Chemokines and Gelatinases in the Aqueous Humor of Patients With Active Uveitis

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• PURPOSE: **To investigate the involvement of the chemokines CXCL10/IP-10, CXCL11/I-TAC, CXCL8/1L-8, CXCL6/GCP-2, CCL3/MIP-1α, and CCL18/PARC, and gelatinases A and B in uveitis.**

• DESIGN: **Prospective, experimental, case-control study.**

• METHODS: **Aqueous humor samples from 30 patients with active uveitis, and 14 control patients and paired serum samples were assayed for chemokines with specific enzyme-linked immunosorbent assays (ELISAs) and for gelatinase levels by quantitative zymography.**

• RESULTS: **In control AH, none of the chemokines was detected. Gelatinase A was detected in all samples, and gelatinase B was detected in only one sample. In patients with uveitis, IP-10 was detected in all AH samples, whereas I-TAC, IL-8, GCP-2, MIP-1α, and PARC were detected in three, 16, six, two, and 12 samples, respectively. IP-10 levels were significantly higher in AH samples than those of serum ($P = .006$). Gelatinase A was detected in 29 AH samples and gelatinase B was detected in 26 samples. Gelatinase A levels were significantly higher in AH samples from patients than those of controls ($P < .0001$). In 11 AH samples, gelatinase B was detected in complex with lipocalin (NGAL). Disease activity correlated significantly with the levels of IP-10 ($r = .627$; $P < .0001$), gelatinase A ($r = .508$; $P = .002$), gelatinase B ($r = .685$; $P < .0001$), and NGAL-gelatinase B complex ($r = .595$; $P < .0001$).**

• CONCLUSIONS: **These data suggest a pathogenic role of the T lymphocyte chemoattractant IP-10 and gelatinases in the recruitment and activity of T cells into the eye in patients with uveitis and in the pathogenesis of uveitis. (Am J Ophthalmol 2004;138:401–411. © 2004 by Elsevier Inc. All rights reserved.)**

UVEITIS IS A CLINICALLY HETEROGENOUS GROUP OF diseases in which intraocular inflammation often leads to decreased vision, retinal destruction, and blindness. T-cell dependent immunologic events are increasingly being considered to play a central role in the pathogenesis of uveitis.[1] The immune system can mount distinct and selective responses according to the type of infection or antigenic challenge. In the context of such heterogeneity, two extremely polarized forms of $CD4^+$ T helper (Th) cell-mediated specific immune responses have been described. After priming, $CD4^+$ Th cells are differentiated into two functional subsets on the basis of their profiles of cytokine production. Th1 cells that produce interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-β activate macrophages and are responsible for cell-mediated immunity and are involved in the pathogenesis of organ-specific autoimmune disorders. In contrast, Th2 cells, which produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, induce strong antibody responses by B cells and induce eosinophil activation, and are responsible for allergic reactions.[2] Evidence suggesting that uveitis is characterized by Th1 cell-mediated immune responses has recently accumulated.[1] Also neutrophils are thought to play a central role in the pathogenesis of Behçet disease.[3] Despite increasing evidence for involvement of T lymphocytes in uveitis, the mechanism of T lymphocyte recruitment into the eye remains incompletely defined. Chemokines and matrix metalloproteinases (MMPs), in particular gelatinase B (MMP-9), play key roles in the migration of leukocytes to sites of inflammation.[4,5]

Chemokines are a superfamily of 8- to 10-KDa secreted proteins that direct the recruitment of leukocytes to sites of inflammation. These chemokines are grouped into the CXC, CC, C, and CX3C subfamilies on the basis of the

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arrangement of the conserved cysteine residue.[4] Recently, a new classification system for the different families of chemokines has been proposed.[6] In general, CC chemokines chemoattract monocytes, eosinophils, basophils, and T lymphocytes. Macrophage inflammatory protein-1α (CCL3/MIP-1α) has been shown to attract T lymphocytes, monocytes, and neutrophils and to induce endothelial cell and eosinophil activation in vivo.[7] Pulmonary and activation-regulated chemokine (CCL18/PARC) shows a specificity for naive resting T lymphocytes.[8] The CXC chemokines further fall into two classes based on the presence or absence of a NH_2–terminal sequence glutamic acid–leucine– arginine (ELR). The ELR-containing CXC chemokines, such as CXCL8/IL-8, and granulocyte chemotactic protein 2 (CXCL6/GCP-2), chemoattract neutrophils and are angiogenic, whereas most non-ELR CXC chemokines chemoattract lymphocytes and are angiostatic. Among the non-ELR CXC chemokines, IFN-γ-inducible protein of 10 KDa (CXCL10/IP-10), and interferon-inducible T-cell α chemoattractant (CXCL11/I-TAC) are IFN-γ inducible and potently chemoattract activated T lymphocytes.[4]

The specific effects of chemokines are mediated by a family of seven transmembrane-spanning G-protein-coupled receptors.[4] T lymphocyte subsets differ in their expression profiles of chemokine receptors. The CXC chemokine receptor 3 (CXCR3) and the CC chemokine receptor 5 (CCR5) are preferentially expressed on human Th1 lymphocytes, whereas Th2 lymphocytes preferentially express CCR8, CCR4, and CCR3.[9] IP-10 and I-TAC specifically interact with the receptor CXCR3 which is nearly exclusively expressed on activated T cells.[4,6] MIP-1α signals by means of the receptors CCR1, CCR3, and CCR5 which are also expressed on activated T cells.[4]

