Differential CXC and CX3C Chemokine Expression Profiles in Aqueous Humor of Patients With Specific Endogenous Uveitic Entities

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PURPOSE. To determine the levels of the neutrophil chemoattractants CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8, the T helper 1 chemoattractants CXCL9, CXCL10 and CXCL11, the lymphoid chemokines CXCL12 and CXCL13 and the soluble form of the transmembrane chemokines CXCL16 and CX3CL1, in aqueous humor samples from patients with specific uveitic entities.

METHODS. Aqueous humor samples from patients with active uveitis associated with Behçet's disease ($n = 13$), sarcoidosis ($n = 8$), HLA-B27–related inflammation ($n = 12$), Vogt-Koyanagi-Harada (VKH) disease ($n = 12$), and healthy controls ($n = 9$) were assayed with the use of a multiplex assay.

RESULTS. All chemoattractant levels were significantly higher in all patients than in the controls. The levels of all neutrophil chemoattractants and CXCL10, CXCL16, and CX3CL1 were significantly higher in nongranulomatous uveitis (Behçet's disease and HLA-B27–associated uveitis) than in granulomatous uveitis (sarcoidosis and VKH disease), whereas the levels of the B cell chemoattractant CXCL13 were significantly higher in granulomatous uveitis than in nongranulomatous uveitis. CXCL13 levels were highest in the patients with VKH disease. CXCL9, CXCL11, and CXCL12 levels did not differ significantly.

CONCLUSIONS. Inflammation in nongranulomatous uveitis appears to be driven by neutrophils and T helper 1 lymphocytes, whereas B lymphocytes may contribute to the inflammatory process in granulomatous uveitis, particularly in VKH disease.

Keywords: uveitis, Behçet's disease, sarcoidosis, HLA-B27, Vogt-Koyanagi-Harada disease

Endogenous uveitis is a clinically heterogenous group of intraocular inflammatory diseases that often lead to decreased vision, retinal destruction, and blindness. Endogenous uveitis often occurs in conjunction with systemic diseases, such as Behçet's disease (BD), sarcoidosis, human leukocyte antigen (HLA)-B27–associated uveitis, and Vogt-Koyanagi-Harada (VKH) disease. The wide heterogeneity in clinical manifestations, causes, and outcomes of the disease continue to be largely unpredictable and unexplained.

However, the recruitment and activation of leukocytes is thought to be essential in the perpetuation of the inflammatory response.[1,2] Inflammatory chemokines are secreted proteins that recruit leukocytes from blood to sites of inflammation via activation of 7-transmembrane-domain G-protein-coupled receptors.[3] Evidence is accumulating that chemokine and chemokine receptor antagonists have strong therapeutic potential for autoimmune diseases. Therapies designed to block the activity or inhibit the production of these mediators and their corresponding receptors are currently being developed.[3]

The chemokine receptors CXCR1 and CXCR2, which are predominantly expressed on neutrophils, drive neutrophil migration and activation in response to their ligands such as growth-related oncogene–α/CXCL1, growth-related oncogene–β/CXCL2, epithelial-derived neutrophil attractant–78/CXCL5, granulocyte chemotactic protein–2/CXCL6, and IL-8/CXCL8. This chemokine network has been suggested to be important to the development of neutrophilic inflammation and is activated in response to TNF-α or IL-1β production. The CXCR3 receptor and its three IFN-γ-inducible ligands, monokine induced by IFN-γ/CXCL9, IFN-γ-inducible protein of 10 kDa/CXCL10, and IFN-inducible T-cell α chemoattractant/CXCL11, have been suggested to serve as critical players in the pathogenesis of T helper 1 (Th1) inflammatory responses.[3] A key phenomenon in the pathogenesis of autoimmune diseases is the formation of ectopic lymphoid aggregates with germinal center-like structures in the inflamed tissues, which contain proliferating B lymphocytes, plasma cells, follicular helper CD4+ T lymphocytes, and a network of follicular dendritic cells.[4–6] The formation and maintenance of ectopic lymphoid structures is dependent on the expression of lymphoid chemokines, such as stromal cell-derived factor-1/CXCL12, and B cell attracting chemokine-1/CXCL13 and their specific receptors CXCR4 and CXCR5, respectively.[4–6] Within the chemokine family, scavenger receptor for phosphatidyl serine and oxidized low-density lipoprotein/CXCL16 and fractalkine/CX3CL1 are exceptional in

