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The Corneal Wound Healing Response: Cytokine-mediated Interaction of the Epithelium, Stroma, and Inflammatory Cells

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Abstract — The corneal wound healing cascade is complex and involves stromal–epithelial and stromal–epithelial–immune interactions mediated by cytokines. Interleukin-1 appears to be a master modulator of many of the events involved in this cascade. Keratocyte apoptosis is the earliest stromal event noted following epithelial injury and remains a likely target for modulation of the overall wound healing response. Other processes such as epithelial mitosis and migration, stromal cell necrosis, keratocyte proliferation, myofibroblast generation, collagen deposition, and inflammatory cell infiltration contribute to the wound healing cascade and are also likely modulated by cytokines derived from corneal cells, the lacrimal gland, and possibly immune cells. Many questions remain regarding the origin and fate of different cell types that contribute to stromal wound healing. Over a period of months to years the cornea returns to a state similar to that found in the unwounded normal cornea.

1. INTRODUCTION

The corneal wound healing response is an exceedingly complex cascade involving cytokine-mediated interactions between the epithelial cells,

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keratocytes of the stroma, corneal nerves, lacrimal glands, tear film, and cells of the immune system. As complex as the response is, it is relatively simple in contrast to wound healing that occurs in skin and organs that contain blood vessels and other components that participate in the process. This response is very similar in different species, although there appear to be some quantitative and qualitative variations in specific processes that comprise the cascade. Within a species there is variability depending on the inciting injury. For example, incisional, lamellar, and surface scrape injuries are followed by typical wound healing responses that are similar in some respects, but different in others. This review article will provide an overview of the cellular interactions that contribute to the corneal wound healing response with emphasis on cytokine regulation of these interactions.

2. EVOLUTIONARY CONTEXT

It is important to view the corneal wound healing response in the context of the types of injuries that were most likely to place selective pressure during evolution. Clearly vision was essential to the survival of most animals and responses would likely have evolved to maintain vision following injury. It seems probable that abrasions from branches, projectiles, and other sources would have been the most common injuries to the vertebrate cornea. The wound healing responses to these variable injuries must retain integrity of the eye, restore the protective epithelial surface, while at the same time maintaining sufficient corneal clarity to provide vision. Infection from ubiquitous viral pathogens such as herpes simplex virus, smallpox virus, adenovirus, and their ancestors may have posed significant threats to the vision and survival of the evolving animals. Thus, pathogens that had the potential to permanently blind would likely have placed great selective pressure on animal species. Systems designed to impede the spread of viral pathogens until the immune response could eradicate the invader could have provided an advantage (Wilson *et al.*, 1997). The responses that occur in the cornea appear to be well designed to accomplish these objectives.

3. OVERVIEW OF THE WOUND HEALING RESPONSE

The epithelium, stroma, and nerves participate in homeostasis of the anterior cornea and ocular surface. The lacrimal glands and tear film also contribute to the maintenance of surface smoothness and integrity important to function of the eye. Following injury, these components participate in an orchestrated response that efficiently restores corneal structure and function in most situations. Many cytokines and receptors modulate the process. Activation of these systems also attracts immune cells that function to eliminate debris and microbes that may breach the injured surface and gain entry to the corneal stroma. Only by considering the individual contributions of each of these components and their interactions can one begin to truly appreciate the beauty and efficiency of the overall response.

4. MASTER REGULATORS OF THE WOUND HEALING RESPONSE

The cascade of responses to injury to the cornea is initiated very rapidly regardless of the type of injury. For example, the keratocyte apoptosis response detected by electron microscopy is so rapid that if one euthenizes a mouse, enucleates the eye, and performs a single scrape across the epithelium prior to plunging the eye into fixative, the anterior keratocytes already show chromatin condensation and other morphologic changes consistent with apoptosis. Thus, the cornea is primed and ready to respond immediately to injury. This would make sense if one of the functions of this response was to retard dissemination of viral pathogens until other defense mechanisms can be rallied.

What are the key modulators that regulate the early events in the wound healing cascade? After working on these problems for many years we have come to believe that there are a few key cytokine modulators that act as ‘‘master

regulators'' of the response. Some of these master regulators and their receptors are likely to be constitutively produced and, therefore, constantly available to initiate the wound healing response. These initiators are likely to be sequestered until needed so that their mischief is not unleashed unduly, at least in the normal cornea. Thus, these master regulators serve as ever-vigilant guardians, silently waiting and on the ready to unleash the cascade at the first sign of trouble.

