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Research Paper

Corneal NF-κB activity is necessary for the retention of transparency in the cornea of UV-B-exposed transgenic reporter mice☆

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Abstract

To determine the dynamics of Nuclear Factor-κB (NF-κB) in murine corneal pathology and the role of NF-κB in maintaining corneal clarity after ultraviolet B radiation insult, transgenic mice containing NF-κB-*luciferase* reporter were exposed to LPS (bacterial lipopolysaccharide), TNF-α (Tumor Necrosis Factor-alpha) or 4 kJ m^{-2} UV-B radiation. NF-κB decoy oligonucleotides were also administered in some of the UV-B experiments. Following various exposure times, the mice were sacrificed and whole eyes or corneal tissues were obtained. Whole eyes were examined for scattering using a point-source optical imaging technique. Tissue homogenates were examined for luciferase activity using a luminometer. TNF-α and LPS-injected NF-κB-luciferase transgenic mice demonstrated 3–10-fold increases in cornea NF-κB with peak activities at 4 and 6 hr post-injection, respectively. Mice exposed to 4 kJ m^{-2} UV-B exhibited a 3-fold increase in NF-κB activity 4 hr post-exposure. The administration of NF-κB-decoy oligonucleotides to mice had the effect of reducing UV-B-induced NF-κB activity in the cornea and significantly increasing the amount of light scattering in UV-B exposed corneas 7 days post-UV-B exposure when compared to sham injected mice. These results indicate that NF-κB is activated in cornea in pathologies that involves increased plasma levels of LPS and TNF-α, as well as direct UV-B exposure, and suggest that NF-κB activation play an essential part in the corneal healing process.
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Keywords: nuclear factor-kappaB; corneal wound healing; decoy oligodeoxynucleotides ultraviolet radiation

1. Introduction

The cornea is challenged with maintaining optical form and clarity, whilst being exposed to the dangers of foreign object contact, bacterial and viral infections, autoimmune diseases, ischemia from non-permeable contact lenses, and ultraviolet (UV) radiation from solar (Shimmura et al., 1996; Meyer-Rochow, 2000) or cosmetic laser sources (Kasetsuwan et al., 1999). Optimally, the cornea responds to these insults with complete healing, resulting in a restoration of the cornea's integrity and clarity. Unfortunately, this is not always the case and the post-injury process can result in conditions such as neovascularization, scarring, and loss of transparency. As with other tissues, the extent (and success) of injury response is clearly contingent on an appropriate cytokine response (reviewed by Torres and Kijlstra, 2001), tailored to the nature and extent of ocular insult.

Direct injury to the cornea can occur from the formation of reactive oxygen species (ROS) due to exposure to UV light or due to natural or anthropomorphic ozone. These noxious stimuli, among others, initiate the release or production of inflammatory cytokines (Yamada et al., 2003). Indirect injury, either near or distal to the eye, also exposes the cornea to inflammatory cytokines as some of these factors can be found in the tears and exposes the cornea to whatever deleterious (cytokines, viri) or beneficial (lactoferrin) constituents may be found within. Tumour necrosis factor-α (TNF-α), for example, can be found in the tears and may initiate a cytokine cascade in the cornea that is not related to any injury to the cornea per se (Slepova et al., 2001). Corneal injury can also result from the activity of a gram-negative bacterial infection of the ocular region, which exposes the cornea to bacterial lipopolysaccharide (LPS). Rat corneal epithelial cells, for example, have been shown to respond to the in vitro administration of LPS with the production of a number of cytokines, including: interleukin (IL)-1β, IL-6 and TNF-α (Jozwiak et al., 2000). Interestingly, experiments involving these cytokines in non-ocular tissues,

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usually demonstrate a subsequent activation of nuclear factor (NF)-κB. NF-κB is dimeric transcription factor typically consisting of p65 (RelA) and p50 subunits and maintains station in the cytoplasm due to its sequestration by an inhibitory protein, IκB. Inflammation and immune events may initiate the dissociation of IκB, permitting the translocation of the now-unbound NF-κB dimer into the nucleus which subsequently binds to the regulatory NF-κB elements in the target genes usually activating genes involved in immune response, tissue repair or apoptosis.

Activation of NF-κB transcription factors is essential for proper inflammatory responses. Such inflammation is a beneficial host response to foreign challenge or tissue injury that leads ultimately to the restoration of tissue structure and function (Karin and Ben-Neriah, 2000; Hoffmann et al., 2002). If NF-κB activation is prolonged, however, inflammation can also contribute to pathogenesis. This is the case not only in many diseases of infectious origin but also in diseases like rheumatoid arthritis, gout, chronic obstructive pulmonary disease, emphysema, asthma, ischemia-reperfusion, ulcerative colitis, Crohn's disease, type 1 and 2 diabetes, and several types of neurodegenerative diseases (Nathan, 2002; Wyss-Coray and Mucke, 2002). More recently, inflammation has also been demonstrated to contribute to some types of cancer and atherosclerosis (Libby, 2002; Vakkila and Lotze, 2004).

