Interleukin-1-β Changes the Expression of Metalloproteinases in the Vitreous Humor and Induces Membrane Formation in Eyes Containing Preexisting Retinal Holes

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Purpose. Proliferative vitreoretinopathy occurs when cells migrate into the vitreous humor, where they proliferate and produce a membrane composed of extracellular matrix. Interleukin-1-beta (IL-1-β) may be involved in these processes because it is chemotactic and mitogenic, and it stimulates metalloproteinase production. In the present study, the effects of intravitreally injected IL-1-β on retinal membrane formation and the associated changes in metalloproteinase content of vitreous humor were examined.

Methods. Rabbit eyes were injected with IL-1-β in a buffer, with or without the prior creation of retinal holes. Control eyes received the buffer alone or no injection, with or without retinal holes. Animals were examined by slit lamp biomicroscopy and indirect ophthalmoscopy for 1 month. Zymography was performed on a portion of vitreous humor to assess collagenase content, and the remaining tissue was subjected to histologic analysis.

Results. Intravascular IL-1-β induced perilimbal vessel engorgement, keratic precipitates, synechiae, flare, lens deposits, optic disk hyperemia, and granulomatous formations that gradually subsided during the first week. Intravitreal injection of IL-1-β in eyes with preexisting retinal holes additionally induced membrane formation. Zymographic analysis of vitreous humor from animals sacrificed 24 hours after IL-1-β injection showed a 100-kd and a 65-kd gelatinase, whereas control vitreous humor contained predominantly a single gelatinase species of approximately 65 kd. Retinal holes did not affect IL-1-β induction of the 100-kd gelatinase.

Conclusions. IL-1-β induces a 100-kd gelatinase in the vitreous humor and epiretinal membrane formation in eyes containing preexisting retinal holes. The presence of retinal holes and abnormal production of cytokines may lead to a cascade of events, including aberrant extracellular matrix remodeling, that result in proliferative diseases of the eye. Invest Ophthalmol Vis Sci. 1994;35:4260-4267.

Proliferative vitreoretinopathy (PVR) is one of the major causes of failure of surgery for the repair of retinal detachments. This pathologic condition can be separated into three distinct phases. The first phase involves an infiltration of cells into the preretinal or subretinal space, or both, where the cells begin to proliferate. The next includes the continuation of cell proliferation and the production of an extracellular matrix (ECM). The final phase involves the contraction of the ECM resulting in retinal detachments.

Studies of PVR membranes taken from human subjects show a complex mixture of cell types, including retinal pigment epithelial (RPE), glial, and fibroblast-like cells. More recent studies of PVR membranes have found inflammatory cells, suggesting that the immune system may be involved. The ECM of PVR membranes includes collagen, laminin, and fibronectin and is similar in composition to the basement membrane of the eye (Bruch’s membrane) maintained by RPE cells. The production and remodeling of ECM, which must occur during
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PVR membrane formation, involves interaction of secretory cells producing membrane components (including collagen) and a family of enzymes (matrix metalloproteinases [MMPs], collagenases).15 Modulation of the finely controlled interaction between the ECM and MMP in inflammation causes cellular proliferation, migration, and tissue morphogenesis15 and may lead to the development of pathologic conditions including PVR.

Interleukin-1-beta (IL-1-β) is involved in the acute-phase response. It is chemotactic for neutrophils, monocytes, and RPE cells in vitro, and it stimulates proliferative responses in many tissues.14,15 This cytokine has been shown to be produced by ocular cells, including RPE and corneal cells,16,17 and has been found in ocular fluids during inflammation, trauma, and PVR.18-20 Moreover, an in vitro model of retinal membrane contraction, as occurs in PVR, is modulated by IL-1-β.21

Previous investigations have noted that locally injected IL-1 elicits an acute inflammation of the anterior segment.22-24 However, in these studies, clinical features such as might be involved in PVR formation in the posterior segment were not studied. The metalloproteinase content of normal and inflamed vitreous humor also has not been previously investigated, yet ECM turnover is likely to be a fundamental process in proliferative eye disease. To mimic the surgical condition, where cytokines are likely to be present, the synergism between retinal surgery and cytokines in membrane formation and MMP production was studied.