Interleukin (IL)-1 seems to fit the requirements of a master regulator with regards to expression and function. First the expression of this cytokine–receptor system will be discussed and then the many functions that are regulated in the keratocytes by IL-1 will be reviewed.

Both IL-1 alpha (Fig. 1) and IL-1 beta mRNAs and proteins are expressed constitutively in the corneal epithelium (and endothelium) (Wilson *et al*., 1994b; Weng *et al*., 1996). IL-1 receptor (binds both IL-1 alpha and IL-1 beta) is also constitutively produced in keratocytes and corneal fibroblasts (cultured keratocytes will be referred to as corneal fibroblasts if they are cultured in serum and cultured keratocytes if they are cultured without serum) (Bereau *et al*., 1993; Fabre *et al*., 1991; Girard *et al*., 1991; Wilson *et al*., 1994c; Beales *et al*., 1999; Jester *et al*., 1999b). Neither IL-1 alpha or IL-1 beta are detectable by immunocytochemistry in keratocytes in the unwounded cornea. However, Fini and coworkers have demonstrated that exposure to IL-1 can induce keratocytes to produce IL-1 via an autocrine loop (Strissel *et al*., 1997a,b; West-Mays *et al*., 1995). Thus, IL-1 protein is detectable in keratocytes or myofibroblasts in the wounded cornea. IL-1 (alpha or beta) does not appear to be released from the epithelium into the stroma in significant amounts in the normal unwounded cornea. Both forms of IL-1 lack signal sequences for transport from the cell and released via cell injury or death (Dinarello, 1994). IL-1 is released from apical epithelial cells undergoing programmed cell death as a part of the normal maturation and turnover of the epithelium and may be present in tears at increased levels in conditions associated with ocular surface injury such as keratoconjunctivitis sicca. (Pflugfelder, 1998; Pflugfelder *et al*., 1999). However, tear IL-1 probably does not pass into the anterior stroma in the absence of epithelial injury or death because of the barrier provided by the intact epithelium. IL-1 is dumped onto the exposed stroma immediately following epithelial injury that is of sufficient magnitude to break down epithelial barrier function or directly damage basal epithelial cells. In some cases, epithelial debris could be tracked into the stroma, for example with a microkeratome cut into the cornea. Once IL-1 penetrates the stroma, it can bind IL-1 receptors on the keratocyte cells and modulate the functions of these cells. Thus, IL-1 is sequestered within the epithelium separated from the stromal cells in the normal unwounded cornea until epithelial injury triggers its release.

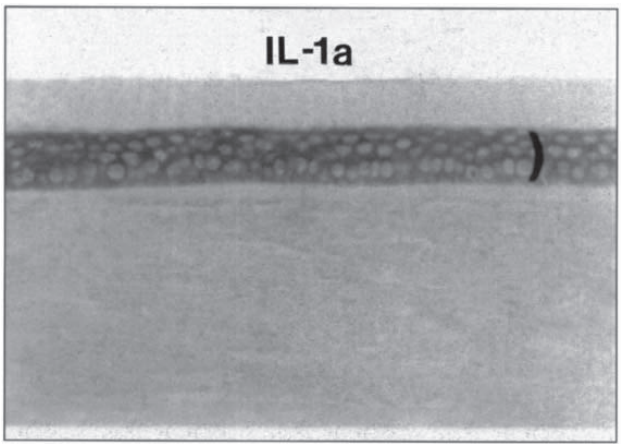

Fig. 1. Interleukin 1-alpha is constitutively produced in the corneal epithelium. This immunocytochemical localization was performed in normal human cornea. Note there is no detectable expression of IL-1 alpha in the keratocytes in the unwounded cornea. Reprinted with permission from Wilson *et al*., (1994), Exp. Eye Res. 59, 63–72, © 1994 by permission of the publisher, Academic Press.

IL-1 has an array of important effects on keratocyte cells related to wound healing. It has been shown to modulate apoptosis of keratocytes and corneal fibroblasts (Wilson *et al*., 1996), although the effect appears to be mediated indirectly via the Fas/Fas ligand system through autocrine suicide (Mohan *et al*., 1997). Since IL-1 alpha also triggers NF-kappa B activation (Mohan *et al*., 2000) it also has negative apoptotic effects on the keratocyte cells and myofibroblast cells that appear during the stromal wound healing response. Thus, the overall effect of IL-1 could be related to the specific milieu of the cell

and the overall input by a number of different cytokines.

IL-1 has negative chemotactic effects on keratocytes and corneal fibroblasts and may have a role in maintaining corneal tissue organization (the morphologic separation of epithelium from stroma in the normal cornea) through this effect (Wilson *et al.*, 1996; Kim *et al.*, 1999a,b; Wilson and Hong, 2000).

