Published in final edited form as:

Exp Eye Res. 2008 May ; 86(5): 753–757. doi:10.1016/j.exer.2008.02.001.

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

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Abstract

Interleukin (IL)-1 α 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 $\times$ field and 13.9 ± 1.2 cells/400 $\times$ 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)-1 α 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-1 α and IL-1 β released from the corneal epithelium.

The purpose of this study was to test the hypothesis that exogenous topical IL-1 receptor antagonist delivered during 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 of 50 mg/kg ketamine and xylazine 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. Epithelial 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-1 α and IL-1 β 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 8 μ m 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 (AlexaFluor 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 $1 \times$ 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 $400\times$ 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/ $400\times$ 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/ $400\times$ 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-1 α 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 proliferation, migration and differentiation (Weng et al., 1997; Wilson et al., 1999), keratocyte production of collagenases and metalloproteinases (Girard et al., 1991; Li et al., 2003; Xue et al., 2003), and keratocyte production of chemokines (Hong et al., 2001). Biswas et al. (2004) also demonstrated that IL-1 receptor antagonist reduced the influx of cells of the innate and adaptive immune response into the cornea in a mouse herpes simplex model.

Other studies have shown effects of IL-1 receptor antagonist on corneal cells. Sun et al. (2006) demonstrated that IL-1 receptor antagonist prevented apoptosis during ex vivo expansion of human limbal epithelial cells cultivated on human amniotic membranes. Ward et al. (2007) found that IL-1 receptor antagonist modulated immediate dynamic behavioral responses by corneal Langerhans cells in response to corneal thermal injury.

In the present study, the data demonstrate conclusively that topical IL-1 receptor antagonist markedly down-regulates the appearance of CD11b-positive bone marrow-derived monocytic cells. In this study, CD11b-positive cells were used as markers for bone marrow-derived cells because of the efficacy of available antibodies in immunocytochemistry, but it is likely the influx of other inflammatory cells is also downregulated, since previous studies demonstrated that IL-1 upregulates expression of numerous chemokines that are chemotactic to a variety of inflammatory cells (Hong et al., 2001). Recently, experiments in rabbits have noted the same effect of IL-1 receptor antagonist on the appearance of CD11b-positive cells in the stroma after corneal epithelial scrape injury (Ramos-Esteban, Chaurasia, Medeiros, and Wilson, unpublished data, 2008).

Recent studies using chimeric mice have demonstrated that waves of bone marrow-derived cells invade the cornea in the first few days after epithelial scrape or other corneal injury in the mouse (Wilson et al., 2004; Carlson et al., 2006, 2007). However, studies have also demonstrated that there are resident CD11b-positive cells present in the unwounded corneal stroma (Mayer et al., 2007). Therefore, although the authors believe it is likely most of the infiltrating cells detected in these experiments are bone marrow-derived cells, we cannot exclude the possibility that some cells are locally recruited CD11b-positive cells.

The concentration of IL-1 receptor antagonist used for topical application in this pilot study (20 mg/ml) is very high. In order for IL-1 receptor antagonist to effectively block IL-1 binding to receptors, the soluble receptor must be in significant excess. This follows from studies demonstrating that IL-1 receptor antagonist binds to the IL-1 R type I receptor with lower affinity than IL-1 α or IL-1 β (Dinarello, 1994; Granowitz et al., 1991). It could be that lower concentrations of IL-1 receptor antagonist would also be effective. This will be explored in ongoing studies of IL-1 receptor antagonist in rabbit refractive surgery models.

In some respects, it is surprising how effective IL-1 receptor antagonist is in blocking inflammatory cell influx into the cornea. Given the importance of this process in combating infection, our expectation was that there would be considerable redundancy in the regulation of inflammatory cell infiltration into the stroma. However, the results of this study suggest that IL-1 α and IL-1 β are the primary modulators of this response, with the redundancy primarily being represented by the expression of the two IL-1 isotypes in corneal epithelial cells.

This study suggests that topical application of IL-1 receptor antagonist could be an important adjuvant to corticosteroids for decreasing inflammatory cell populations in the cornea in a variety of conditions. Thus, in vision threatening conditions, such as corneal transplant rejection and severe diffuse lamellar keratitis after laser in situ keratomeliosis, topical IL-1 receptor antagonist could effectively reduce further inflammatory cell migration into the corneal stroma and improve resolution of the inflammatory condition. Clinical studies in the future may address these potential applications of IL-1 receptor antagonist.