MATERIALS AND METHODS

Intraocular Effects of Interleukin-1-β

Rabbits weighing between 4 and 5 pounds were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Eyes were dilated using a 1:1 solution of 1% phenylephrine hydrochloride/2.5% cyclopentolate hydrochloride. Intramuscular ketamine was used to maintain anesthesia. For all intravitreal injections, 250 U of recombinant human IL-1-β (Eli Lilly, Indianapolis, IN) in 0.1 ml balanced saline solution (BSS) were used. The activity of IL-1-β was determined by a thymocyte proliferation assay using cells from C3H/HeJ mice. One unit of IL-1-β is defined as that which results in 50% of maximum thymidine incorporation.25

In experiment 1, six animals were anesthetized, a contact lens and operating microscope. The contralateral eye was used as a control and received 0.1 ml of BSS alone. This group was examined at 2, 4, 8, and 24 hours before they were killed. Eyes were enucleated and bisected. Half of each eye was used to obtain samples for zymography, and the remaining half was used for histology. Tissue used for histology was fixed with 10% phosphate buffered formaldehyde at room temperature, dehydrated in a graded ethanol series, and embedded in paraffin wax. Eight-micron sections were stained with hematoxylin and eosin.

In experiment 2, 16 animals were anesthetized, eyelids were retracted, and a retrobulbar injection of 2% lidocaine was given. The conjunctiva was retracted, and a 25-gauge sclerotomy was performed just above the equator, allowing access to the globe’s interior. Two or three retinal holes (approximately a half-disc diameter) were created by aspiration inferior to the medullated nerve fiber layer using a 30-gauge cannula. Holes were created in both eyes of 11 rabbits and in a single eye of the remaining 5 rabbits. Two of the eyes were excluded from the study because of excessive hemorrhaging after the surgical procedure. All animals were allowed to heal for 1 week. The rabbits were anesthetized again with ketamine hydrochloride. Experimental eyes were injected with IL-1-β in BSS. Controls either were injected with BSS or were not injected. Six animals (six eyes with IL-1-β and retinal holes and six eyes with BSS and retinal holes) were killed after 24 hours to collect vitreous samples for zymography. The remaining 10 animals were examined each day for 1 week and once per week thereafter for approximately 1 month. This resulted in four groups of eyes that were examined before euthanasia and sample collection for zymography and histology, as follows: (1) IL-1-β and holes [six eyes]; (2) BSS and holes [six eyes]; (3) holes without injection [four eyes]; and (4) IL-1 without holes [four eyes].

Metalloproteinase Activity of Vitreous

Samples of vitreous humor were centrifuged at 30,000g for 10 minutes, and the supernatant fraction containing soluble proteins was collected and frozen at -70°C until further study. The presence of gelatinases was determined using zymography. Before separation by SDS-polyacrylamide gel electrophoresis, the vitreous supernatant fractions were incubated for 1 hour in either 0.1 M p-amino phenylmercuric acetate (APMA) or a sample buffer containing 0.05 M tris, 1.0% SDS, 10% glycerol, and 0.1% bromophenol blue. In the case of APMA incubation, the sample buffer was added immediately before loading the samples onto the gel. The supernatant fractions were then separated at 25 mA in either a 7% or a 12% polyacrylamide gel containing 1 mg pigskin gelatin/ml (Sigma, St. Louis, MO). The gel was washed twice in a 50-mM Tris buffer (pH 7.5) containing 2.5% Triton X-100, then rinsed once and incubated for 18 hours at 37°C in a 50 mM Tris buffer (pH 7.5) containing 5 mM.
CaCl₂ to allow enzymatic degradation of the gelatin. Gels were stained with Coomassie blue R-250 and de-stained with methanol:acetic acid:water (5:1:4). Unstained bands were observed in regions of gelatinase activity. Standard molecular weight markers (Bio-Rad, Richmond, CA) containing myosin, β-galactosidase, phosphorylase b, bovine serum albumin, and ovalbumin were included in each gel. Molecular weights of the enzymes found by zymography were determined by first-order regression using the standards as the reference points.

RESULTS

Preliminary studies demonstrated that IL-1-β alone elicited posterior inflammation accompanied by an increase in vitreous protein levels at 24 hours.¹⁶ Protein levels (mean mg/ml ± SD) were 5.08 ± 1.59 (n = 12) for IL-1-β-injected eyes, 1.51 ± 0.81 (n = 16) for BSS-injected eyes, and 1.36 ± 0.44 (n = 22) for uninjected eyes. Analysis of variance of these sample sets showed a highly significant difference between the IL-1-β-injected set and BSS-injected set (P < 10⁻⁶). There was no significant difference found between the BSS-injected set and the uninjected set. These preliminary experiments involving periods of 1 month showed only trace inflammatory responses of eyes from experiment 2 showed that the inflammation reached its maximum within 3 days and subsided within 1 week. These eyes also exhibited white aggregates located in the mid to inferior vitreous body. These aggregates peaked in size from 6 to 9 days. The aggregates were completely absorbed by the end of the study in 8 of 10 eyes. However, highly refractile points were present in the vitreous body, suggesting cells or unabsorbed breakdown products. The remaining two eyes contained only remnants of the initial aggregates.

