Ocular Inflammation Stimulated by Intravitreal Interleukin-8 and Interleukin-1

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Interleukin-8 (IL-8), a cytokine with neutrophil chemotactic and activating properties, is known to be stimulated by IL-1. Fischer rats are more resistant to inflammation than Lewis rats probably due to a higher corticosteroid stress response. To determine the role of IL-8 in ocular inflammation, the effect of intravitreal injection of IL-8 was compared with that of IL-1 in both Lewis and Fischer rats. The IL-8, IL-1α, or sterile balanced salt solution (control) was injected into one eye of each animal. Both IL-8 and IL-1α caused inflammation in the eye of both strains, as detected by leukocyte counts of the anterior chamber and histopathologic examination. The eyes of animals injected with a cytokine had significantly higher numbers of leukocytes compared with eyes of control animals. Histopathologic examination confirmed these findings. The IL-1α induced inflammation more consistently and more severely than the most effective dose of IL-8. This finding agreed with the concept of IL-1 initiating a cascade of inflammatory mediators including IL-8, which acts more specifically on a smaller population of leukocytes. A contralateral response was observed in the uninjected eye of experimental and control animals. The contralateral response in animals receiving the cytokines was significantly greater than that in controls. Lewis rats show a higher inflammatory response to the injections than do the Fischer rats. These data suggest that IL-8 may be active as one component in neutrophil-mediated ocular inflammation. Invest Ophthalmol Vis Sci 32:1534–1539, 1991

Accumulation of leukocytes in a tissue is thought to be the result of the release of specific chemotactic mediators in the area of inflammation. One of these factors, interleukin-8 (IL-8), selectively activates polymorphonuclear neutrophils and causes chemotaxis of T-lymphocytes. It has also been referred to as monocyte-derived neutrophil chemotactic factor, neutrophil-activating peptide-1, and neutrophil-activating factor. This substance has the ability to cause the chemotaxis of neutrophils, neutrophil shape change, exocytosis of neutrophilic granules, surface expression of adhesion molecules, respiratory burst, and a rise in cytosolic Ca²⁺ concentrations in the neutrophil. Peripheral blood monocytes, alveolar macrophages, endothelial cells, fibroblasts, epithelial cells, hepatoma cells, and retinal pigment epithelial cells all are capable of elaborating IL-8. Lipopolysaccharide is a stimulus for the first three types of cells, and tumor necrosis factor α and IL-1 (α and β) are both independent stimuli for all seven cell types. In addition IL-8 has in vivo inflammatory activity in the skin.

A very effective inducer of intraocular inflammation in the rabbit eye, IL-1 is known to be extremely sensitive to inflammatory stimuli. It is the initiating factor in the production of a cascade of inflammatory mediators, one of which is IL-8. Furthermore, IL-1 itself has no in vitro neutrophil chemotactic activity.

To elucidate the mechanisms of ocular inflammation and the possible involvement of IL-8, human recombinant IL-8 was injected intravitreally into Lewis and Fischer 344 rats, and the resulting inflammation was compared with that caused by intravitreal injections of human recombinant IL-1α. The IL-8 caused an inflammation characterized by the influx of neutrophils into various anterior ocular compartments of the injected eye, but IL-1α initiated inflammation much more consistently and severely than IL-8. In addition the experiments with the Fischer rats showed that if only one eye was injected with a cytokine, the contralateral eye will still respond with significant inflammation.

Materials and Methods

Animals/Procedures

Fifty male Lewis and Fischer 344 rats were purchased from Charles River Laboratories (Raleigh,
NC) and used at age 7–9 weeks. They were treated in accordance with the ARVO Resolution on the Use of Animals in Research. The animals were anesthetized with a combination of ketamine (10 mg) and xylazine (8 mg) given intramuscularly and proparacaine given topically to the eye. To preclude a rise in intraocular pressure and compression of the ocular tissue, before the intravitreal injection, paracentesis of the anterior chamber was done. A volume of 10 μl was then injected using an Hamilton 50-μl syringe and a 30-gauge needle inserted through the pars plana into the central vitreous. After the injection gentaminic ophthalmic ointment was applied. Two experimental designs were used: (1) 16 animals were injected with sterile balanced salt solution (BSS) in the right eye and IL-8 in the left eye or (2) they were injected in the right eye only with BSS (8 rats), IL-8 (18 rats), or IL-1 (8 rats), and the contralateral eye was not injected.