Acknowledgements

Supported in part by US Public Health Service grants EY010056 and EY015638 from the National Eye Institute and Research to Prevent Blindness, New York, NY. Dr. Wilson is the recipient of a Research to Prevent Blindness Physician-scientist Award.

References

- Biswas PS, Banerjee K, Zheng M, Rouse BT. Counteracting corneal immunoinflammatory lesion with interleukin-1 receptor antagonist protein. *J. Leukoc. Biol* 2004;76:868–875. [PubMed: 15258192]

- Carlson EC, Drazba J, Yang X, Perez VL. Visualization and characterization of inflammatory cell recruitment and migration through the corneal stroma in endotoxin-induced keratitis. *Investig. Ophthalmol. Vis. Sci* 2006;47:241–248. [PubMed: 16384969]
- Carlson EC, Lin M, Liu CY, Kao WW, Perez VL, Pearlman E. Keratocan and lumican regulate neutrophil infiltration and corneal clarity in lipopolysaccharide-induced keratitis by direct interaction with CXCL1. *J. Biol. Chem* 2007;282:35502–35509. [PubMed: 17911102]
- Dinarello CA. The interleukin-1 family: 10 years of discovery. *FASEB J* 1994;8:1314–1325. [PubMed: 8001745]
- Girard MT, Matsubara M, Fini ME. Transforming growth factor-beta and interleukin-1 modulate metalloproteinase expression by corneal stromal cells. *Investig. Ophthalmol. Vis. Sci* 1991;32:2441–2454. [PubMed: 1651296]
- Granowitz EV, Clark BD, Mancilla J, Dinarello CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. *J. Biol. Chem* 1991;266:14147–14150. [PubMed: 1830582]
- Hong J-W, Liu JJ, Lee J-S, Mohan RR, Mohan RR, Woods DJ, He Y-G, Wilson SE. Pro-inflammatory chemokine induction in keratocytes and inflammatory cell infiltration into the cornea. *Investig. Ophthalmol. Vis. Sci* 2001;42:2795–2803. [PubMed: 11687520]
- Kennedy MC, Rosenbaum JT, Brown J, Planck SR, Huang X, Armstrong CA, Ansel JC. Novel production of interleukin-1 receptor antagonist peptides in normal human cornea. *J. Clin. Invest* 1995;95:82–88. [PubMed: 7814649]
- Li DQ, Shang TY, Kim HS, Solomon A, Lokeshwar BL, Pflugfelder SC. Regulated expression of collagenases MMP-1, -8, and -13 and stromelysins MMP-3, -10, and -11 by human corneal epithelial cells. *Investig. Ophthalmol. Vis. Sci* 2003;44:2928–2936. [PubMed: 12824233]
- Mayer WJ, Irschick UM, Moser P, Wurm M, Huemer HP, Romani N, Irschick EU. Characterization of antigen-presenting cells in fresh and cultured human corneas using novel dendritic cell markers. *Investig. Ophthalmol. Vis. Sci* 2007;48:4459–4467. [PubMed: 17898266]
- Mohan RR, Liang Q, Kim W-J, Helena MC, Baerveldt F, Wilson SE. Apoptosis in the cornea: further characterization of Fas–Fas ligand system. *Exp. Eye Res* 1997;65:575–589. [PubMed: 9464190]
- Narayanan S, Glasser A, Hu YS, McDermott AM. The effect of interleukin-1 on cytokine gene expression by human corneal epithelial cells. *Exp. Eye Res* 2005;80:175–183. [PubMed: 15670796]
- Okamoto M, Takagi M, Kutsuna M, Hara Y, Nishihara M, Zhang MC, Matsuda T, Sakanaka M, Okamoto S, Nose M, Ohashi Y. High expression of interleukin-1beta in the corneal epithelium of MRL/lpr mice is under the control of their genetic background. *Clin. Exp. Immunol* 2004;136:239–244. [PubMed: 15086386]
- Shin JS, Kim CW, Kwon YS, Kim JC. Human beta-defensin 2 is induced by interleukin-1beta in the corneal epithelial cells. *Exp. Mol. Med* 2004;36:204–210. [PubMed: 15272231]
- Sun CC, Su Pang JH, Cheng CY, Cheng HF, Lee YS, Ku WC, Hsiao CH, Chen JK, Yang CM. Interleukin-1 receptor antagonist (IL-1RA) prevents apoptosis in ex vivo expansion of human limbal epithelial cells cultivated on human amniotic membrane. *Stem Cells* 2006;24:2130–2139. [PubMed: 16741227]
- Tran MT, Tellaetxe-Isusi M, Elner V, Strieter RM, Lausch RN, Oakes JE. Proinflammatory cytokines induce RANTES and MCP-1 synthesis in human corneal keratocytes but not in corneal epithelial cells. Beta-chemokine synthesis in corneal cells. *Investig. Ophthalmol. Vis. Sci* 1996;37:987–996. [PubMed: 8631642]
- Ward BR, Jester JV, Nishibu A, Vishwanath M, Shalhevet D, Kumamoto T, Petroll WM, Cavanagh HD, Takashima A. Local thermal injury elicits immediate dynamic behavioural responses by corneal Langerhans cells. *Immunology* 2007;120:556–572. [PubMed: 17250587]
- Weng J, Mohan RR, Li Q, Wilson SE. IL-1 upregulates keratinocyte growth factor and hepatocyte growth factor mRNA and protein production by cultured stromal fibroblast cells: interleukin-1 beta expression in the cornea. *Cornea* 1997;16:465–471. [PubMed: 9220246]
- Wilson SE, Lloyd SA, He YG. Glucocorticoid receptor and interleukin-1 receptor messenger RNA expression in corneal cells. *Cornea* 1994a;13:4–8. [PubMed: 8131405]