Clinical Observations

During the first 24 hours, eyes injected with IL-1-β (six eyes from experiment 1 and 10 eyes from experiment 2) showed an intense inflammatory response in the anterior segment while all controls showed little or no reaction. The presence of a hole made no difference in the early clinical observations. After 4 hours, the perilimbal blood vessels (those surrounding the cornea just below the limbus) of the IL-1-β-injected eyes began to show signs of engorgement (Fig. 1A). This steadily increased and peaked at 24 hours, after which it slowly decreased and finally resolved by the end of the second week. Punctate keratic precipitates denoted by small grayish flecks of protein and inflammatory cells located on the endothelium were seen in 9 of 16 IL-1-β-injected eyes by 24 hours (Fig. 1A). By the end of the first week, 8 of 10 IL-1-β-injected eyes had developed keratic precipitates that resolved by 8 days. Anterior chamber flare including protein, fibrous material, and a few cells steadily increased over time. At 8 hours, the flare reached moderate to severe levels. At 24 hours, anterior chamber flare was so extreme in two animals that a small hemorrhage in the iris caused an accumulation of blood in the lower portion of the anterior chamber. At 24 hours, all 16 IL-1-β-injected eyes had fixed clumps of fibrous material attached to the anterior lens capsule (Fig. 1B). Adhesions of the iris to the anterior lens capsule (posterior synechiae) were present in 11 of 16 eyes after 1 week (Fig. 1C). Synechiae were severe in 8 eyes (encompassing >25% of the pupil) and moderate in 8 eyes (<25% of the pupil). All IL-1-β-injected eyes showed “iris pattern blur” representing iris edema and engorged capillaries but inflammatory lens opacities or corneal edema could not be found.

Initial examinations using indirect ophthalmoscopy of eyes that received IL-1-β from experiments 1 and 2 showed an inflammatory response within 24 hours that included vitreous haze and disk hyperemia. Delayed observations of eyes from experiment 2 showed that the inflammation reached its maximum within 3 days and subsided within 1 week. These eyes also exhibited white aggregates located in the mid to inferior vitreous body. These aggregates peaked in size from 6 to 9 days. The aggregates were completely absorbed by the end of the study in 8 of 10 eyes. However, highly refractile points were present in the vitreous body, suggesting cells or unabsorbed breakdown products. The remaining two eyes contained only remnants of the initial aggregates.

The posterior segment of all eyes not injected with IL-1-β from experiments 1 and 2 showed minimal response to the injection of IL-1-β. The vitreous body contained only remnants of the initial aggregates. The vitreous body showed a minimal cellular infiltrate remaining after 1 month, and these cells were mainly monocytes. The membranes formed in IL-1-injected eyes containing retinal holes originated from the holes and extended to other areas of the retina, forming traction (Fig. 4A). The

Histopathology

IL-1-β-injected eyes, irrespective of the presence of a retinal hole, contained a cellular infiltrate in the anterior and posterior segments. At 24 hours, the infiltrate included predominantly neutrophils, with some plasma cells and lymphocytes. Sections of an eye 7 days after IL-1-β injection showed a cellular aggregate opposing the internal limiting membrane (Fig. 3A). Higher magnification showed this aggregate to be a granulomatous formation composed of monocytes, red blood cells, and ascites (Fig. 3B). Eyes with holes injected with BSS or not injected showed minimal response to the injection consisting of a few mononuclear cells.

Eyes without retinal holes injected with IL-1-β showed a minimal cellular infiltrate remaining after 1 month, and these cells were mainly monocytes. The membranes formed in IL-1-injected eyes containing retinal holes originated from the holes and extended to other areas of the retina, forming traction (Fig. 4A). The
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FIGURE 1. Slit lamp photographs of the anterior segment of eyes 24 hours after injection with IL-1β (A) showing keratic precipitates (arrow) and perilimbal vessel engorgement (arrowhead), (B) white fibrous anterior capsular deposits, and (C) synechiae.