TABLE 1. Detection Ranges of the Different Analytes in the Multiplex Assay

Analyte	Detection Range, pg/ml
CXCL1	3.89–2910
CXCL2	4.47–5162
CXCL5	13.24–27982
CXCL6	0.83–985
CXCL8	0.42–2413
CXCL9	5.57–18409
CXCL10	1.12–4834
CXCL11	0.13–595
CXCL12	9.6–10968
CXCL13	0.34–1405
CXCL16	0.34–1358
CX3CL1	4.38–17381

that they can be found in both membrane-bound and soluble forms. The soluble forms are generated from the former by the proteolytical activities of, for example, a disintegrin and metalloproteinase 10 and a disintegrin and metalloproteinase 17. As transmembrane molecules on the surface of endothelial cells, CXCL16 and CX3CL1 act as adhesion molecules that can interact with their receptors CXCR6 and CX3CR1, respectively, which are expressed on leukocyte subtypes. The released soluble forms function as potent leukocyte chemoattractants. CXCL16 and CX3CL1 preferentially act on Th1 lymphocytes rather than Th2 cells.[3]

The magnitude and pattern of the inflammatory chemoattractant responses in different types of endogenous uveitis remain unclear. Analysis of the aqueous humor (AH) from patients with specific clinical entities of endogenous uveitis may help in generating novel biomarkers for different phenotypes of uveitis. Once the causal role of specific molecules for particular disease entities is established, these may serve as potential targets for selective therapy. We therefore screened the AH from patients with active uveitis associated with BD, sarcoidosis, HLA-B27–related intraocular inflammation and VKH disease for the presence of the inflammatory chemoattractants CXCL1, CXCL2, CXCL5, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL16, and CX3CL1.

PATIENTS AND METHODS

Patients with active uveitis seen at the outpatient clinic of King Abdulaziz University Hospital (Ridyadh, Saudi Arabia) were included in the study. Patients who had undergone elective cataract extraction at King Abdulaziz University Hospital with no prior history of uveitis served as the control group. Control samples were obtained intraoperatively before opening the anterior chamber. Patients with uveitis were examined using slit-lamp biomicroscopy, indirect ophthalmoscopy, fluorescein angiography, and indocyanine green angiography. Diagnoses were made using established clinical criteria, with supporting laboratory evidence as needed.[7,8] HLA-B27–associated uveitis was diagnosed based on HLA typing of peripheral blood cells and typical ocular manifestations of unilateral acute anterior uveitis. In each patient, the uveitis activity was graded according to the criteria of the Standardization of the Uveitis Nomenclature Working group grading scheme.[9] None of the patients was on topical or systemic therapy on presentation.

AH (100–200 µl) was aspirated from each patient by means of limbic paracentesis with the use of a 27-gauge needle attached to a tuberculin syringe after the application of the 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. AH samples from patients with uveitis were obtained before therapy. All procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients and the controls. The study was approved by the Research Center, College of Medicine, King Saud University.

Chemokine Assays

The chemokine expression profile in AH samples was determined using the Bio-Plex chemokine panel of BIORAD (Hercules, CA, USA). This technique relies on antibody-coated, nonmagnetic beads and allows to concomitly measure the levels of many different analytes in a single sample. We analyzed AH for the presence of CXCL1, CXCL2, CXCL5, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL16, and CX3CL1 following the manufacturer's instructions, and the results were generated using the Bio-Plex 200 system and software. The experimental detection range of the different analyses is indicated in Table 1.

Statistical Analysis

Data management was preliminarily performed using Excel 2013 (Microsoft, Redmond, WA, USA), and all parameters were then analyzed using SPSS version 20.0 software (IBM, Armonk, NY, USA). Descriptive statistics of the continuous variables were presented as means ± SEM or SD and fold increase, where the fold increase was calculated by dividing the inflammatory chemoattractant levels with the control levels. The Kruskal-Wallis test was used to compare the data distribution within different disease categories. The Mann-Whitney test was then used to compare two independent groups. A P value less than 0.05 indicated statistical significance.