Cytokine

Human IL-8 was produced in Escherichia coli by recombinant DNA technology and purified by means of CM-Sepharose CL-6B column chromatography (Pharmacia, Gaithersburg, MD) and gel filtration on Toyopearl HW-55 (Tosoh, Tokyo, Japan). The IL-8 was diluted in endotoxin-free sterile BSS (Endosol; Entravision, Tosoh, Tokyo, Japan) and injected intravitreally in total doses of 1 pg, 100 pg, 10 ng, and 1 μg (0.125 fmol, 12.5 fmol, 1.25 pmol, and 125 pmol, respectively). Human recombinant IL-1α was diluted in the same BSS, and 5 ng (0.3 pmol = 100 U) was injected intravitreally. Both the stock rIL-8 and rIL-1 was verified to be endotoxin free by limulus assay.

Cell Counts, Histology, and Protein Concentration

The animals were killed with CO2 at different intervals after injection, and 6 μl of aqueous humor was aspirated. Leukocytes in a volume of 2 μl were stained with Wright's stain and were counted. The eyes from animals not used for aspiration were enucleated and fixed in 4% glutaraldehyde and 10% formalin. Sections through the vertical plane were stained with conventional hematoxylin and eosin. The results of the histologic examination were graded on the following scale: trace, inflammatory cells in ocular tissues (ciliary body, iris, or cornea), but not in anterior chamber; +1, < 80 inflammatory cells in anterior chamber; +2, 80–120 inflammatory cells in anterior chamber; +3, 120–150 inflammatory cells in anterior chamber; and +4, > 150 inflammatory cells in anterior chamber and vitritis.

Results

The intravitreal injection of 10 ng of IL-8 produced a significant influx of neutrophils into the anterior chamber of the rat eye 20 hr after injection, as seen in 20 rats. Contralateral eyes injected with BSS also showed leukocyte infiltration, but the eyes injected with IL-8 had significantly more leukocytes than the control eye in 10 of 11 animals (Fig. 1). Histologic examination of 12 rats confirmed that the infiltrating cells were almost exclusively polymorphonuclear neutrophils. In IL-8-injected eyes these cells can be found in the iris, ciliary body, cornea, posterior chamber, and anterior chamber (Fig. 2). Histopathologic testing at subsequent times after injection showed that the neutrophils were entering the anterior chamber from the blood vessels in the trabecular meshwork, the iris, and the ciliary body and that there were higher grades in some animals with eyes injected with IL-8 compared with other animals receiving BSS only. Others showed similar degrees of ocular inflammation (Table 1).

Neutrophil infiltration into the anterior chamber after injection of various amounts of IL-8 followed a bell-shaped curve. The most effective dose of IL-8 was 10 ng, and 1 μg caused an inflammation similar to the lowest dose tested (1 pg), as noted in 12 animals (Fig. 3).

We further evaluated the kinetics of neutrophil migration into the eye after IL-8 injection. The first neutrophils to enter the anterior chamber appeared 4–6 hr after the injection of 10 ng of IL-8. The peak activity of intravitreal IL-8 was 10–24 hr after the injec-
tion, and IL-8-induced neutrophil infiltration was subsiding at 48 hr (Fig. 4).

When BSS, IL-1, or IL-8 was injected into one eye and the contralateral eye left uninjected, both eyes of all animals had some degree of inflammation. The IL-1 caused the most consistently severe inflammation in the anterior segment of the injected eye, and vitritis was noted in the eye of almost every animal (Figs. 5, 6). The IL-1-induced inflammation was also characterized by a mainly neutrophil infiltration. In contrast IL-8 induced an anterior segment inflammation which was intermediate in severity between the BSS controls and IL-1. The Lewis rats had a much greater response to the injection itself as can be seen by comparing the BSS injections in both strains (Fig.

6). The experiments in which only one eye was injected also demonstrated the presence of a contralateral response to all injections, including the BSS. This contralateral response was characterized by a mild inflammatory cellular infiltration in the iris, ciliary body, cornea, and posterior and anterior chambers. In the Fischer rats, the cytokines elicited a greater contralateral response than the BSS; both the cell count data and the histopathologic grading demonstrated this (Fig. 6 and Table 1). This difference between the cytokines and the BSS was not apparent in the Lewis strain due to the inflammation caused by the injection of BSS alone, which overshadowed that produced by the cytokines.

Discussion

Our study found that intravitreal injection of human IL-8 induces significant neutrophil infiltration

Table 1. Histopathological grading of eyes injected with cytokines

<table>
<thead>
<tr>
<th>Animal</th>
<th>BSS eye</th>
<th>Uninjected eye</th>
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<td>1</td>
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<td>3</td>
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<tr>
<td>4</td>
<td>1+</td>
<td>1+</td>
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<tr>
<td>BSS</td>
<td>10 ng IL-8</td>
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<td>11</td>
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<td>12</td>
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All animals were Fischer 344 rats enucleated at 20 hr after intravitreal injection of indicated substance.
in the iris, ciliary body, cornea, posterior chamber, and anterior chamber of rats. At present, rat IL-8 has not yet been isolated. However, a rat neutrophil chemoattractant was recently found to have close structural similarity to human IL-8, suggesting that human IL-8 and rat IL-8 may be able to act across species barriers.12