The RPE cell layer had a highly cystic appearance, indicating hyperplasia. Higher magnification of the membrane (Fig. 4B) showed that it involved retinal and inflammatory elements. Several distinct cell types were seen within a lightly staining extracellular matrix. These included inflammatory cells with darkly staining nuclei covering a major portion of the cytoplasm, spindle-shaped cells indicating cells of fibroblast or RPE origin, and rounded cells with lightly staining nuclei indicating glial cells, possibly Müller cells, from the inner nuclear layer. Control eyes with retinal holes injected with BSS remained free of membranes (Fig. 4C), but four eyes with retinal

FIGURE 2. Fundus photographs of eyes containing preexisting retinal holes. (A) An eye injected 1 month earlier with IL-1β. A membrane has formed over the hole and extends into the vitreous body, forming adhesions with other portions of the retina. (B) An eye injected with balanced saline solution. The retinal hole is defined by the upturned edges of the neural retina. Notice that the hole remains clear and the retinal pigment epithelial cell layer remains undisturbed.
holes showed signs of basement membrane thickening and some disruption of the RPE cell layer; fibrosis was seen in one control eye.

**Zymography**

The formation and remodeling of collagen-containing membranes in eyes treated with IL-1-β after retinal hole formation must require synthesis and degradation of ECM components, particularly collagen. Primary among the enzymes involved in such processes is a series of MMPs. Zymography was used to determine the effect of intravitreal injection of IL-1-β on the collagen-degrading MMPs of the vitreous humor (Fig. 5). Normal vitreous humor contains almost ex-
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FIGURE 5. Zymography of gelatinolytic activity in vitreous humor from rabbit eyes with 1-month-old retinal holes without injection (lane 1), without retinal holes 1 month after the injection of IL-1-β (lane 2), with retinal holes 1 month after balanced saline solution injection (lane 3), with retinal holes 1 month after the injection of IL-1-β (lane 4), without retinal holes 24 hours after balanced saline solution injection (lane 5), and without retinal holes 24 hours after IL-1-β injection (lane 6). Lane 7: High molecular weight standards. Samples were separated using a 7% SDS-PAGE gel containing a gelatin substrate. After incubation, bands of gelatinase activity were detected by staining with Coomassie blue. The presence of gelatinases is indicated by clear bands.

clusively a 65-kd gelatinase, whereas two predominant forms of gelatinase (100 kd and 65 kd) could be demonstrated in vitreous humor 24 hours after the injection of IL-1-β. Retinal holes were found to have no effect on MMP production (Fig. 6). One month after injection of IL-1-β, vitreous humor contained predominantly the 65-kd enzyme; the induced 100-kd enzyme disappeared (Fig. 5).

Zymographic analysis suggested that the MMPs might have been involved in the formation of retinal membranes; however, retinal hole formation alone had no effect on MMP production, and IL-1-β alone did not produce membranes. It appears, therefore, that MMP induction alone is insufficient to cause the changes observed.

SDS used in zymographic analysis activates MMP proenzymes by a conformational change. To determine if the MMPs detected in samples of vitreous humor were in their active or proenzyme form, the supernatant fractions were incubated in the presence of APMA (Fig. 7). Activation of the proenzyme with APMA involves autoproteolytic cleavage of a peptide fragment from the amino-terminus, resulting in size reduction. Supernatant fractions incubated in APMA before gel separation contained a mixture of the 65-kd form and a smaller form. Those not incubated showed predominantly the 65-kd form. This confirms that the 65-kd enzyme present in vitreous humor was largely present in a latent form. The 100-kd enzyme also showed a size reduction after APMA incubation, suggesting it, too, was present in a latent form.

DISCUSSION

The neural retina may act as a barrier preventing access of vitreous humor components, including growth

FIGURE 6. Zymography of gelatinolytic activities in vitreous humor from rabbit eyes with preexisting retinal holes enucleated 24 hours after intravitreal injection of IL-1-β (lanes 2, 4, and 6) or balanced saline solution lanes (1, 3, and 5). Lane 7: Molecular weight standards. Preexisting retinal holes did not affect MMP induction. MMP = matrix metalloproteinase.

