Synthesis of Type II Interleukin-1 Receptors by Human Corneal Epithelial Cells but not by Keratocytes

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**Purpose.** The purpose of this study was to determine whether human corneal epithelial cells and keratocytes synthesize both the soluble and membrane forms of the type II IL-1 receptor (IL-1RII).

**Methods.** Primary cell cultures of human corneal epithelial cells and keratocytes were established from human corneas. RT-PCR was used to analyze cell cultures for expression of IL-1RII mRNA. The capacity of corneal cells to synthesize membrane-bound IL-1RII was determined by immunofluorescence microscopy, whereas ELISA was used to quantify synthesis of soluble IL-1RII after IL-1α and TNF-α stimulation.

**Results.** Corneal epithelial cells expressed IL-1RII mRNA. The cells also stained positive for membrane-bound IL-1RII, and media harvested from epithelial cell cultures contained up to 50 pg/ml of soluble IL-1RII. Both IL-1α and TNF-α significantly enhanced the amounts of soluble IL-1RII released from epithelial cell surfaces. In contrast to epithelial cells, corneal keratocytes did not express IL-1RII mRNA. Membrane-bound IL-1RII was not detected on keratocytes, nor was soluble IL-1RII detected in culture media harvested from these cells.

**Conclusions.** Human corneal epithelial cells but not corneal keratocytes synthesize both membrane and soluble forms of IL-1RII. Because both forms of IL-1RII can function as IL-1α antagonists, the results suggest that human corneal epithelial cells but not corneal keratocytes have evolved the capacity to dampen IL-1α responses through the production of IL-1RII. (Invest Ophthalmol Vis Sci. 2001;42:701–704)

Interleukin (IL) 1 is a cytokine that mediates inflammatory responses through interactions with type 1 IL-1 receptors (IL-1R) found on nearly all tissue and organ systems of the body. Binding of IL-1R to its ligand leads to the initiation of a signal transduction cascade that results in synthesis of a multitude of proinflammatory mediators. Because of the variety of inflammatory molecules inducible by IL-1, prolonged exposure to this cytokine can have serious pathologic consequences.

Therefore, many cells synthesize IL-1 inhibitors, which have been postulated to play a role in downregulating IL-1 activity. One of these molecules is called IL-1 receptor antagonist (IL-1RA). This protein antagonizes IL-1 activity by blocking type I receptors. A second IL-1 antagonist is the type II IL-1 receptor (IL-1RII). IL-1RII is synthesized as a membrane-bound IL-1 ligand that is missing the signal-transducing cytoplasmic domain found in the type 1 receptor. After its synthesis, matrix metalloproteinases rapidly cleave the receptor from cell surfaces causing it to be shed into the milieu. Both membrane-bound and secreted IL-1RII can bind IL-1 and prevent its interaction with the signal transducing IL-1RI. The corneal surface is composed of a layer of transparent epithelial cells resting on a stratum of connective tissue possessing fibroblast-like cells called keratocytes. Corneal epithelial cells can initiate inflammatory reactions at eye surfaces through their capacity to store IL-1α in the cytoplasm in a biologically active form where it is available for passive release whenever epithelial cell membranes are disrupted by disease or injury. It has recently been reported that human corneal epithelial cells secrete IL-1RA. In this study, we demonstrate that human corneal epithelial cells but not corneal keratocytes synthesize IL-1RII.

**Materials and Methods**

**Preparation of Corneal Epithelial Cell and Kerocyte Cultures**

Corneas were obtained from the National Disease Research Interchange (Philadelphia, PA) or the Alabama Donor Eye Bank (Mobile, AL) and processed within 4 days of enucleation. Human corneal epithelial and keratocytes were harvested from corneal donors and plated with equal density in 25-cm² flasks as described previously. Epi-thelial cell and keratocyte cultures were used for experiments when they were approximately 90% confluent.

