Interferon-Induced Protein 10 and Interleukin 8

C-X-C Chemokines Present in Proliferative Diabetic Retinopathy

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Objective: To determine vitreous levels of interleukin 8 (IL-8) and interferon-induced protein 10 (IP-10), which are members of the C-X-C chemokine family that promote and inhibit neovascularization, respectively.

Methods: We measured the levels of IL-8 and IP-10 by specific enzyme-linked immunosorbent assays in the vitreous from 30 patients with proliferative diabetic retinopathy (PDR) and 10 control patients undergoing vitrectomy for idiopathic macular holes or idiopathic macular puckers.

Results: Detectable levels of IL-8 were found in 23 of 24 patients with active PDR, 4 of 6 patients with inactive PDR, and 6 of 10 controls. Levels of IL-8 were significantly increased in vitreous samples from the patients with active PDR (P=.02) when compared with vitreous samples from the controls. The IL-8 levels detected in vitreous samples from patients with inactive PDR were not significantly elevated over those found in the control samples. Interferon-induced protein 10 was detected in the vitreous samples from 23 of 24 patients with active PDR, all patients with inactive PDR, and 9 of 10 controls. Significant elevations of IP-10 were measured in samples from patients with active PDR (P=.004) and in those with inactive PDR (P=.00) over those from controls. In addition, levels of IP-10 were significantly elevated in vitreous samples from patients with inactive PDR compared with vitreous samples from patients with active PDR (P=.02).

Conclusion: Both IL-8 and IP-10 participate in the pathogenesis of PDR.


OBJECTIVE neovascularization is a complication of diabetes mellitus that results in substantial morbidity and blindness. Elucidation of the mechanisms underlying initiation, propagation, and treatment-associated regression of retinal neovascularization remains an important goal for the development of targeted therapies for diabetic retinopathy. Previous studies have identified several potential angiogenic agents that may participate in the initiation of vascularization. Among these are cytokines, including growth factors, and proinflammatory mediators, including the C-X-C chemokine, interleukin 8 (IL-8). In previous studies we identified significantly elevated levels of IL-8 in vitreous removed from patients with proliferative diabetic retinopathy (PDR). Moreover, we identified IL-8 as an angiogenic agent that may be reproduced by resident cells within the eye. In recent studies, we have shown that glycated serum proteins may enhance IL-8 production by resident retinal cells. Together, these findings suggest that IL-8 may be involved in the pathogenesis of PDR.

Another C-X-C chemokine, interferon-induced protein 10 (IP-10), is a closely related chemokine that lacks the neutrophil chemoattractant and angiogenic properties of IL-8. Interferon-induced protein 10 inhibits angiogenesis in vivo at least in part by antagonizing the functions of IL-8. Previous studies have demonstrated that IP-10 both prevents IL-8–induced neovascularization in a corneal pocket model of angiogenesis and that IP-10 inhibits IL-8–induced endothelial chemotaxis. The lack of neutrophil chemoattractant and its angiostatic properties in vivo seem to be due to the lack of an N-terminal ELR (Glu-Leu-Arg) motif responsible for chemokine receptor binding and activation.

In view of these features of IP-10, we analyzed vitreous removed from patients with active and inactive PDR to determine whether IP-10 was present to a significant degree. We measured IL-8 in the same vitreous samples so that the levels of these 2 C-X-C chemokines could be...
MATERIALS AND METHODS

VITREOUS SPECIMENS

Vitreous samples from 40 patients were collected in accord with the guidelines and recommendations of the institutional review board of the University of Michigan, Ann Arbor. Undiluted vitreous samples were obtained from 30 eyes undergoing pars plana vitrectomy for PDR with progressive fibrovascular proliferation, tractional retinal detachment, and/or nonclearing vitreous hemorrhage. Vitreous from 10 eyes undergoing uncomplicated pars plana vitrectomy for idiopathic macular pucker, idiopathic macular hole, or aqueous misdirection served as controls. All eyes from which control vitreous samples were obtained were without clinical signs of inflammation prior to surgery. No patients received preoperative perioculostatic or systemic corticosteroids. All vitreous samples were obtained prior to starting intraocular infusion of fluid. Vitreous samples were immediately placed in sterile, 1.5-mL polypropylene tubes on ice, snap-frozen with liquid nitrogen, and stored at −70°C until assayed. Prior to enzyme-linked immunosorbent assay (ELISA), the vitreous samples underwent sonication (Sonic & Materials Inc, Danbury, Conn) for 10 seconds at one-tenth total power to completely disrupt vitreous and to yield a uniform sample for assay. Sonication was performed with each sample immersed in an ice bath and each was then maintained continuously on ice until ELISA to prevent loss of antigenic activity.

The clinical ocular findings were graded at the time of vitrectomy for the presence and amount of vitreous hemorrhage, tractional retinal detachment, previous panretinal photocoagulation, and presence or absence of patent new vessels present on the retina or optic disc (Table). Patients with active PDR were graded as such on the basis of visible patent new vessels present on the retina or optic disc or their absence (inactive PDR).