NF-κB activation and activity are therefore tightly controlled by a number of mechanisms that limit the excessive and prolonged production of pro-inflammatory mediators, which can cause tissue damage. IκB kinase α (IKKα), IκB kinase β (IKKβ) (which both inhibit NF-κB expression) and the de-ubiquitinating enzyme A20 play important roles in limiting the duration and magnitude of NF-κB signaling, and of the resolution of inflammation (Hoffmann et al., 2002; Wertz et al., 2004; Lawrence et al., 2005).

NF-κB is known to demonstrate both pro- and anti-apoptotic actions in a number of processes, including, early development (Odgren et al., 2003), tissue remodeling (Faucheux et al., 2001) and wound healing (Loncar et al., 2003). Elements of pro- or anti-apoptosis may be manifested by NF-κB in the cornea as there are a number of reports of NF-κB-like activity in the cornea, including recent descriptions by Wilson et al. (2004) on the similarities of corneal wound healing to bone turn-over, which include the aforementioned NF-κB aspects. Experiments by Cho et al. (1999) exposing bovine corneal endothelial cells to ROS does provide some insight into the possible role of NF-κB in the cornea. Through their use of light-activated riboflavin and rose bengal, Cho's group found that, in the bovine corneal endothelial cells, there was an initial apoptotic, and subsequently necrotic, series of events taking place. Cellular apoptotic and necrotic events are usually associated with NF-κB, and various intermediate steps can determine an ultimate apoptotic or necrotic trajectory. Although Cho's group did not describe NF-κB in the cornea per se, similar experiments performed on the lens do provide evidence for the ocular NF-κB activity. Exactly how the cornea may respond to injury may also be deduced from lens experiments as this epithelial tissue is also found in an avascular environment. Investigations by Dudek et al. (2001) used TNF-α and H_2O_2 as stressors in a lens epithelial cell line, and showed a strong NF-κB activation in this in vitro experiment, whereas our own group has recently demonstrated the in vivo activation of NF-κB in lens epithelial tissue by using stressors such as LPS (bacterial lipopolysaccharide), TNF-α and UV-B (ultraviolet-'B': ca. 300 nm) radiation (Alexander et al., 2003). Given the avascularity of both the cornea and the lens, and, as they are, in part, exposed to the same intraocular humor, it is reasonable to presume that NF-κB activity in the cornea may share some similarities with lens NF-κB activity. Further evidence of this is provided by murine cornea scrape-wounding experiments (Wilson et al., 2004) in which cultured corneal fibroblasts and keratinocytes were shown to express receptor activator of NF-κB (RANK) and corneal stromal cells exhibited the presence of RANK ligand (RANKL). Many of the cited experiments are predominantly in vitro, and thus, an in vivo approach in the further determination of the role of corneal NF-κB activity in corneal injury would provide a better understanding of how the cornea responds to oxidative stress or injury and provide insight into the possible use of NF-κB-altering agents in order to preserve or regain corneal transparency compromised by disease or injury.

In the present study, we induced systemic oxidative stress to the eyes of transgenic NF-κB-luciferase mice by treating them with TNF-α and LPS to characterize local NF-κB activation in intact native cornea: We also induced externally-directed oxidative stress by exposing the eyes of both transgenic and wild mice to UV-B radiation and examined these eyes for the response and effect of NF-κB and NF-κB-decoy oligonucleotides on the state of corneal clarity 7 days after UV-B exposure.

2. Methods

2.1. Materials

The anesthetics, fentanyl citrate and midazolam, were purchased from Janssen (Beerse, Belgium) and Roche (Basel, Switzerland), respectively. Reporter lysis buffer was obtained from Promega (Madison, WI), and luciferin substrate was purchased from Biothema (Dalaro, Sweden). Murine TNF-α was procured from R&D Systems (Minneapolis, MN), and LPS (*Escherichia coli* serotype 055:B5) was purchased from Sigma (St Louis, MO). Phosphorothioated NF-κB oligodeoxynucleotides were purchased from Eurogentec (Seraing, Belgium) and the TransIt gene delivery system was obtained from TransIt (Mirus, Panvera, WI).

2.2. Transgenic mice

The transgenic mice used in this experiment were as those used in previous experiments (Carlsen et al., 2002). These mice were heterozygous 3x-κB-*luc* mice with a (C57BL/6J x CBA/J) genetic background. These experimental animals were housed in a controlled light (12 l:12d), humidity and

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