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Topical interleukin-1 receptor antagonist inhibits inflammatory cell infiltration into the cornea

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Abstract

Interleukin (IL)-1α and β are important modulators of many functions of corneal epithelial and stromal cells that occur following injury to the cornea, including the influx of bone marrow-derived inflammatory cells into the stroma attracted by chemokines released from the stroma and epithelium. In this study, we examined the effect of topical soluble IL-1 receptor antagonist on bone marrow-derived cell influx following corneal epithelial scrape injury in a mouse model. C57BL/6 mice underwent corneal epithelial scrape followed by application of IL-1 receptor antagonist (Amgen, Thousand Oaks, CA) at a concentration of 20 mg/ml or vehicle for 24 h prior to immunocytochemical detection of marker CD11b-positive cells into the stroma. In two experiments, topical IL-1 receptor antagonist had a marked effect in blocking cell influx. For example, in experiment 1, topical IL-1 receptor antagonist markedly reduced detectible CD11b-positive cells into the corneal stroma at 24 h after epithelial injury compared with the vehicle control (3.5 ± 0.5 (standard error of the mean) cells/400× field and 13.9 ± 1.2 cells/400× field, respectively, $p < 0.01$). A second experiment with a different observer performing cell counting had the same result. Thus, the data demonstrate conclusively that topical IL-1 receptor antagonist markedly down-regulates CD-11b-positive monocytic cell appearance in the corneal stroma. Topical IL-1 receptor antagonist could be an effective adjuvant for clinical treatment of corneal conditions in which unwanted inflammation has a role in the pathophysiology of the disorder.

Keywords: corneal wound healing; interleukin-1; bone marrow-derived cells; keratocytes; receptor antagonist; monocytes

1. Introduction

Interleukin (IL)-1α and β are produced constitutively by the corneal epithelium and released into the tear film and stroma by epithelial cell injury or death (Wilson et al., 1994a,b, 2001; Weng et al., 1997; Okamoto et al., 2004). Subsequently, IL-1 modulates cellular functions by binding to IL-1 type I and type II receptors expressed by keratocytes and other corneal cells (Dinarello, 1994; Wilson et al., 1994a,b). Functions in the corneal stroma that have been found to be regulated by interleukin-1 include modulation of the Fas/Fas ligand system triggering keratocyte apoptosis (Wilson et al., 1996; Mohan et al., 1997), keratocyte production of paracrine mediators hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) that regulate epithelial proliferation, migration and differentiation (Weng et al., 1997), keratocyte production of collagenases and metalloproteinases (Girard et al., 1991; Li et al., 2003; Xue et al., 2003), and keratocyte production of chemokines (Tran et al., 1996; Hong et al., 2001). IL-1 also modulates production of cytokine and of other important proteins like beta-defensin 2, collagenases and stromelysins by corneal epithelial cells (Li et al., 2003; Narayanan et al., 2005; Shin et al., 2004). IL-1 receptor antagonist is also produced by corneal epithelial and keratocyte cells (Kennedy et al., 1995), presumably to regulate the effects of IL-1α and IL-1β released from the corneal epithelium.

The purpose of this study was to test the hypothesis that exogenous topical IL-1 receptor antagonist delivered during

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the early period following epithelial scrape injury can be used to modulate inflammatory infiltration into the cornea.

2. Materials and methods

2.1. Animal protocols and reagents

Female C57BL/6 mice (10- to 12-week-old) were purchased (Jackson Labs, Bar Harbor, ME) and used in this experiment. Animal use was approved by the Animal Research Committee at the Cleveland Clinic, Cleveland, OH. All animals were treated according to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Mice were treated with intraperitoneal injection off 50 mg/kg ketamine and xylazene 5 mg/kg for deep anesthesia and 1 drop of 1% proparacaine for local anesthesia prior to epithelial injury to the cornea. Any eye that developed infection during the first 24 h after surgery was excluded.

In two experiments, animals were divided into three groups: (1) naive unwounded cornea, (2) epithelial scrape plus topical balanced salt solution control, and (3) epithelial scrape plus topical IL-1 receptor antagonist, with 6–8 eyes completing the study in each group.

Epithelial injury was produced by scraping the cornea with a #64 Beaver blade (Becton–Dickinson, Franklin Lakes, NJ) while viewing the eye through an operating microscope. Eepithelial scrape injuries removed all central corneal epithelium except a 0.5 mm rim at the limbus.