IL-1 is the primary regulator or hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) production by keratocytes (Weng *et al.*, 1996). HGF and KGF are classical mediators of stromal-epithelial interaction that are produced by keratocytes and myofibroblasts to regulate proliferation, motility, and differentiation of epithelial cells (Wilson *et al.*, 1994a). Thus, IL-1 released by injured corneal epithelial cells triggers production of HGF and KGF by the keratocytes to regulate the repair process of the corneal epithelial cells.

IL-1 upregulates expression of collagenases, metalloproteinases, and other enzymes by keratocytes (Strissel et al., 1997a,b; West-Mays *et al.*, 1995). These enzymes have an important role in remodeling of collagen during corneal wound healing. IL-1 and TNF alpha also upregulate the expression of some chemokines such as IL-8, RANTES, and monocyte chemotactic protein (MCP)-1 in keratocytes and corneal epithelial cells (Tran *et al.*, 1996; Takano *et al.*, 1999). IL-1 also potentiates the chemotactic effect of platelet-derived growth factor (PDGF) on corneal fibroblasts.

Thus, IL-1 directly regulates several critical processes that contribute to wound healing. This, along with the distribution of expression of IL-1 and IL-1 receptor in the cornea and the sequestration of IL-1 in the absence of injury, suggest a role for IL-1 as a master regulator of the corneal wound healing response.

There may be other master regulator cytokines that function to initiate the early wound healing response. For example, PDGF is expressed by corneal epithelial cells and the keratocytes express the PDGF receptors (Denk and Knorr, 1997; Andresen and Ehlers, 1998; Kamiyama *et al.*, 1998; Kim *et al.*, 1999a,b). PDGF is found at very high levels in the epithelial basement membrane. This localization and sequestration is likely related to the heparin-binding property of PDGF. PDGF is released into the stroma after damage to the epithelium and underlying basement membrane. PDGF modulates corneal fibroblast proliferation, chemotaxis, and possibly differentiation (Denk and Knorr, 1997; Andresen and Ehlers, 1998; Kamiyama *et al.*, 1998; Kim *et al.*, 1999a,b). Tumor necrosis factor (TNF) alpha could also participate (Mohan *et al.*, 2000). Less is known about the function of TNF alpha and other cytokines that could also serve as master regulators in the corneal wound healing response.

5. THE CORNEAL WOUND HEALING CASCADE

Studies performed over the past decade have revealed numerous processes that comprise the overall wound healing cascade in the cornea. It is helpful to outline the steps in this response prior to discussing individual components of the response. It must be appreciated that many of these events occur simultaneously and, therefore, the ''cascade'' should be viewed as such only in rough terms. It is clear that some events proceed others. For example, keratocyte apoptosis is the earliest stromal response that can be detected following epithelial injury and other components of the cascade appear to follow. Figure 2 provides the rough framework of the cascade. It is not intended to be comprehensive and some processes that are clearly important to the overall wound healing response are not depicted in the figure. Subsequent sections will concentrate on individual processes in the wound healing cascade with emphasis on cytokine mediation.

6. KERATOCYTE APOPTOSIS AND NECROSIS

Work in our laboratory demonstrated that the disappearance of keratocytes that followed epithelial injury was mediated by apoptosis (Wilson *et al.*, 1996). Studies have suggested that this regulated cell death is mediated by cytokines released from the injured epithelium such as IL-1