The matrix metalloproteinases represent a family of zinc-containing endopeptidases that are recognized as key enzymes both for normal extracellular matrix turnover and for the exaggerated extracellular matrix breakdown associated with pathologic conditions including tumor cell invasion and metastasis, angiogenesis, inflammatory reactions, wound healing, and scar formation.[10,11] Gelatinases A and B (the 65-KDa to 75-KDa matrix metalloproteinase-2 and the 85-KDa to 96-KDa matrix metalloproteinase-9, respectively) cleave denatured collagens (gelatins) and other matrix proteins.[10] Because of their unique and broad substrate specificity, their involvement in leukocyte migration, and their role in other chronic inflammatory and autoimmune diseases,[5,11,12] we hypothesized that excessive production of gelatinases may play a role in the pathogenesis of uveitis.

To address mechanisms of uveitis pathogenesis and identify putative molecular targets for therapeutic intervention, we measured the levels of the T lymphocyte chemoattractants IP-10, I-TAC, MIP-1α, and PARC, the neutrophil chemoattractants IL-8, and GCP-2, and gelatinases A and B in the aqueous humor from 30 patients with active uveitis and 14 control subjects who had undergone elective cataract surgery.

DESIGN

THIS IS A PROSPECTIVE, EXPERIMENTAL, CASE-CONTROL study of chemokines and gelatinases levels in the aqueous humor from patients with active uveitis and control subjects.

PATIENTS AND METHODS

• **PATIENTS AND CONTROL SUBJECTS:** Thirty patients with active uveitis seen at the outpatient clinic of King Abdulaziz University Hospital were included in the study. The patients were 20 males and 10 females. The mean age was 30.1 ± 12.4 years (range, 14 to 54 years). All the patients were examined to determine the nature of their disease and associated systemic illnesses. The patients with uveitis were divided between those with disease associated and not associated with systemic diseases. The first group included 25 patients. Thirteen of these had Vogt-Koyanagi-Harada (VKH) disease, 10 had Behçet disease, and two had ankylosing spondylitis. The second group included five patients. Three of these had idiopathic uveitis and two had chronic endophthalmitis after extracapsular cataract extraction and intraocular lens implantation caused by *Propionibacterium acnes*. Fourteen patients who had undergone elective cataract extraction with no prior history of uveitis, served as a control group. Patients were examined using slit-lamp biomicroscopy, indirect ophthalmoscopy, and fluorescein angiography. In each patient the uveitis activity was graded using Forrester and associates grading.[13] Anterior chamber cells were clinically graded on a 0 to 5 scale as follows: 0 = < 5 cells/field, 1+ = 5 to 10 cells/field, 2+ = 11 to 20 cells/field, 3+ = 21 to 50 cells/field, 4+ = >50 cells/field, 5+ = hypopyon formation. Three patients with Behçet disease had hypopyon on presentation. None of the patients was on topical or systemic therapy on presentation. The clinical features of the 30 patients with uveitis included in this study are shown in Table 1.

Aqueous humor (100 to 200 μl) was aspirated from each patient by means of limbic paracentesis using a 27 gauge needle attached to a tuberculin syringe after the application of topical local anesthetic oxybuprocaine hydrochloride 0.4% (Benoxinate, Chauvin Pharmaceuticals Ltd., Kingston, United Kingdom). The procedure was performed under a surgical microscope. The samples were snap frozen and maintained at −70 C until use. All aqueous humor samples from patients with uveitis were obtained before therapy. In four patients, repeated aqueous humor samples were obtained 7 days after treatment. To address whether chemokines detected in the aqueous

TABLE 1. Clinical Features and Chemokine and Gelatinase Levels in Patients With Uveitis