Recombinant human IL-1 receptor antagonist (supplied by Amgen, Thousand Oaks, CA, USA) was applied topically (1 drop at 20 mg ml^{-1}) to the mouse corneas immediately after cornea scrape injury and at 4-h intervals until 24 h post-injury. This dosage of IL-1 receptor antagonist was selected so that a vast excess of the antagonist over the cytokine in the stroma would be likely in the stroma after topical application. The IL-1 receptor antagonist used in this experiment is effective in blocking both IL-1α and IL-1β receptor binding (Granowitz et al., 1991). Animals assigned to the vehicle control group receive balanced salt solution drops at the same intervals.

Twenty-four hours after initial injury, the animals were placed under general anesthesia and euthanized with 100 mg/kg intraperitoneal pentobarbital. The mouse eyes were then harvested and frozen to −80 °C in Optimal Cutting Temperature (OCT) compound (Tissue-Tek, Torrance, CA). Cryostat sections were cut at 8μm thickness and mounted on microscope slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA).

2.2. Immunohistochemistry

A previously described method for immunocytochemistry was used in both experiments (Hong et al., 2001). Briefly, anti-mouse CD11b (BD Pharmingen, cat #550282) was used as the primary antibody at a 1:50 concentration and incubation was performed in a moistened dark chamber at room temperature for 90 min. Goat anti-rat IgG (AlexaFlour 488, Molecular Probes, Eugene, OR, cat #A11006) was used as secondary antibody at 1:100 concentration and was incubated at room temperature in moistened dark chamber for 30 min. Antibodies were diluted using 1× phosphate buffered saline (PBS) and 1% bovine serum albumin (BSA, Molecular Probes) was used as a blocking agent in both the IL-1 receptor antagonist and vehicle control solutions. Final mounting of tissue sections was done with mounting medium with DAPI (Vectashield, Burlingame, CA).

2.3. Microscopy, cell counting, and statistical analysis

Stained sections were examined using fluorescence microscopy and the number of cells per 400× microscopic field were counted. Positively-stained cells were counted from six non-overlapping regions of the central stroma in each tissue section, and the results from six sections of central cornea were counted and averaged to give the value for each animal. Data were then analyzed using statistical software (StatView, Cary, NC) with a Bonferroni/Dunn adjustment for repeated measures using ANOVA analysis (alpha value 0.05 for significance).

3. Results

Topical IL-1 receptor antagonist was highly effective in blocking the influx of CD11b-positive monocytic cells, used as a marker for inflammatory cell infiltration. In the first experiment, topical IL-1 receptor antagonist markedly reduced the influx of CD11b-positive bone marrow-derived cells into the corneal stroma at 24 h after epithelial injury (Fig. 1). CD11b-positive cells/400× field were 13.9 ± 1.2 [S.E.M.] ($n = 6$), 3.5 ± 0.5 ($n = 8$), and 5.1 ± 0.3 ($n = 6$) in the BSS-treated control, IL-1 receptor antagonist-treated, and unwounded control eyes, respectively. The difference between the BSS-treated control group and the IL-1 receptor antagonist or unwounded control group was significant ($p < 0.01$). There was no significant difference between the IL-1 receptor antagonist-treated and the unwounded control groups.

Similarly, in the second experiment performed with a second observer performing cell counts, IL-1 receptor antagonist markedly reduced CD11b-positive cells in the corneal stroma at 24 h after injury (Fig. 2). CD11b-positive cells/400× field were 19.1 ± 2.0 ($n = 7$), 5.8 ± 0.8 ($n = 7$), and 3.5 ± 0.8 ($n = 4$) in the BSS-treated control, IL-1 receptor antagonist-treated, and unwounded control eyes, respectively. The difference between the BSS-treated control group and the IL-1 receptor antagonist or unwounded control group was significant ($p < 0.01$). There was no significant difference between the IL-1 receptor antagonist-treated and the unwounded control groups.

4. Discussion

Interleukin-1α and β modulate several important components of the stromal response to injury, including Fas/Fas ligand-mediated regulation of keratocyte apoptosis (Wilson et al., 1996; Mohan et al., 1997), keratocyte production of paracrine mediators hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) that regulate epithelial