RESULTS

Patients

AH samples were obtained from 13 patients with BD, 8 with sarcoidosis, 12 with HLA-B27–associated uveitis, and 12 with VKH disease. A total of 9 patients who had undergone cataract extraction served as the control group. Table 2 presents the demographic and clinical characteristics of the study participants (obtained at the time of sample collection).

Levels of the Neutrophil Chemoattractants CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 in AH Samples

CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 were detected in all AH samples from patients with uveitis and the controls. When considering the entire patient group, these chemokine levels were significantly higher in the AH of patients than in controls (Table 3). CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 levels in BD ($P < 0.001$ for all comparisons), sarcoidosis ($P = 0.001$, $P = 0.013$, $P = 0.004$, $P = 0.002$, and $P = 0.002$, respectively), HLA-B27–associated uveitis ($P < .001$ for all comparisons) and VKH disease ($P < 0.001$, $P = 0.029$, $P = 0.271$, $P = 0.003$, and $P = 0.006$, respectively) were significantly increased when compared with the controls except for CXCL5 in VKH disease (Fig. 1).

The neutrophil chemoattractant levels in the four disease groups and controls were compared to determine the

TABLE 2. Demographic and Clinical Characteristics of All Participants at Presentation

Characteristic	Controls, *n* = 9	Behçet's Disease, *n* = 13	Sarcoidosis, *n* = 8	HLA-B27, *n* = 12	VKH Disease, *n* = 12
Sex					
Male, *n* = 34; *n* (%)	5 (55.5)	13 (100)	5 (62.5)	5 (41.7)	6 (50.0)
Female, *n* = 20; *n* (%)	4 (44.4)	0 (0.0)	3 (37.5)	7 (58.3)	6 (50.0)
Age in years, mean ± SD [range]	58.2 ± 9.1 [41-70]	28.9 ± 4.2 [23-36]	25.1 ± 6.5 [17-35]	34.3 ± 9.3 [22-50]	28.3 ± 7.2 [18-42]
Anterior chamber reaction					
≤2+, *n* = 14; *n* (%)		5 (38.5)	3 (37.5)	2 (17.0)	4 (33.3)
>2+, *n* = 31; *n* (%)		8 (61.5)	5 (62.5)	10 (83.0)	8 (66.7)
Visual acuity					
≥20/40, *n* = 24; *n* (%)		2 (15.4)	8 (100)	9 (75.0)	5 (41.7)
<20/40, *n* = 21; *n* (%)		11 (84.6)	0 (0.0)	3 (25.0)	7 (58.3)
Presence of posterior synechiae, *n* = 28; *n* (%)		6 (46.2)	6 (75.0)	8 (66.7)	8 (66.7)

VKH, Vogt-Koyanagi-Harada.

differences in the neutrophil chemoattractant expression profiles of the various clinical entities of uveitis (Kruskal-Wallis test), and the results are shown in Figure 1. CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 levels in AH samples differed significantly between patients with BD, sarcoidosis, HLA-B27–associated uveitis, and VKH disease and the controls ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.001$, $P < 0.001$, respectively). Pairwise comparisons (Mann-Whitney test) indicated that CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 levels were significantly increased in patients with BD when compared with patients with VKH disease ($P = 0.003$, $P = 0.011$, $P = 0.0011$, $P = 0.006$, and $P = 0.026$, respectively). In addition, higher CXCL1 levels were measured in patients with BD than in the patients with sarcoidosis ($P = 0.008$). CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 levels were significantly higher in patients with HLA-B27–associated uveitis than in patients with sarcoidosis ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.049$, and $P = 0.001$, respectively) and VKH disease ($P < 0.001$ for all comparisons).

Levels of the Th1 Lymphocyte Chemoattractants CXCL9, CXCL10, and CXCL11 in AH Samples

CXCL9, CXCL10, and CXCL11 were detected in all samples from patients with uveitis and controls. When the entire patient group was considered, CXCL9, CXCL10, and CXCL11 levels were significantly higher in the AH of patients than in controls (Table 3). The same phenomenon persisted for each disease group ($P < 0.001$ for all comparisons; Fig. 1).

To evaluate the distribution of CXCL9, CXCL10, and CXCL11 levels among the four disease groups and controls, we used the Kruskal-Wallis test, and the results are shown in Figure 1. Chemokine levels in AH samples differed significantly ($P < 0.001$ for all comparisons). Pairwise comparisons (Mann-Whitney test) indicated that CXCL10 levels in BD were significantly higher than in patients with VKH disease ($P = 0.037$).