		Chemokine Concentrations (ng/ml)												Gelatinase Concentrations in AH (Scanning Units)		
		IP-10		I-TAC		IL-8		GCP-2		MIP-1α		PARC				
Diagnosis	Activity	AH	S	AH	S	AH	S	AH	S	AH	S	AH	S	Gelatinase A	Gelatinase B	NGAL-gelatinase B complex
VKH																
1	1+	0.9	–	ND	–	ND	–	ND	–	ND	–	ND	–	252	26	0
2	1+	0.4	–	ND	–	ND	–	ND	–	ND	–	4	–	508	329	0
3	4+	3.6	ND	ND	1.12	0.47	ND	ND	0.2	ND	ND	156	22	1,170	1,321	0
4	4+	7	ND	ND	0.82	0.45	ND	ND	ND	0.1	ND	63	19.5	901	1,139	0
5	2+	1.6	–	ND	–	0.13	–	ND	–	ND	–	ND	–	272	466	0
6	2+	1.04	–	ND	–	ND	–	ND	–	ND	–	2.15	–	366	548	0
7	1+	0.54	–	ND	–	ND	–	ND	–	ND	–	ND	–	176	29	0
8	1+	1.4	–	ND	–	ND	–	ND	–	ND	–	ND	–	173	33	0
9	3+	2.25	ND	ND	0.74	0.15	6.5	ND	ND	ND	0.1	8.4	17.2	504	718	10.4
10	3+	2.77	ND	ND	0.82	0.43	6.8	ND	ND	ND	0.1	68	22	1,286	1,220	32
11	1+	3.35	0.2	ND	0.84	ND	ND	ND	ND	ND	ND	ND	10.5	219	0	0
12	3+	3.44	ND	ND	1.12	0.35	ND	ND	ND	ND	ND	ND	15.5	256	23	0
13	3+	6.39	–	ND	–	0.15	–	ND	–	ND	–	ND	–	253	72	0
Belçet disease																
14	2+	5.6	–	ND	–	ND	–	ND	–	ND	–	ND	–	380	0	0
15	3+	7.1	–	ND	–	ND	–	ND	–	ND	–	ND	–	382	272	19
16	5+	17.8	ND	ND	1.4	ND	ND	ND	ND	ND	ND	ND	9.1	498	251	24
17	5+	14.7	ND	0.3	1.04	7	ND	0.42	0.22	ND	ND	ND	22.2	0	3,294	0
18	2+	3.42	–	ND	–	ND	–	ND	–	ND	–	ND	–	255	0	0
19	5+	9.08	–	0.3	–	0.75	–	0.96	–	ND	–	8.6	–	1,291	1,289	343
20	3+	15.1	ND	ND	0.58	3.1	0.72	0.93	ND	ND	0.13	ND	22.5	178	204	36
21	4+	15.5	0.1	ND	0.66	0.19	9.47	ND	ND	ND	0.63	ND	31.5	448	650	143
22	1+	5.37	ND	ND	0.82	ND	ND	ND	ND	ND	ND	ND	19.5	234	0	0
23	3+	2.51	0.68	ND	1.04	ND	ND	ND	ND	ND	ND	ND	34	383	185	0
Ankylosing spondylitis																
24	3+	5.2	ND	ND	0.94	0.13	ND	ND	ND	ND	ND	2.5	32	503	452	0
25	2+	2.35	–	0.4	–	0.12	–	ND	–	ND	–	16.5	–	1,037	728	0
Idiopathic uveitis																
26	3+	4.4	–	ND	–	ND	–	0.26	–	ND	–	9	–	534	784	55
27	4+	8.8	–	ND	–	2.3	–	ND	–	0.14	–	5.9	–	563	539	26
28	1+	0.18	–	ND	–	ND	–	ND	–	ND	–	ND	–	290	188	0
Chronic endophthalmitis																
29	4+	1.7	ND	ND	0.92	2.1	1.7	0.36	ND	ND	ND	61	42	573	1,176	542
30	4+	0.46	ND	ND	0.66	2.68	5.7	0.43	ND	ND	0.45	ND	9.1	545	1,069	429

IP-10 = interferon-γ-inducible protein of 10 KDa (CXCL10); I-TAC = interferon-inducible T-cell α chemoattractant (CXCL11); IL-8 = interleukin-8 (CXCL8); GCP-2 = granulocyte chemotactic protein 2 (CXCL6); MIP-1α = macrophage inflammatory protein-1α (CCL3); PARC = pulmonary and activation-regulated chemokine (CCL18); NGAL = neutrophil gelatinase B-associated lipocalin; AH = aqueous humor; S = paired serum sample; VKH = Vogt-Koyanagi-Harada disease; ND = not detected.

TABLE 2. Antibody Pairs (Source and Concentration) of the ELISAs Used in This Study

ELISA	Antibody	Clonality (Species)	Source*	Antibody concentration (ng/ml)
IL-8	Capturing	Polyclonal (goat)	Rega Institute[14]	1,000
	Secondary	Monoclonal (mouse)	R & D Systems	50
GCP-2	Capturing	Polyclonal (rabbit)	Peprotech	400
	Secondary	Monoclonal (mouse)	R & D Systems	250
PARC	Capturing	Polyclonal (goat)	R & D Systems	200
	Secondary	Polyclonal (rabbit)	Peprotech	100
IP-10	Capturing	Monclonal (mouse)	R & D Systems	500
	Secondary	Polyclonal (goat)	R & D Systems	1,700
I-TAC	Capturing	Polyclonal (rabbit)	Peprotech	670
	Secondary	Monoclonal (mouse)	R & D Systems	500

*Location of manufacturers: R & D Systems Europe Ltd., Abingdon, United Kingdom; Peprotech Inc., Rocky Hill, New Jersey.

humor could originate from the circulation, we measured the levels of chemokines in 15 paired serum samples. Six of these had Behçet disease, six had VKH disease, one had ankylosing spondylitis, and two had chronic endophthalmitis.

All procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients and the control subjects. The study was approved by the Research Center, College of Medicine, King Saud University.

• **CHEMOKINE ASSAYS:** Chemokine concentrations were determined by an MIP-1α ELISA purchased from BioSource International Inc. (Camarillo, California) or specific sandwich ELISAs developed in our laboratory (IL-8, GCP-2, PARC, IP-10, and I-TAC). The commercial MIP-1α ELISA was performed following the manufacturer's guidelines. ELISAs were developed in our laboratory using a standard preparation of the chemokine. We used the natural purified chemokines IL-8 and GCP-2[14] or commercial recombinant I-TAC (Peprotech Inc., Rocky Hill, New Jersey), IP-10 (Peprotech Inc.), and PARC (R & D Systems Europe Ltd., Abingdon, United Kingdom). The antibody pairs for the inhouse developed ELISAs are indicated in Table 2. Experimentally, different dilutions of several antibodies were combined with a broad concentration curve of the antigen. The best primary (coating) antibody and secondary (detection) antibody, as well as the optimal dilution combination were then determined and the concentration range of chemokine yielding a linear standard curve was established. In the following step, known concentrations of the standard chemokine preparation were added to serum to check whether this biologic fluid interfered in the detected chemokine concentrations. The lowest chemokine concentration that could be detected in this way was referred to as the detection limit of the ELISA. The protocol for ELISA was described earlier.[14]

Detection limits for IL-8, GCP-2, PARC, MIP-1α, IP-10, and I-TAC were 0.1 ng/ml, 0.2 ng/ml, 2 ng/ml, 0.1 ng/ml, 0.03 ng/ml, and 0.2 ng/ml, respectively.