**Amplification of IL-1 Receptor mRNAs by RT-PCR**

Total cellular RNA was isolated from cell cultures by the acid guanidinium thiocyanate-phenol-chloroform extraction method as previously described. Primers for the polymerase chain reaction (PCR) were selected with the aid of OLGIGO primer selection software (Eccles Institute for Human Genetics and Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT) running on the National Cancer Institute-Frederick Cancer Research Center Vax 6620 (Frederick, MD). Reverse primers were selected to be complementary to exon sequences within the human IL-1RI, IL-1RII, or glyceraldehyde-3-phosphate dehydrogenase (GAPD) coding regions. Primers that span at least one exon-exon boundary in the target mRNA sequence were chosen for use in RT-PCR. The following primer sequences were used, and the RT-PCR product sizes indicated in parentheses: human IL-1RI primers (495-bp product): mRNA, forward 5'-GGT GAC TCC TCC CTC CG-3; human IL-1RI primers (411-bp product): mRNA, forward 5'-CTC GGATT ATT ATG ATG CCC TG-3; and mRNA reverse 5'-TAT AAG GCC ACA CAA GTC CGG-3; human IL-1RI primers (411-bp product): mRNA, forward 5'-TAT AAG GCC ACA CAA GTC CGG-3; human IL-1RI primers (411-bp product): mRNA, forward 5'-ATG ATT ATG ATG CTG AGA AGC-3; reverse 5'-TAT AAG GCC ACA CAA GTC CGG-3; human GAPD primers (417-bp product): mRNA forward 5'-CCA AAA GGG TCA TCA TCT CCG G-3; reverse 5'-ATT TGG CAG GTT TTT CTA GAC GC-3.

DNA complementary to total cellular RNA was made using a GeneAmp RNA PCR kit (Perkin Elmer, Norwalk, CT) according to the manufacturer’s specifications. All RT-PCR products were amplified using thermocycles consisting of 30 seconds at 95°C, 30 seconds at 65°C, and 2 minutes at 72°C. Preliminary experiments were performed to establish the number of cycles of amplification needed to generate products within the exponential amplification phase of the RT-PCR. A duplicate RNA sample was amplified by PCR with the reverse transcription step omitted to verify that the RNA samples were free of detectable levels of genomic DNA. After completion of the PCR am-
Epithelial cells  Keratocytes

IL-1RI mRNA  
1:1  1:5  1:25  1:125

IL-1RII mRNA  
1:1  1:5  1:25  1:125

dilution factor

**FIGURE 1.** Analysis of IL-1 receptor mRNA synthesis in human corneal epithelial cells and keratocytes. Confluent monolayers of human corneal epithelial cells and keratocytes were grown for 12 hours in fresh medium. Total RNA was harvested from cell monolayers, serially diluted 1:5, and reverse-transcribed to cDNA. The reverse transcription products were further amplified using PCR primers specific for either IL-1RI or IL-1RII cDNA.

Immunohistochemical Analysis of Cell Surface Type II IL-1 Receptors

Keratocytes and epithelial cells were plated to Laboratory-Tek II tissue culture eight-chamber, coverslips (Nalge Nunc International, Naperville, IL). After an overnight incubation, cultures were rinsed twice with ice-cold PBS. Potential cell surface Fc receptors were blocked by incubating the cell cultures with 10 μg human IgG in 150 μl PBS for 15 minutes at room temperature. Next, 0.1, 1.0, or 10 μg biotinylated anti-human IL-1RII polyclonal antibody (BAF263; R&D Systems, Minneapolis, MN) diluted in phosphate-buffered saline containing 2% bovine serum album was added to culture chambers. Fluorescein-conjugated avidin was used as the secondary detection reagent. Primary and secondary labeling was carried out at 4°C for 1.5 hours and 4°C for 1 hour, respectively, before final washing with 1× RD1 wash buffer (R&D Systems). Stained cells were analyzed by fluorescence microscopy using an Olympus inverted microscope (Melville, NY).

**Analysis of Soluble IL-1RII Protein Synthesis**

Medium was aspirated from cell cultures and replaced with 2 ml medium or 2 ml medium containing either human rIL-1α (R&D Systems) or human rTNF-α (Genzyme, Cambridge, MA). At preselected times postinduction, medium was removed and frozen at −20°C for subsequent analysis. Soluble IL-1RII receptor levels were measured by ELISA kits obtained from R&D Systems following the manufacturer’s instructions. The lower limit of IL-1RII detection for this ELISA was 10 pg/ml. Colorimetric results were read at 450 nm using an EL308 microplate reader (Biotek Instruments, Winooksi, VT). Significance differences in IL-1RII synthesis were determined by using small sample paired t-statistics. P ≤ 0.05 was considered significant.