Levels of the Lymphoid Chemokines CXCL12 and CXCL13 in AH Samples

CXCL12 and CXCL13 were detected in all samples. The levels were significantly higher in the entire patient group than in controls (Table 3). The same phenomenon persisted for each disease group ($P < 0.001$ for all comparisons; Fig. 1).

The chemokine levels in the four disease groups and controls were compared (Kruskal-Wallis test; Fig. 1). CXCL12 and CXCL13 levels differed significantly between BD, sarcoidosis, HLA-B27–associated uveitis, VKH disease, and controls ($P = 0.001$ and $P < 0.001$, respectively). Pairwise comparisons (Mann-Whitney test) indicated that no statistically significant difference between disease groups could be obtained for

TABLE 3. Comparisons of Mean Inflammatory Chemoattractant Levels Between Patients and Controls

	All Patients, *n* = 45, pg/ml, Mean ± SEM [Range] (Fold Increase)†	Controls, *n* = 9, pg/ml, Mean ± SEM [Range]	*P* Value, Mann-Whitney Test
CXCL1	6645 ± 1410 [33-23283] (112)	59 ± 15 [31-142]	<0.001*
CXCL2	391 ± 122 [35-4311] (9)	43 ± 6 [35-85]	<0.001*
CXCL5	3591 ± 519 [499-14593] (6.6)	548 ± 33 [499-753]	<0.001*
CXCL6	882 ± 250 [7-5761] (126.1)	7.0 ± 0.0 [7-7]	<0.001*
CXCL8	1789 ± 651 [11-19310] (78.5)	23 ± 9 [3-82]	<0.001*
CXCL9	4736 ± 909 [44-30072] (72.9)	65 ± 12 [44-139]	<0.001*
CXCL10	26823 ± 2361 [1630-38680] (703.8)	38 ± 15 [9-148]	<0.001*
CXCL11	215 ± 116 [2-4770] (176.0)	1.2 ± 0.1 [1-2]	<0.001*
CXCL12	1779 ± 158 [301-5808] (6.1)	291 ± 109 [76-820]	<0.001*
CXCL13	1825 ± 507 [9-11244] (586.5)	3.1 ± 0.1 [3-4]	<0.001*
CXCL16	3887 ± 561 [182-10870] (5.0)	771 ± 275 [95-2699]	0.001*
CX3CL1	1718 ± 417 [48-9484] (25.3)	68 ± 9 [35-108]	<0.001*