• **ZYMOGRAPHY:** Gelatinase levels in aqueous humor samples were determined by gelatin zymography after prepurification, and quantification was done using scanning densitometry as described earlier.[15,16] The gelatinase levels were expressed as arbitrary laboratory scanning units.

• **STATISTICAL ANALYSIS:** Statistical analyses were performed using SPSS 10.01 software, (SPSS Inc., Chicago, Illinois), and Epi Info 6, 6.02 (Centers for Disease Control & Prevention). The data were analyzed using the nonparametric Mann-Whitney test, the nonparametric Wilcoxon signed-rank test, χ^2 test, Fisher exact test, χ^2 for linear trend test, Spearman rank correlation coefficient, and Pearson correlation coefficient, where appropriate. The differences were considered significant if the P value was <0.05.

RESULTS

• **CHEMOKINE CONCENTRATIONS:** The chemokine levels detected are shown in Table 1, and the results are summarized in Table 3. None of the chemokines studied was detected in the 14 aqueous humor samples of control group.

IP-10 was detected in all aqueous humor samples from patients with active uveitis (median, 3.43 ng/ml). The detected levels of IP-10 were significantly greater in the aqueous humor of Behçet patients than in VKH patients

TABLE 3. Summary Data for Chemokine Levels

	IP-10		I-TAC		IL-8		GCP-2		MIP-1α		PARC	
	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)	No. of Samples With Detectable Levels (%)	Levels Detected (ng/ml) (Median)
AH of controls (n = 14)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Paired serum samples (n = 15)	3 (20)	0.2	15 (100)	0.84	6 (40)	6.1	2 (13.3)	0.21	5 (33.3)	0.13	15 (100)	22
AH of all uveitis patients (n = 30)	30 (100)	3.43	3 (10)	0.3	16 (53.3)	0.44	6 (20)	0.425	2 (6.67)	0.12	12 (40)	8.8
p value	<0.0001*	0.006**	<0.0001*	0.002**	0.2336	0.01**	0.458	0.071	0.032	0.857	<0.0001*	0.217
AH of VKH patients (n = 13)	13 (100)	2.25	ND	ND	7 (53.8)	0.35	ND	ND	1 (7.7)	0.1	6 (46)	35.7
AH of Behçet patients (n = 10)	10 (100)	8.09	2 (20)	0.3	4 (40)	1.925	3 (30)	0.93	ND	ND	1 (10)	8.6
AH of ankylosing spondylitis patients (n = 2)	2 (100)	3.77	1 (50)	0.4	2 (100)	0.125	ND	ND	ND	ND	2 (100)	9.5
AH of idiopathic uveitis patients (n = 3)	3 (100)	4.4	ND	ND	1 (33)	2.3	1 (33)	0.26	1 (33)	0.14	2 (66)	7.45
AH of chronic endophthalmitis patients (n = 2)	2 (100)	1.08	ND	ND	2 (100)	2.39	2 (100)	0.345	ND	ND	1 (50)	61

Statistically significant at 5% level of significance. *P* value*: incidence of detection of chemokines in aqueous humor samples from patients with uveitis vs paired serum samples (Fisher exact test). The *P* value for the incidence of IL-8 detection was calculated using Chi-square test; *P* value**: chemokine concentrations in aqueous humor samples from patients with uveitis versus paired serum concentrations (Mann-Whitney test). *P* = .001:IP-10 levels in the aqueous humor of Behçet's patients and VKH patients (Mann-Whitney test); IP-10 = interferon-γ-inducible protein of 10 KDa (CXCL10); I-TAC = interferon-inducible T-cell α chemoattractant (CXCL11); IL-8 = interleukin-8 (CXCL8); GCP-2 = granulocyte chemotactic protein 2 (CXCL6); MIP-1α = macrophage inflammatory protein-1α (CCL3); PARC = pulmonary and activation-regulated chemokine (CCL18).

(median, 8.09 vs 2.25 ng/ml; $P = .001$, Mann-Whitney test). The detected IP-10 levels were higher in Behçet patients with hypopyon than in Behçet patients without hypopyon, but the difference was not statistically significant (median, 14.7 vs 5.6 ng/ml; $P = .183$, Mann-Whitney test). IP-10 was detected at low levels in only three of 15 paired serum samples (median, 0.2 ng/ml). The detected aqueous humor concentrations of IP-10 were significantly higher than serum concentrations ($P = .006$, Mann-Whitney test).

I-TAC was detected in only three aqueous humor samples at lower levels than IP-10 (median, 0.3 ng/ml) and in all serum samples (median, 0.84 ng/ml). The detected levels in the serum were significantly higher than in the aqueous humor ($P = .002$, Mann-Whitney test).

