electron microscopy, it can be noted that chromatin condensation, cell shrinkage, and budding of apoptotic bodies begin immediately after epithelial injury (Wilson et al., 1996a,b). It cannot be emphasized enough how important it is to confirm TUNEL assay results using another method when studying a new system if at all possible. Unfortunately, under some circumstances, the TUNEL assay also labels cells undergoing necrosis where there is random degradation of deoxyribonucleic acid (DNA). There are two important examples of this that have been noted in our laboratory. The first is when TUNEL labeling of cells in the anterior stroma after epithelial scrape injury or epithelial scrape with photorefractive keratectomy continues to be noted for a week or more after the injury even though transmission electron microscopy shows that by a few days after injury almost all the cells that are continuing to die are undergoing necrosis (Mohan et al., 2003). The second example is when the femtosecond laser is used to make a lamellar cut in the stroma, without injury to the epithelium, keratocytes surrounding the cut label with the TUNEL assay and are found to be dying only by necrosis when the tissue is studied with transmission electron microscopy (Netto et al., in press). Thus, confirmation of TUNEL assay results in a particular system is critical. The gold standard technique for this remains transmission electron microscopy. There is some hope that other methods, such as immunocytochemical

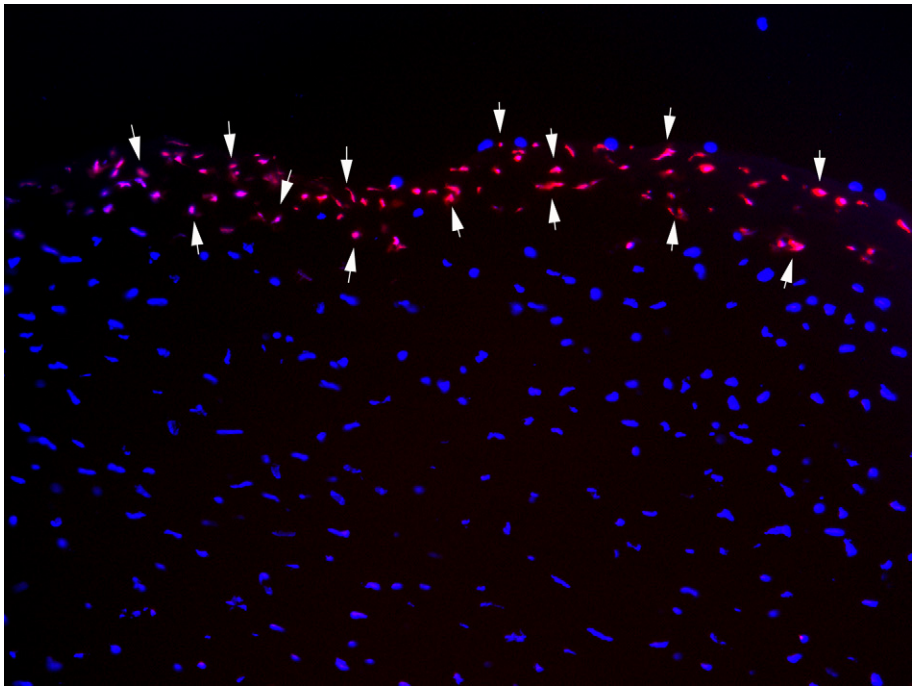

Fig. 1. Superficial keratocyte apoptosis (red label indicated by arrows) at 4 h after epithelial scrape and −9 diopter photorefractive keratectomy injury in a rabbit cornea detected with a fluorescent TUNEL assay. Intact nuclei of residual keratocytes are labeled blue with DAPI. Magnification 600×.