* Statistically significant at 5% level of significance.
† Fold increase compared with controls.
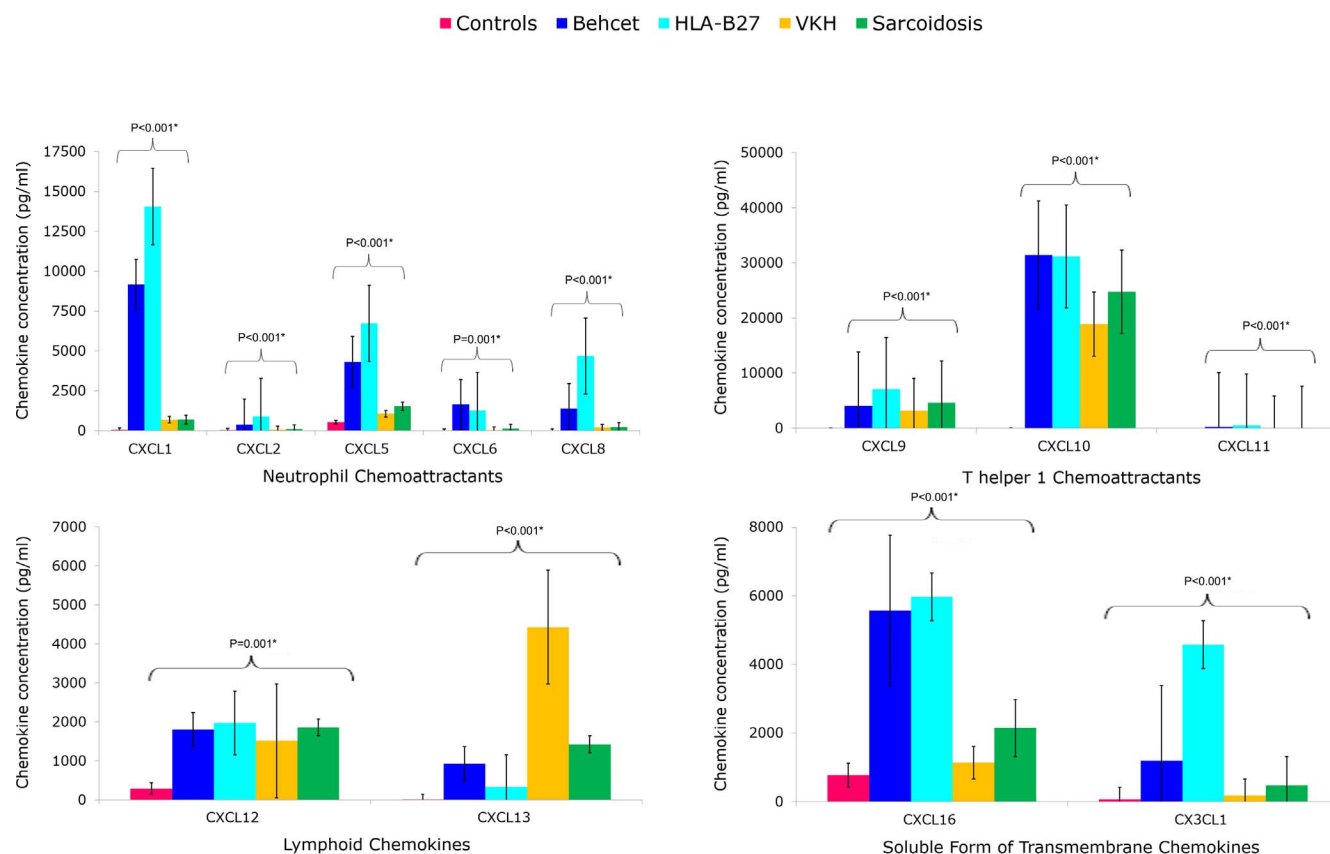
FIGURE 1. Comparisons of mean ($\pm$ SEM) inflammatory chemoattractant levels in the aqueous humor of controls and patients with Behçet's disease, HLA-B27–associated uveitis, Vogt-Koyanagi-Harada (VKH) disease, and sarcoidosis (Kruskal-Wallis test). *Statistically significant at 5% level of significance.

CXCL12. CXCL13 levels in VKH disease and sarcoidosis were significantly higher than the levels in BD ($P < 0.001$ and $P = 0.025$, respectively). In addition, CXCL13 levels were significantly increased in VKH disease when compared with HLA-B27–associated uveitis ($P = 0.001$). However, CXCL13 levels did not differ significantly between sarcoidosis and HLA-B27–associated uveitis ($P = 0.069$) or between VKH and sarcoidosis ($P = 0.089$).

Levels of the Soluble Form of Transmembrane Chemokines CXCL16 and CX3CL1 in AH Samples

CXCL16 and CX3CL1 were detected in all samples, and their levels were significantly enhanced in the uveitis patients when compared with the controls (Table 3). The same phenomenon persisted for BD ($P < 0.001$ and $P = 0.003$, respectively), sarcoidosis ($P = 0.054$ and $P = 0.001$, respectively), HLA-B27–associated uveitis ($P = 0.001$ and $P < 0.001$, respectively), and VKH disease ($P = 0.047$ and $P = 0.004$, respectively) except for CXCL16 in sarcoidosis (Fig. 1).

CXCL16 and CX3CL1 levels differed significantly between BD, sarcoidosis, HLA-B27–associated uveitis, VKH disease, and controls ($P < 0.001$ for both comparisons; Kruskal-Wallis test; Fig. 1). Pairwise comparisons (Mann-Whitney test) indicated that more CXCL16 and CX3CL1 was produced in BD than in VKH patients ($P < 0.001$ and $P = 0.011$, respectively). In addition, CXCL16 levels in BD were significantly higher than in sarcoidosis ($P = 0.013$). The expression of CXCL16 and CX3CL1 was increased in HLA-B27–associated uveitis when compared with sarcoidosis ($P = 0.03$ for both comparisons) and VKH disease ($P < 0.001$ for both comparisons). In

addition, enhanced CX3CL1 levels were detected in sarcoidosis when compared with VKH disease ($P = 0.006$).