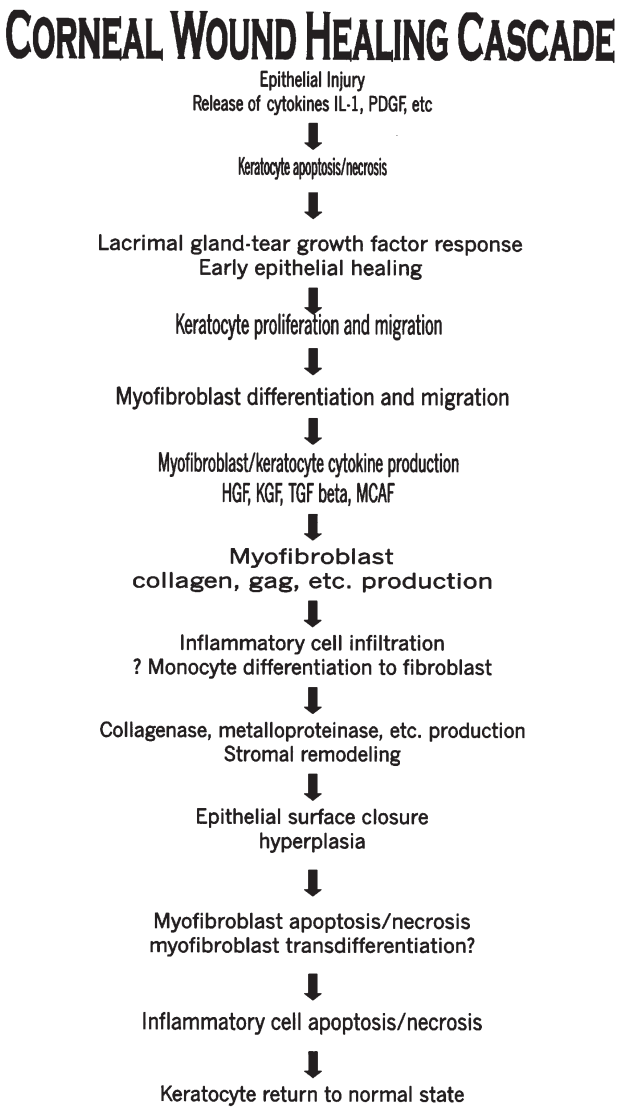

Fig. 2. Schematic diagram indicating some of the events that occur in the corneal wound healing response that occurs following corneal epithelial injury or surgical procedures such as PRK or LASIK. Note that this is a simplified scheme and not all events that may be important are included. While there is some indication of sequence (for example keratocyte apoptosis is the first observable event following injury) many of the events occur simultaneously in the cornea.

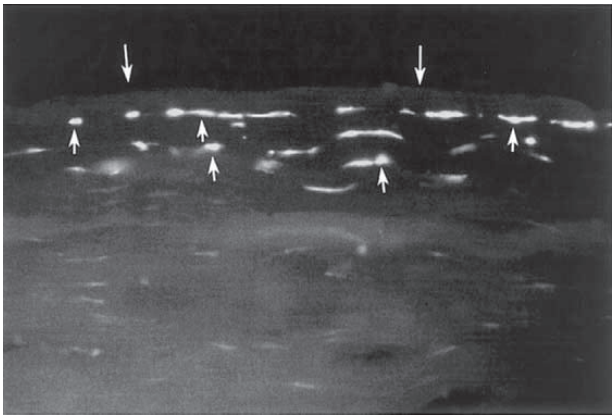

Fig. 3. Keratocyte apoptosis detected with the TUNEL assay at 4 h after epithelial scrape injury in the human cornea. The scrape injury was produced 4 h prior to enucleation for intraocular melanoma. Note staining of the superficial keratocytes (small arrows) beneath Bowman's layer (large arrows). This experiment was approved by the Institutional Review Board at the University of Washington, Seattle, WA. Magnification 200 ×.

and TNF alpha (Wilson *et al*., 1996; Mohan *et al*., 1998, 2000). It is largely unknown how these different pro-apoptotic cytokine pathways interact to determine whether signaling dictates cell death or some other response in individual cells. Likely there is redundancy of the systems and possibly a requirement for context before a death signal is recognized and acted upon. In some cases, more than one of these systems may be involved. For example, the effect of the IL-1 system on keratocyte apoptosis may be mediated indirectly through the Fas–Fas ligand system via autocrine suicide (Mohan *et al*., 1997). Many types of epithelial injury will induce keratocyte apoptosis. Some of the triggers include mechanical scrape (Wilson *et al*., 1996), corneal surgical procedures like photorefractive keratectomy (PRK) and laser *in situ* keratomeliusis (LASIK) (Helena *et al*., 1998), viral infection (Wilson *et al*., 1997), incisions (Helena *et al*., 1998), and even pressure applied with an instrument on the epithelial surface (Wilson, 1997). Recent experiments have confirmed that apoptosis occurs in the keratocytes underlying Bowman's layer in the human eye when the epithelium has a scrape injury (Fig. 3, Ambrosio *et al*., unpublished data).

It was previously noted that keratocyte apoptosis is the first observable stromal response following epithelial injury (Wilson *et al*., 1996). The earliest changes are noted at the electron microscopic level. It takes a few minutes longer (up to 30 min) before apoptosis can be detected with the TUNEL assay. Thus, DNA fragmentation detected by the TUNEL assay takes somewhat longer to develop and has been found to be most prominent at approximately 4 h after the scrape injury in mice and rabbits (Wilson *et al*., 1996, 1998).

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