ASSAY FOR IL-8 AND IP-10

Enzyme-linked immunosorbent assays were performed on dilutions of each of the 40 vitreous samples. Antigenic IL-8 and IP-10 were quantitated using a modification of a double-ligand ELISA method as previously described using specific antibody to IL-8 and IP-10. Specific human IL-8 and IP-10 antibodies were produced in rabbits as previously described by immunization of rabbits with corresponding cytokines with complete Freund adjuvant. Briefly, flat-bottomed 96-well microtiter plates (Nunc Immuno-Plate I 96-F; Dynatech Laboratories, Alexandria, Va) were coated with either rabbit antihuman IL-8 or IP-10 antibodies (0.9 mg in 100 mL of 0.1-mol sodium carbonate, pH 9.6) for 16 hours at 4°C and washed with phosphate buffered saline, pH 7.5, containing 0.5% polysorbate-20 (washing buffer, Tween 20, Sigma, St Louis, Mo). Nonspecific binding sites were blocked with 2% fetal calf serum in washing buffer and incubated 1 hour at 37°C. Plates were rinsed 4 times with washing buffer and diluted samples (100 mL) in duplicate samples were added following incubation for 90 minutes at 37°C. Plates were washed 4 times, biotinylated anti-IL-8 or anti-IP-10 antibodies (final concentration, 1:2000) were added, and plates were incubated for 90 minutes at 37°C. Plates were washed 4 times and streptavidin-peroxidase conjugate (Bio-Rad Laboratories, McLean, Va) added and incubated for 30 minutes at 37°C. Plates were washed 4 times and chromagen substrate (Bio-Rad Laboratories) added. The plates were incubated at room temperature to the desired extinction and the reaction was terminated with 3% oxalic acid solution. Plates were read at 405 nm in an ELISA reader. Standards included 0.5-log dilutions of recombinant IL-8 (Genzyme, Cambridge, Mass) or IP-10 (R&D Systems, Minneapolis, Minn) from 5 pg to 150 ng per well. This ELISA method consistently detected cytokine concentrations of more than 10 pg/mL and did not cross-react with macrophage inflammatory protein 1 α/β, RANTES, interleukin 1 α/β, interleukin 2, interleukin 4, interleukin 6, interleukin 7, tumor necrosis factor γ, epithelial-derived neutrophil activating protein 78, growth-related oncogene α/β/γ, granulocyte-macrophage colony-stimulating factor, or neutrophil-activating protein 2.

Statistical analysis results were expressed as nanograms of cytokine protein per milliliter of vitreous (mean ± SEM). Unpaired 2-tailed Student t tests were used to determine whether the amount of cytokine in vitreous from patients with active or inactive PDR differed from that from controls. P≤.05 was considered statistically significant.

RESULTS

Interleukin 8 (>10 pg/mL) was detected in 27 (90%) of 30 vitreous samples from patients with PDR, including 23 of 24 patients with active PDR and 4 of 6 patients with inactive PDR, as well as in 6 (60%) of 10 vitreous samples from controls (Figure 1). Levels of IL-8 were significantly increased in patients with PDR (22.2 ± 3.9 ng/mL; P=.04) when compared with controls (7.5 ± 2.3 ng/mL) (Figure 2). Samples from patients with active PDR (24.7 ± 4.5 ng/mL; P=.02) also had significantly elevated IL-8 levels when compared with those from controls (Figure 2). Vitreous samples from patients with inactive PDR (11.6 ± 5.2 ng/mL; P=.44) did not differ significantly from those from controls (Figure 2). Vitreous levels of IL-8 from patients with active PDR were not significantly different from those from patients with inactive PDR (P=.1) (Figure 2).

Interferon-induced protein 10 (>10 pg/mL) was detected in 29 (97%) of 30 vitreous samples from patients with PDR, including 23 of 24 samples from patients with active PDR and 6 of 6 samples from patients with inactive PDR (Figure 3). Interferon-induced protein 10 was also detected in 9 (90%) of 10 control vitreous samples (Figure 3). Levels of IP-10 were significantly increased in vitreous samples from patients with PDR (11.7 ± 1.1 ng/mL; P=.001) as well as in those from the subgroups of active (10.4 ± 1.2 ng/mL; P=.004) and inactive (16.7 ± 2.6 ng/mL; P=.00) PDR when compared with samples from
controls (4.6 ± 0.9 ng/mL) (Figure 4). The levels of IP-10 in samples from patients with active PDR did significantly differ from those levels found in samples from patients with inactive PDR (P = .02) (Figure 4).

No significant difference between levels of IL-8 or IP-10 and the presence or severity of vitreous hemorrhage or tractional retinal detachment in PDR was found (data not shown). Additionally, preoperative panretinal photocoagulation did not affect either vitreous IL-8 (P = .32) or IP-10 (P = .86) levels when compared with samples from patients without preoperative panretinal photocoagulation.