Inflammatory Chemoattractant Levels in Nongranulomatous Uveitis and Granulomatous Uveitis

When patients were divided into those with nongranulomatous uveitis (BD and HLA-B27–associated uveitis; $n = 25$) and those with granulomatous uveitis (sarcoidosis and VKH disease; $n = 20$), CXCL1, CXCL2, CXCL5, CXCL6, CXCL8, CXCL10, CXCL16, and CX3CL1 levels in nongranulomatous uveitis were significantly higher than those in granulomatous uveitis. On the other hand, CXCL13 levels in granulomatous uveitis were significantly higher than those in nongranulomatous uveitis. No statistically significant difference could be obtained for CXCL9, CXCL11, or CXCL12 (Table 4).

DISCUSSION

In the present study, we add novel data on the relative expression of the neutrophil chemoattractants in the AH from patients with specific uveitic entities. Among the neutrophil chemoattractants studied, the highest absolute production levels were reached for CXCL1 and CXCL5, whereas lower levels of CXCL2, CXCL6, and CXCL8 were measured. CXCL1, CXCL2, and CXCL5 activate CXCR2, whereas CXCL6 and CXCL8 activate both CXCR1 and CXCR2.[3] These findings suggest that CXCL1 and CXCL5 are important chemoattractants for neutrophils in endogenous uveitis. However, levels

TABLE 4. Comparisons of Mean Inflammatory Chemoattractant Levels Between Nongranulomatous Uveitis (Behçet's Disease and HLA-B27–Associated Uveitis) and Granulomatous Uveitis (Sarcoidosis and Vogt-Koyanagi-Harada Disease)

	Nongranulomatous Uveitis, $n = 25$, pg/ml, Mean $\pm$ SEM (Fold Increase)†	Granulomatous Uveitis, $n = 22$, pg/ml, Mean $\pm$ SEM (Fold Increase)†	P Value, Mann-Whitney Test
CXCL1	**11607 $\pm$ 2101 (196.0)**	691 $\pm$ 266 (11.7)	<0.001*
CXCL2	631 $\pm$ 207 (14.5)	88 $\pm$ 11 (2.0)	<0.001*
CXCL5	5467 $\pm$ 716 (10.0)	1245 $\pm$ 274 (2.3)	<0.001*
CXCL6	**1466 $\pm$ 393 (209.5)**	71 $\pm$ 23 (10.2)	<0.001*
CXCL8	**3036 $\pm$ 1109 (133.3)**	213 $\pm$ 83 (9.3)	<0.001*
CXCL9	5508 $\pm$ 1534 (84.7)	3770 $\pm$ 707 (58.0)	0.615
CXCL10	31295 $\pm$ 2713 (821.2)	21232 $\pm$ 3803 (557.1)	0.020*
CXCL11	**363 $\pm$ 201 (297.2)**	20 $\pm$ 4 (16.5)	0.169
CXCL12	1882 $\pm$ 220 (6.5)	1650 $\pm$ 228 (5.7)	0.392
CXCL13	656 $\pm$ 466 (210.7)	***3228 $\pm$ 881 (1037.5)***	<0.001*
CXCL16	5765 $\pm$ 801 (7.5)	1540 $\pm$ 327 (2.0)	<0.001*
CX3CL1	**3055 $\pm$ 694 (45.0)**	310 $\pm$ 62 (4.6)	<0.001*

Bold values indicate chemokines that are at least 10-fold more upregulated in nongranulomatous uveitis compared to granulomatous uveitis. Bold values in italics indicate the highest upregulated chemokine in granulomatous uveitis.
* Statistically significant at 5% level of significance.
† Fold increase compared with controls.

measured for the other neutrophil chemoattractants are also biologically significant. In addition, the levels of all neutrophil chemoattractants studied were significantly higher in non-granulomatous uveitis associated with BD and HLA-B27–related inflammation than those in granulomatous uveitis associated with sarcoidosis and VKH disease. These observations are consistent with previous reports showing that neutrophil infiltration and activation are important pathophysiological features in BD and HLA-B27–associated diseases.[10-13] Therefore, inhibition of neutrophil chemotaxis by blocking CXCR1 and CXCR2 could provide a novel therapeutic strategy in the management of acute uveitis associated with HLA-B27–related inflammation and BD. Preclinical and clinical studies demonstrated that SCH527123, a selective antagonist of the human CXCR1 and CXCR2 receptors, markedly reduced neutrophil activation and recruitment and attenuated neutrophil-mediated inflammation in several models of acute inflammation.[3,14,15]