Our data demonstrated that the ocular inflammation induced by the intravitreal IL-8 occurs 10–24 hr after the injection. Both rat strains showed a similar inflammatory response to the IL-8. The time course of intraocular IL-8 was much longer than has been reported for this cytokine injected into the dermis.6 In the dermis IL-8 causes the influx of neutrophils in 30 min, and the neutrophils are leaving by 3 hr. In the eye it is not until after 48 hr that the IL-8-induced inflammation subsides. Morphologic differences between the vitreous and the dermis probably account for the disparate time courses. The dermis is a much...
more vascular tissue with better circulation than the vitreous gel. Intravitreal IL-8 followed a classic bell-shaped dose–response curve with the peak dose lying at approximately 10 ng. The drop in activity at higher doses was most likely explained by the saturation of the system and loss of a chemotactic gradient. Alternatively there may be a block of the receptor sites or an inhibitor produced with high-dose administration of IL-8 to cause the less effective chemotaxis.

In our experiment a total volume of 10 μl of fluid was injected into the vitreous, which contains 50 μl. After paracentesis to decompress the eye, this volume may not affect the intraocular pressure and may decrease the effect induced by this relatively large injection. Care was also taken to minimize any mechanical damage and inflammatory stimulus due to the technique.

As has been shown in the rabbit eye, intravitreal IL-1α stimulated a very strong anterior segment inflammation in both rat strains studied. Vitritis was also observed in almost all animals. The stronger response to IL-1 compared with IL-8 agreed with current hypotheses on the role that these two inflammatory mediators play. The IL-1 has a more general function which includes direct activation of certain populations of immune cells, stimulation of the production of other cytokines such as IL-8, induction of arachidonic acid metabolism, and proinflammatory effects on local resident cells such as fibroblasts, endothelial cells, and synovial cells, but it is not chemotactic. On the other hand, IL-8 has been found to have much more specific activities which are limited to activation and chemotaxis of neutrophils and chemotaxis of T-lymphocytes. Therefore IL-1 triggers a stronger inflammatory reaction because of the cascade it initiates.

The Lewis rats responded to the injections with significantly more inflammation than did the Fischer rats in this preliminary experiment, and this difference between the two rat strains also has been seen in other experimental inflammatory models. These include both experimental autoimmune uveoretinitis (EAU) and streptococcal cell wall arthritis. In EAU and streptococcal cell wall arthritis, the susceptibility of the Lewis strain is much greater than the Fischer strain. The Fischer rats have an hypothalamic-pituitary-adrenal axis which is more effective in producing stress-related corticosteroids than do Lewis rats, and these hormones play a key role in modulating inflammation. In this study the response between the two strains was less marked than normally expected in another experimental model. This may be explained by the postulated mechanism of action of the corticosteroids which suppress inflammation by blocking the production of inflammatory mediators. When preformed inflammatory mediators such as IL-1 or IL-8 were injected as active molecules, the corticosteroids could not block their action as effectively. Thus, approximately equal inflammation occurred in both strains in the case of cytokine injections. In contrast, the inflammation induced by the trauma of the injection depended on the production of endogenous mediators, and this production may be suppressed more effectively in the Fischer rats.

The contralateral reaction observed in the Fischer rats is one that has been observed in other experimental conditions. Yew et al. reported a contralateral reaction to hypertonic saline injections in mice. The reason for this phenomenon is still unknown. In our system, the cytokines elicited a much stronger contralateral response than did the BSS injections in the Fischer rats. Small amounts of IL-8 could reach the contralateral eye through the blood circulation or a neuronal reflex induced by the injection might produce inflammation there. The inflammation in the contralateral eye is, therefore, somewhat dependent on the degree of inflammation in the injected or traumatized eye. This relationship agrees with the lack of a difference among the contralateral reactions to the cytokines and the BSS in the Lewis. The BSS injections in the Lewis rats cause so much inflammation from the penetrating site that the contralateral inflammation is similar to that of a contralateral reaction to a cytokine injection.

Since exogenous IL-8 is active in the eye, it may be involved in general in ocular inflammation. Cytokines such as IL-1 and tumor necrosis factor α stimulate a more general inflammatory reaction by causing the activation of various cells which then, among their other functions, release a cascade of other cytokines. One of the cytokines that is produced is IL-8, and it is the IL-8 which is a more direct activator of the neutrophils and, as has been recently described, T-lymphocytes. It is the variable cytokines with more specific functions, such as IL-8, which mediate some of the effects of the more general cytokines. To understand the role of IL-8 in ocular inflammation, it will be necessary to study the production of endogenous IL-8 in the eye.

Key words: interleukin-8, neutrophil chemotaxis, interleukin-1, anterior uveitis, ocular inflammation

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References