IL-8 was detected in 16 aqueous humor samples (median, 0.44 ng/ml). The detected IL-8 levels were higher in Behçet patients than in VKH patients, but the difference was not significant (median, 1.925 vs 0.35 ng/ml; $P = .073$, Mann-Whitney test). IL-8 was detected in six serum samples (median, 6.1 ng/ml). The detected serum concentrations of IL-8 were significantly higher than aqueous humor concentrations ($P = .01$, Mann-Whitney test).

GCP-2 was detected in six aqueous humor samples (median, 0.425 ng/ml) and in two serum samples (median, 0.21 ng/ml). The difference between the detected aqueous humor and serum levels was not significant ($P = .071$, Mann-Whitney test).

MIP-1α was detected in two aqueous humor samples (median, 0.12 ng/ml) and in five serum samples (median, 0.13 ng/ml). The difference between the detected aqueous humor and the serum levels was not significant ($P = .857$, Mann-Whitney test).

PARC was detected in 12 aqueous humor samples (median, 8.8 ng/ml). The detected aqueous humor levels of PARC in patients with VKH disease were higher than in the aqueous humor of patients with other types of noninfectious uveitis, but the difference was not significant (median, 35.7 vs 8.6 ng/ml; $P = .622$, Mann-Whitney test). PARC was detected in all serum samples (median, 22 ng/ml). The difference between the detected aqueous humor and the serum levels was not significant ($P = .217$, Mann-Whitney test). Levels of PARC in aqueous humor samples were higher than in the serum in three patients with VKH (156, 63, and 68 ng/ml, respectively), and in one patient with chronic endophthalmitis (61 ng/ml). The detected aqueous humor levels of PARC in patients with VKH disease were higher than in serum, but the difference was not statistically significant ($P \geq .999$, Mann-Whitney test).

• **ZYMOGRAPHY:** The gelatinase levels detected in the aqueous humor samples from patients with uveitis are shown in Table 1. Gelatinase A was detected in all 12 control aqueous humor samples tested (median, 166.5 scanning units). Gelatinase B was detected in only one (8.33%) of the 12 control samples tested (44 scanning units). Gelatinase A was detected in 29 aqueous humor samples from patients with uveitis (median, 382.5 scanning units) (Figure 1). Gelatinase A levels in the aqueous humor samples from patients with uveitis were significantly higher than the levels found in control samples ($P < .0001$, Mann-Whitney test). Gelatinase A levels in Behçet patients and in VKH patients did not differ significantly (median, 381 vs 272 scanning units; $P = .927$, Mann-Whitney test). Gelatinase B was detected in 26 aqueous humor samples (86.7%) from patients with uveitis (median, 390.5 scanning units) (Figure 1). The incidence of detection of gelatinase B in aqueous humor samples from patients with uveitis was significantly higher than the incidence of detection in normal controls ($P < .0001$, Fisher exact test). The difference between gelatinase B levels in Behçet patients and in VKH patients was not significant (median, 227.5 vs 329 scanning units; $P = .738$, Mann-Whitney test).

In 11 aqueous humor samples from patients with uveitis, gelatinase B was detected as a covalent complex with neutrophil gelatinase B-associated lipocalin (NGAL) suggesting that neutrophils were a significant source of gelatinase B in these samples.[17] Five of these had Behçet disease, two had VKH disease, two had idiopathic uveitis, and two had chronic endophthalmitis.

Among patients with Behçet disease, compared with aqueous humor samples from patients without hypopyon, samples from patients with hypopyon had insignificantly higher levels of gelatinase A (median, 498 vs 380 scanning units; $P = .517$, Mann-Whitney test), gelatinase B (median, 1289 vs 329 scanning units; $P = .067$, Mann-Whitney test), and NGAL-gelatinase B complexes (median, 24 vs 0.0 scanning units; $P = .517$, Mann-Whitney test).

• **CORRELATION STUDIES:** The levels of IP-10, and gelatinase B in aqueous humor samples from patients with uveitis correlated significantly with the disease activity in all patients, in patients with uveitis associated with systemic disease, in patients with Behçet disease, and in patients with VKH disease. Gelatinase A levels correlated significantly with the disease activity in all patients, in patients with uveitis associated with systemic disease, and in patients with VKH disease. NGAL-gelatinase B complex levels correlated significantly with disease activity in all patients, in patients with uveitis associated with systemic disease and in patients with Behçet disease (Table 4).

There was a significant correlation between the incidence of IL-8 detection in aqueous humor samples from patients with uveitis and increased disease activity, increased levels of gelatinase A, increased levels of gelatinase B, and increased levels of NGAL-gelatinase B complexes. There was a significant correlation between the incidence of GCP-2 detection and increased disease activity, in-

TABLE 4. Relationships Between Levels of IP-10, Gelatinase A, Gelatinase B, and NGAL-gelatinase B Complex in Aqueous Humor Samples From Patients with Uveitis and Disease Activity