FIGURE 7. Zymography of gelatinolytic activities in vitreous humor from rabbit eyes enucleated 24 hours after IL-1-β injection (lane 1, after APMA incubation; or lane 2, after SDS incubation) or balanced saline solution (lane 3, after APMA incubation; or lane 4, after SDS incubation). Lane 5 shows high molecular weight standards. Supernatant fractions were separated on a 12% gelatin substrate gel. Note the decrease in molecular weight of the gelatinase activity in fractions incubated in APMA, indicating that the metalloproteinases are normally present predominantly in a latent form.
factors, to the pigment epithelium. It may also maintain the integrity of the underlying cell layers. Because the formation of PVR membranes may result from a surgical breach of the neural retina, attempts have been made to mimic this process in an animal model. However, damage to the barrier in the form of created retinal holes, although able to cause RPE cell proliferation, does not allow membrane formation. This indicates that factors other than components of vitreous humor have access to the RPE layer for membrane induction. Such factors might result from the inflammatory aspect of PVR. In PVR, cytokine production is elevated. It has been well established that the intravitreal injection of interleukin-1 produces inflammatory changes, including a cellular infiltrate in the eye, but most studies have emphasized changes in the anterior segment of the eye. In view of the well-known chemotactic and mitogenic activity of IL-1, the possible synergistic effects of the presence of a retinal hole and IL-1-β in the promotion of retinal membranes were studied.

As expected, cell and flare were observed by slit lamp and indirect ophthalmoscopy in eyes injected with IL-1-β. The acute response consisted predominantly of neutrophils, as reported by others, and the inflammation elicited by the cytokine did not differ in eyes with or without holes. However, membranes developed only in eyes containing a retinal hole and IL-1-β, suggesting the cooperation of both inflammatory and vitreous humor factors in the cellular migration and proliferation that is typical of PVR. Nevertheless, it should be noted that there are limitations in the model system used in this study. In humans, membranes can form because of tears in the retina, but this was not true of the rabbits used in these experiments. This may reflect anatomic differences between rabbit and human eyes, especially with regard to the vasculature. In humans, retinal blood vessels are abundant. In rabbits, the retinal vessels are restricted to the medullary rays—a medullated nerve fiber layer originating from the optic disk and extending nasally and temporally in two broad white bands. In the present work, holes were created inferiorly to the medullated nerve fiber layer—an area devoid of retinal blood vessels.

The process of membrane formation involves cellular proliferation and the production of a disorganized ECM containing constituents similar to Bruch’s membrane. It is likely that this ECM is produced and remodeled by RPE and other cells that constitute the epiretinal membrane. Among the enzymes involved in these processes are the MMPs. Moreover, a variety of studies have linked IL-1 production to MMP induction and to membrane contraction, the most devastating event of PVR. It is therefore possible that the effects of IL-1-β in the presence of a retinal hole are mediated by the synthesis of new MMPs; indeed, two predominant gelatinases (the 100-kd and the 65-kd forms) were found in vitreous humor 24 hours after the injection of IL-1-β, whereas control vitreous humor contained predominantly the 65-kd form. Samples taken 1 month after IL-1-β administration showed the vitreous humor had returned to the control state. However, the additional presence of a retinal hole did not affect the MMPs observed, and a retinal hole alone did not affect MMP production. Thus, induction of the 100-kd MMP by IL-1 cannot alone explain membrane formation in the model system used. Furthermore, both MMPs were present predominantly in a latent form. It is likely that these enzymes exist as an extracellular pool and become activated at specific sites where membrane remodeling occurs, therefore making detection of the patent enzyme difficult.

Our finding of IL-1 induction of MMP reflects similar results with a cultured cell system. RPE cells normally produce a 65-kd collagenase constitutively, whereas IL-1-β causes RPE cells to produce a 100-kd collagenase. However, the situation in the eye may be more complex, and it is possible that other ocular cell types and infiltrating leukocytes contribute to the MMP content of vitreous humor.

In mature animals, extracellular matrix remodeling is a continuous process for cells that form a basement membrane, and it is important in maintaining the integrity of the basement membrane and cell–extracellular matrix contacts. In disease states, the precisely controlled events that result in the formation of correct cellular interactions may go astray. For example, in metastatic neoplasia, cells produce an excess of MMPs, which loosens cellular contacts with surrounding cells and the basement membrane, thus allowing the invasion of other tissues. The presence of retinal holes from trauma, in conjunction with the abnormal production of cytokines (including IL-1-β), may under certain circumstances lead to a cascade of events in the eye—including cell migration, proliferation, and matrix remodeling—similar to that seen in neoplasia and, indeed, in normal wound healing.

**Key Words**
proliferative vitreoretinopathy, interleukin, retina, metalloproteinase, rabbit

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**References**
2. Clarkson JG, Green WR, Massof D. A histopathologic
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1. Alexander JP, Bradley JMB, Gabourel JD, Acott TS.