**RESULTS**

RNA was isolated from epithelial cells and keratocytes and analyzed by RT-PCR for IL-1RI and IL-1RII mRNAs. To compare levels of gene expression, serial dilutions of the RNA samples were made before running RT reactions. It was found that epithelial cells synthesize similar amounts of both IL-1RI and IL-1RII mRNA (Fig. 1). In contrast, IL-1RII mRNA was never detected in keratocytes even though these cells were synthesizing IL-1RI mRNA. To determine whether IL-1RII mRNA was translated into membrane-bound receptors, epithelial cells were analyzed by immunofluorescence microscopy for the presence of membrane IL-1RII protein (Fig. 2A). A strong punctate staining reaction was detected on these cells, which covered the entire surface. In contrast, only background levels of fluorescence were seen on keratocytes (Fig. 2B). These results indicate that corneal epithelial cells but not corneal keratocytes synthesize membrane-bound IL-1RII.

It is known that IL-1RII can be shed from cell surfaces and that proinflammatory mediators enhance their release.8,18–20 To determine whether corneal epithelial cells shed IL-1RII, supernatants from epithelial cell cultures were assayed for soluble IL-1RII by ELISA. In addition, we assayed supernatants harvested from TNF-α- and IL-1α-stimulated epithelial cells for soluble IL-1RII to determine whether these two proinflammatory cytokines stimulate shedding. It was found that epithelial cells constitutively release approximately 50 pg/ml of soluble IL-1RII into the medium (Fig. 3). When cultures were exposed to increasing concentrations of TNF-α, soluble IL-1RII production was enhanced in a dose–response fashion. Stimulation of the cells with 500 U/ml TNF-α enhanced IL-1RII release up to
10-fold above background. IL-1α also significantly stimulated IL-1RII release but to a lesser degree than that induced by TNF-α (2.5-fold). When similar experiments were performed on keratocyte cultures, soluble IL-1RII protein was not detected (data not shown). These results indicate that membrane-bound IL-1RII is shed from the corneal epithelial cell surfaces in a soluble form and that the amounts of soluble IL-1RII released is enhanced by proinflammatory mediators.

**DISCUSSION**

The predominate producers of IL-1RII have been reported to be monocytes, B cells, and polymorphonuclear leukocytes. In this study, it was found that human corneal epithelial cells also produce IL-1RII, which can be shed from the surface of cells in significant amounts. In contrast, neither IL-1RII mRNA nor protein was synthesized by human corneal keratocytes. These results indicate that cells in the epithelial but not stromal layer of the cornea have the capacity to produce both the membrane and soluble forms of IL-1RII.

Work in several laboratories suggests that both membrane and soluble forms of IL-1RII can regulate IL-1 activity by binding to cell-free IL-1, thus inhibiting its capacity to interact with the signal transducing IL-1R. For example, cultured keratinocytes have been found to have significantly diminished responses to IL-1α when they are genetically engineered to express IL-1RII on their cell surface. In studies with soluble IL-1RII, it has been found that incubation of fibroblasts with soluble IL-1RII inhibits the capacity of exogenously added IL-1 to induce prostaglandin E2 and IL-6 synthesis. In addition to inhibiting IL-1 by direct interaction, the membrane form of IL-1RII can also indirectly inhibit IL-1α activity by interfering with IL-1RI-mediated signal transduction. This inhibitory mechanism is dependent on the fact that IL-1R must bind to an IL-1 receptor accessory protein to form a functional signaling receptor complex. Membrane-bound IL-1RII inhibits this interaction by binding to the IL-1 receptor accessory protein preventing its interaction with IL-1RI.

Previous work has shown that human corneal epithelial cells are less responsive than keratocytes to IL-1α. Because IL-1α is rapidly released from diseased or damaged corneal cells, the reduced responsiveness of these cells to IL-1α may play a role in limiting destructive inflammatory responses after superficial injury to immediate corneal surfaces. How epithelial cells regulate their responsiveness IL-1α is not clear. The capacity of epithelial cells to secrete IL-1RA may be one mechanism to account for diminished responses of epithelial cells to IL-1α. The fact that IL-1α and TNF-α significantly enhance IL-1RII secretion suggests that IL-1RII levels can be rapidly increased during corneal inflammation. One could speculate, therefore, that synthesis of IL-1RII provides an additional mechanism whereby corneal epithelial cells can dampen IL-1α activity in the epithelial layer of the cornea after disease or injury.

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