Variables	All Patients	Uveitis Associated With Systemic Disease	VKH	Behçet Disease
IP-10	$r = .627$	$r = .746$	$r = .803$	$r = .692$
	$P < .0001$*	$P < .0001$*	$P < .0001$*	$P = .013$*
Gelatinase A	$r = .508$	$r = .441$	$r = .699$	$r = .424$
	$P = .002$*	$P = .014$*	$P = .004$*	$P = .111$
Gelatinase B	$r = .685$	$r = .644$	$r = .682$	$r = .890$
	$P < .0001$*	$P < .0001$*	$P = .005$*	$P < .0001$*
NGAL-gelatinase B complex	$r = .595$	$r = .528$	$r = .297$	$r = .578$
	$P < .0001$*	$P = .003$*	$P = .162$	$P = .04$*

*Statistically significant correlation at 5% level of significance (Spearman rank correlation coefficient).
IP-10 = interferon-γ-inducible protein of 10 KDa (CXCL10); NGAL = neutrophil gelatinase B-associated lipocalin; VKH = Vogt-Koyanagi-Harada disease.

creased levels of gelatinase B, and increased levels of NGAL-gelatinase B complexes. There was also a significant correlation between the incidence of PARC detection and increased levels of gelatinase A, and increased levels of gelatinase B (Table 5).

There was a significant correlation between gelatinase A levels and gelatinase B levels in all patients, and in patients with VKH disease. There was a significant correlation between IP-10 levels and gelatinase B levels in patients with uveitis associated with systemic disease (Table 6).

• **EFFECT OF TREATMENT:** One week after topical corticosteroid treatment in one patient with ankylosing spondylitis, topical and systemic corticosteroid treatment in two patients with VKH disease, and topical and systemic corticosteroid treatment combined with systemic cyclosporine A therapy in one patient with Behçet disease, there was a marked clinical improvement. This was associated with a statistically insignificant reduction in aqueous humor IP-10 levels (median, 6.1 vs 0.89 ng/ml; $P = .068$, Wilcoxon test), gelatinase A levels (median, 702 vs 399.5; $P = .144$, Wilcoxon test), and gelatinase B levels (median, 795.5 vs 54; $P = .109$, Wilcoxon test).

DISCUSSION

T-CELL-DEPENDENT IMMUNOLOGIC EVENTS ARE INCREASingly being considered to play a central role in the pathogenesis of uveitis,[1] but the mechanisms responsible for T-cell recruitment are only poorly understood. Among several potentially relevant T-cell chemoattractants investigated, the CXC chemokine IP-10 was detected in all aqueous humor samples from patients with active uveitis. Conversely, none of the aqueous humor samples from controls contained detectable levels of IP-10. IP-10 levels in the aqueous humor from patients with uveitis significantly correlated with clinical disease activity. Furthermore, IP-10 was detected at low levels in only three of the paired serum samples tested and levels in aqueous humor of patients with uveitis were significantly higher than those in serum suggesting that IP-10 in aqueous humor reflected local production, and that there was a chemotactic gradient that favors the migration of T cells from the blood to the eye. These data suggest a pathogenic role of IP-10 in the recruitment of activated T cells into the eye in patients with uveitis and support the notion that Th1 cells play a critical role in the pathogenesis of uveitis. Our results are in agreement with a previous report that demonstrated IP-10 detection in all aqueous humor samples from seven patients with acute idiopathic anterior uveitis.[18] Additionally, several studies reported that IP-10 mRNA and protein were upregulated in the eyes of animals with experimental autoimmune uveoretinitis (EAU).[19,20]

IP-10 is known to be a strong ligand for the CXCR3 receptor on activated T cells.[4,6] In addition to chemotactic activity, IP-10 induces T-cell adherence to activated human endothelial cells.[21] Because CXCR3 is predominantly expressed on Th1 cells,[9] it seems likely that IP-10 contributes to the selective recruitment of Th1 cells to sites of inflammation with high IFN-γ production. Moreover, a recent study demonstrated that IP-10 drives naive $CD4^+$ T cells into Th1 polarization.[22] Several studies demonstrated a possible role for IP-10 in Th1 cell-mediated autoimmune clinical conditions, such as multiple sclerosis,[23] rheumatoid arthritis,[24] Sjögren syndrome,[25] Grave disease,[26] Hashimotos thyroiditis,[26] and autoimmune hepatitis.[27] In these diseases, levels of IP-10 correlated with the tissue infiltration of T lymphocytes supporting the hypothesis

TABLE 5. Relationships Between the Incidence of IL-8, GCP-2, and PARC Detection in Aqueous Humor Samples From Patients With Uveitis (n = 30) and the Variables Investigated

Variables	IL-8	GCP-2	PARC
Disease activity			
1+–2+ (n = 12)	2 (16.67%)	0 (0%)	3 (25%)
3+ (n = 9)	6 (66.67%)	2 (22.2%)	4 (44.4%)
4+–5+ (n = 9)	8 (88.9%)	4 (44.4%)	5 (55.5%)
	$P = .00099$*	$P = .01295$*	$P = .158$
IP-10 (ng/ml)			
<5.0 (n = 18)	8 (44.4%)	3 (16.67%)	8 (44.4%)
5.0–9.99 (n = 8)	5 (62.5%)	1 (12.5%)	4 (50%)
≥10.0 (n = 4)	3 (75%)	2 (50%)	0 (0%)
	$P = .2164$	$P = .26059$	$P = .22067$
Gelatinase A (scanning units)			
<500 (n = 18)	6 (33.3%)	2 (11.1%)	1 (5.56%)
500–999 (n = 8)	6 (75%)	3 (37.5%)	7 (87.5%)
≥1000 (n = 4)	4 (100%)	1 (25%)	4 (100%)
	$P = .00615$*	$P = .26059$	$P < .001$*
Gelatinase B (scanning units)			
<500 (n = 17)	5 (29.41%)	1 (5.88%)	2 (11.76%)
500–999 (n = 6)	4 (66.67%)	1 (16.67%)	5 (83.33%)
≥1000 (n = 7)	7 (100%)	4 (57.14%)	5 (71.43%)
	$P = .00148$*	$P = .00686$*	$P = .002$*
NGAL-gelatinase B Complex (scanning units)			
0 (n = 19)	8 (42.11%)	1 (5.26%)	6 (31.58%)
1–99 (n = 7)	4 (57.14%)	2 (28.6%)	4 (57.14%)
≥100 (n = 4)	4 (100%)	3 (75%)	2 (50%)
	$P = .0453$*	$P = .00018$*	$P = .30795$