- Wilson SE, Schultz GS, Chegini N, Weng J, He Y-G. Epidermal growth factor, transforming growth factor alpha, transforming growth factor beta, acidic fibroblast growth factor, basic fibroblast growth factor, and interleukin-1 proteins in the cornea. *Exp. Eye Res* 1994b;59:63–72. [PubMed: 7530663]
- Wilson SE, He Y-G, Weng J, Li Q, McDowall AW, Vital M, Chwang EL. Epithelial injury induces keratocyte apoptosis: hypothesized role for the interleukin-1 system in the modulation of corneal tissue organization and wound healing. *Exp. Eye Res* 1996;62:325–328. [PubMed: 8795451]
- Wilson SE, Liu JJ, Mohan RR. Stromal-epithelial interactions in the cornea. *Prog. Retin. Eye Res* 1999;18:293–309. [PubMed: 10192515]
- Wilson SE, Mohan RR, Mohan RR, Ambrósio R Jr, Hong J, Lee J. The corneal wound healing response: cytokine-mediated interaction of the epithelium, stroma, and inflammatory cells. *Prog. Retin. Eye Res* 2001;20:625–637. [PubMed: 11470453]
- Wilson SE, Mohan RR, Netto MV, Perez V, Possin D, Huang J, Kwon R, Alekseev A. RANK, RANKL, OPG, and M-CSF expression in stromal cells during corneal wound healing. *Investig. Ophthalmol. Vis. Sci* 2004;45:2201–2211. [PubMed: 15223796]
- Xue ML, Wakefield D, Willcox MD, Lloyd AR, Di Girolamo N, Cole N, Thakur A. Regulation of MMPs and TIMPs by IL-1beta during corneal ulceration and infection. *Investig. Ophthalmol. Vis. Sci* 2003;44:2020–2025. [PubMed: 12714639]

Fig. 1. Influx of CD11b-positive inflammatory cells into the cornea at 24 h after epithelial scrape injury in eyes treated with topical IL-1 receptor antagonist or vehicle. A is a native unwounded cornea and only a single CD11b-positive cell is noted (arrow). In the vehicle treated control (B), large numbers of CD11b-positive cells are noted in the corneal stroma (arrows). However, in the IL-1 receptor treated cornea (C), there is a marked reduction in the number of infiltrating CD11b-positive cells (arrows). Magnification 400 $\times$.

Fig. 2.

Plot of data from experiment 2 on the effect of IL-1 receptor antagonist vs BSS vehicle control on CD11b-positive cell infiltration into the cornea after corneal epithelial scrape injury. Native unwounded control eyes are also included. *Indicates a statistically significant difference compared to the balanced salt solution-treated control.