Our data suggest that levels of C-X-C chemokines are significantly increased in PDR. The levels of both of these C-X-C chemokines were substantially elevated in patients with PDR over those detected in control samples from patients with idiopathic macular pucker, macular holes, or aqueous misdirection, all ostensibly noninflammatory entities. The significantly increased levels of IL-8 we detected in specimens from patients with PDR were attributable to those specimens removed from patients with active PDR, a finding consistent with the proangio-
genic nature of this chemokine. In contrast, significant elevations of IP-10 were detected in the subgroups of patients with active and inactive PDR. Levels of IP-10 were found to be significantly higher in vitreous from patients with inactive PDR when compared with vitreous from patients with active PDR.

Previous studies have documented the presence of IL-8 in PDR. To our knowledge, however, this is the first report of IP-10 in human eyes in general and in diabetes in particular. We found significantly elevated levels of IP-10 to be present in patients with active and inactive PDR (Figure 3 and Figure 4). The detection of IP-10, a known angiostatic C-X-C chemokine, might certainly be expected in both subgroups insofar as patients with active PDR include those whose PDR may be at the stage when involution has begun. Moreover, most of these patients had undergone panretinal laser photoocoagulation for their disease, possibly leading to elevated IP-10 levels as their PDR underwent involution. This is suggested by the samples from patients with inactive PDR which, though few, suggest that IP-10 may in fact be elevated during PDR involution. The detection of IP-10 in these eyes raises the possibility that IP-10 may be involved in the pathogenesis of PDR, particularly in its involution.

Our control group consisted of vitreous from eyes with idiopathic macular pucker, macular hole, or aqueous misdirection. It is likely that substantial IL-8 and IP-10 detected in some of the vitreous samples from eyes with these retinal disorders that are not normally associated with significant leukocytic infiltration may indeed have been due to an inflammatory component in these cases. Leukocytes, vascular endothelial cells, and retinal pigment epithelial cells (data not shown) are among the possible sources for the IP-10 measured in our vitreous samples. It is possible that IP-10, like IL-8, may be increased in the vitreous when these cells are exposed to inflammatory mediators such as interleukin 1, tumor necrosis factor, and/or interferon \( \gamma \), which are known to be present in eyes of patients with PDR.

Breakdown of the blood-retina barrier is an important feature of the pathophysiology of PDR. Significant serum levels of IL-8 or IP-10 have not been detected in either normal patients or patients with diabetes mellitus. In support of this, we found no correlation between the presence or degree of vitreous hemorrhage and vitreous IL-8 or IP-10 (Table). Therefore, even in

Figure 1. Distribution of interleukin 8 (IL-8) levels in vitreous from patients with proliferative diabetic retinopathy (PDR) and control vitreous samples. Only 3 of 30 PDR samples and 4 of 10 control samples had nondetectable (<10 pg/mL) levels of IL-8.

Figure 2. Mean ± SEM of interleukin 8 (IL-8) in vitreous from all patients with proliferative diabetic retinopathy (PDR) as well as the subgroups of active PDR and inactive PDR and control vitreous samples. Levels of IL-8 were significantly elevated (asterisk) above control IL-8 levels in vitreous from combined PDR (P = .04) and patients with active PDR (P = .02).

Figure 3. Distribution of interferon-induced protein 10 (IP-10) levels in vitreous from patients with proliferative diabetic retinopathy (PDR) and control vitreous samples. Only 1 of 30 PDR samples and 1 of 10 control samples had nondetectable (<10 pg/mL) levels of IP-10. Most control vitreous samples contained low, but significant, levels of IP-10. The PDR samples exhibited a broader distribution of IP-10 levels.

Figure 4. Mean ± SEM levels of interferon-induced protein 10 (IP-10) in vitreous from all patients with proliferative diabetic retinopathy (PDR) as well as in subgroups with active and inactive PDR. Levels of IP-10 for all groups were significantly elevated (asterisk) above control specimens (P < .01).
the presence of the the breakdown of the blood-retina barrier or vitreous hemorrhage, it is likely that the levels of IL-8 and IP-10 that we measured in the vitreous samples reflect only intraocular production. A variety of proangiogenic cytokines have been proposed as possible mediators of angiogenesis in PDR. These include IL-8 and growth factors, most notably vascular endothelial growth factor. These potentially angiogenic cytokines may act together in the pathogenesis of neovascularization in PDR. Interleukin 8 alone or in combination with other cytokines, including growth factors such as vascular endothelial growth factor and transforming growth factor β, may promote intraocular angiogenesis and fibrocellular proliferation, respectively. To our knowledge, no other selective angiogenic cytokine has been described in the eye. The role of IP-10 in the pathogenesis of PDR and the treatment-induced involution of PDR remains to be elucidated. Future studies may be necessary to determine whether the IP-10 we measured occurs naturally in the evolution of PDR or is elicited in response to treatment.

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REFERENCES


A look at the past . . .

Bed Bug.

The Cimex lectularis, rubbed up with salt and woman’s milk, was highly esteemed in antiquity as a poultice for the eyes. The blood of this creature was also employed to prevent the return of eyelashes after epilation.