*Statistically significant at 5% level of significance (Chi-square for linear trend test).
IL-8 = interleukin-8 (CXCL8); GCP-2 = granulocyte chemotactic protein 2 (CXCL6); PARC = pulmonary and activation-regulated chemokine (CCL18); IP-10 = interferon-γ-inducible protein of 10 KDa (CXCL10); NGAL = neutrophil gelatinase B-associated lipocalin.

TABLE 6. Relationships Between IP-10 and Gelatinases A and B Levels in Aqueous Humor Samples From Patients With Uveitis

Variables	All Patients	Uveitis Associated With Systemic Diseases	VKH	Behçet Disease
Gelatinase A and gelatinase B	$r = .328$	$r = .314$	$r = .937$	$r = -.62$
	$P = .038$*	$P = .063$	$P < .001$*	$P = .432$
IP-10 and gelatinase A	$r = -.347$	$r = -.062$	$r = .323$	$r = .045$
	$P = .403$	$P = .383$	$P = .141$	$P = .450$
IP-10 and gelatinase B	$r = .284$	$r = .346$	$r = .318$	$r = .406$
	$P = .064$	$P = .045$*	$P = .145$	$P = .122$

*Statistically significant correlation at 5% level of significance (Pearson correlation coefficient).
IP-10 = interferon-γ-inducible protein of 10 KDa (CXCL10).

that IP-10 plays an important role in the recruitment of these cells to sites of inflammation. IP-10 detection in the aqueous humor from patients with active uveitis may reflect the Th1-weighted immune response in uveitis and is in line with the hypothesis that IFN-γ produced by Th1 lymphocytes leads to the production of IP-10 that could play a role in the extravasation of activated T lymphocytes from blood to eye. In the present study, IP-10 levels were

significantly higher in patients with Behçet disease than in patients with VKH disease. Therefore, it is tempting to speculate that Th1-type immune responses are more potent in patients with Behçet disease compared with patients with VKH disease.

MIP-1α,[7] and PARC[8] promote T-cell chemotaxis and thus may cooperatively stimulate the migration and accumulation of T cells. In this study, however, the difference between PARC levels in the aqueous humor and serum was not significant, and MIP-1α was detected in only two aqueous humor samples from patients with active uveitis. Similar results were obtained by Verma and associates[18] who did not detect MIP-1α in aqueous humor samples from seven patients with acute idiopathic anterior uveitis. Conflicting results were obtained in animal models of EAU. MIP-1α mRNA and protein were upregulated in EAU,[19,28] and it has been reported that MIP-1α plays a major role in the recruitment of leukocytes into the ocular lesions in EAU.[29] In contrast, Keino and associates[20] reported that MIP-1α mRNA was not expressed in EAU at any of the time points tested. A possible explanation for this could be the different type of disease manifestation in the mouse and rat EAU model. In addition to T cells, neutrophils are thought to play a central role in the pathogenesis of Behçet disease. Neutrophils from patients with Behçet disease are hyperactivated and reveal enhanced chemotaxis.[3] The chemokines IL-8,[30] and GCP-2[31] selectively chemoattract neutrophils and induce the release of gelatinase B from these cells. However, in the present study, IL-8 was detected in only four and GCP-2 in only three aqueous humor samples from patients with Behçet disease. Therefore, it is possible that other neutrophil chemoattractants[4] are involved in neutrophil recruitment in Behçet disease.

In this study, gelatinase A was expressed constitutively in all aqueous humor samples from control subjects, whereas the inducible gelatinase B was detected in only one of the control aqueous humor samples. Aqueous humor levels of gelatinase A were significantly increased in active uveitis compared with controls, and gelatinase B was detected in 26 of 30 aqueous humor samples from patients with active uveitis. Furthermore, gelatinase A and gelatinase B levels correlated significantly with the clinical disease activity in patients with active uveitis. Our data are in general agreement with previous reports, which have demonstrated that only gelatinase A was constitutively expressed in the aqueous humor of disease-free subjects,[32] and normal rats.[33] Conversely, in aqueous humor samples from 16 patients with chronic uveitis, gelatinase A levels increased, gelatinase B was detected, and the levels correlated significantly with the activity of uveitis.[32] Similarly, in an animal model of endotoxin-induced uveitis, the aqueous humor samples contained elevated levels of gelatinase A and gelatinase B. Immunohistochemistry showed gelatinase A and gelatinase B expression in ciliary epithelium and aqueous humor neutrophils.[33] Taken together, these results provide strong evidence of a major role of gelatinases in the pathogenesis of uveitis.

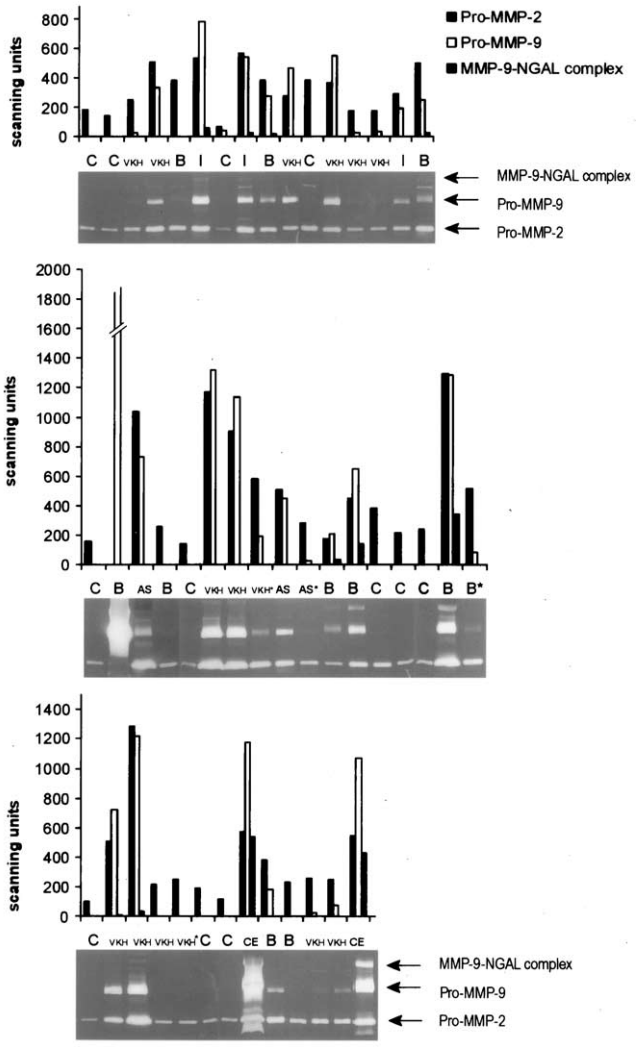

FIGURE 1. Gelatinase zymography of aqueous humor samples from patients with active uveitis (VKH = Vogt-Koyanagi-Harada disease; B = Behçet disease; I = idiopathic; AS = ankylosing spondylitis; CE = chronic endophthalmitis; and C = control patients). Gelatinase levels are visualized as the clear lysis zones on a background of stained residual gelatin substrate. Scanning units were determined by densitometry of individual lysis zones and are indicated for the different molecular forms. Within one gel analysis, the samples can be compared quantitatively and the same scanning unit setting was applied for different zymography gels. One sample (second from left, middle panel) contained excessive pro-MMP-9 levels out of the analytical range. Therefore, the level of this sample is indicated as exceeding 1,800 scanning units. * = post-treatment samples; MMP-2 = matrix metalloproteinase-2 (gelatinase A); MMP-9 = matrix metalloproteinase-9 (gelatinase B); NGAL = neutrophil gelatinase B-associated lipocalin.

Gelatinase B is produced mainly by neutrophils but is also produced by various other blood-derived cell types,

such as monocytes, macrophages, lymphocytes, and dendritic cells.[5] In the present study, the concomitant presence of gelatinase B in complex with NGAL in 11 of 30 aqueous humor samples from patients with active uveitis suggests that the neutrophil is a significant source of the gelatinase B in these samples.[17] Gelatinase B plays a role in normal development but its expression has also been associated with a wide variety of pathologic processes, including autoimmune diseases.[12,34] The mechanism of action of gelatinase B in the pathogenesis of autoimmunity may be through the cleavage of substrate proteins into immunodominant epitopes. Further processing and presentation of the protein fragments on major histocompatibility complex class II molecules on the surface of antigen-presenting cells activates autoreactive T cells.[12,34] Additionally, gelatinase B is an important effector molecule for the migration of leukocytes.[5] Since one of the main components of endothelial basement membrane is type IV collagen, which is specifically cleaved by gelatinase B,[35,34] gelatinase B might increase the extravasation leukocytes into the eye through basement membrane zone underlying the endothelial cell layer. Gelatinase B could also render basement membranes vulnerable and increase vascular permeability leading to disruption of the blood-ocular barrier in patients with uveitis. Additionally, Rosenberg and associates[36] showed that gelatinase A is also able to open the blood-brain barrier. Moreover, recently, Hangai and associates[37] demonstrated that the exposure of a collagen type IV cryptic epitope through cleavage by gelatinase B was proposed to be a mechanism for endothelial cell migration and neovascularization. Therefore, selective inhibition of gelatinase B should be considered a potential therapy in patients with uveitis.

In conclusion, considering the T-cell attracting and stimulating capacity of IP-10 and the importance of T cells in the pathogenesis of uveitis, our current study indicates that this CXC chemokine plays an important role as a mediator of T-cell recruitment and activation in uveitis. Additionally, our findings suggest a pathogenic role of gelatinase activity in uveitis. A strategy for blocking chemokines or chemokine receptors and treatment with specific matrix metalloproteinase inhibitors could be a new approach for the treatment of uveitis.

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Biosketch

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