Because CXCR3 is predominantly expressed on activated Th1 cells, it seems that CXCL9, CXCL10, and CXCL11 contribute to the selective recruitment of Th1 cells to sites of inflammation with high IFN-γ production.[3] In the present study, the levels of the three CXCR3 receptor ligands CXCL9, CXCL10, and CXCL11 in the AH of uveitis patients were significantly enhanced when compared with controls. The levels of CXCL10 were about 5- and 100-fold higher than those of CXCL9 and CXCL11, respectively. Our findings suggest that among the CXCR3 receptor chemokine ligands, CXCL10 is the predominant chemoattractant for Th1 cells in endogenous uveitis. In addition, CXCL10 levels were significantly higher in patients with nongranulomatous uveitis than in patients with granulomatous uveitis. These data suggest the involvement of a Th1 immune response in the pathogenesis of endogenous uveitis, particularly acute nongranulomatous uveitis associated with HLA-B27–related inflammation and BD. Several studies demonstrated that the CXCL10–CXCR3 axis plays an important role in the Th1 immune response, which is responsible for many organ-specific autoimmune diseases and that the determination of high levels of CXCL10 is a marker of Th1-oriented immune responses.[16] Our findings suggest that CXCR3 antagonists may serve as a new strategy for treatment of endogenous uveitis, particularly nongranulomatous uveitis. Recent studies demonstrated that therapy with potent small-molecule CXCR3 antagonists showed significant efficacy in animal models of autoimmune diseases, including rheumatoid arthritis and multiple sclerosis, and in the prevention of transplant rejection.[3,17-19]

Cleavage of the transmembrane chemokines CXCL16 and CX3CL1 is associated with the inflammatory cascade and, therefore, the soluble form of the molecules may serve as inflammatory markers.[20] CXCL16 and CX3CL1 are induced by the Th1 cytokines IFN-γ and TNF-α and preferentially act on Th1 lymphocyte subsets. They may, therefore, further enhance Th1-associated inflammatory responses.[20-22] Indeed, the CXCL16 and CX3CL1 receptors CXCR6[23] and CX3CR1,[24] respectively, have been found to have preferential expression in Th1 cells compared to Th2 cells. Accumulated evidence points toward a role of the chemokine pathways CXCL16–CXCR6[25-27] and CX3CL1–CX3CR1[24,28,29] in several inflammatory conditions. Furthermore, it was demonstrated that CXCR6 and CXCR3 act coordinately in mediating T cell activation and recruitment into inflamed synovial tissue.[26] In addition, CXCL16[30] and CX3CL1[31] expressed on the endothelium triggers platelet activation and adhesion to the endothelium via their receptors, suggesting a role for CXCL16 and CX3CL1 in vascular inflammation and thrombo-occlusive diseases.

Our subgroup analysis showed that CXCL16 and CX3CL1 levels were 3- to 10-fold increased in patients with BD and HLA-B27–associated uveitis compared to sarcoidosis and VKH disease. These findings suggest that CXCL16 and CX3CL1 might serve as novel inflammatory biomarkers for acute nongranulomatous uveitis associated with BD and HLA-B27–related inflammation and that the Th1 immune response is more potent in BD and HLA-B27–associated uveitis. Our data also suggest that the CXCL16–CXCR6 and CX3CL1–CX3CR1 pathways could be potentially useful therapeutic targets in acute nongranulomatous uveitis. Indeed, blockade of CXCL16[25] and CX3CL1[32] ameliorated collagen-induced arthritis and reduced the inflammatory infiltrate in the synovium.

In a previous report, we demonstrated that the levels of the B cell chemoattractant CXCL13 measured in patients with VKH disease largely exceeded those in patients with BD or HLA-B27–associated uveitis.[33] In the present study, the concentrations of CXCL13 in the AH were quantified in a new set of AH samples. The results were consistent with and confirmed our previous data in that higher concentrations of CXCL13 levels were measured in AH from patients with VKH disease. These findings suggest that CXCL13 might serve as an
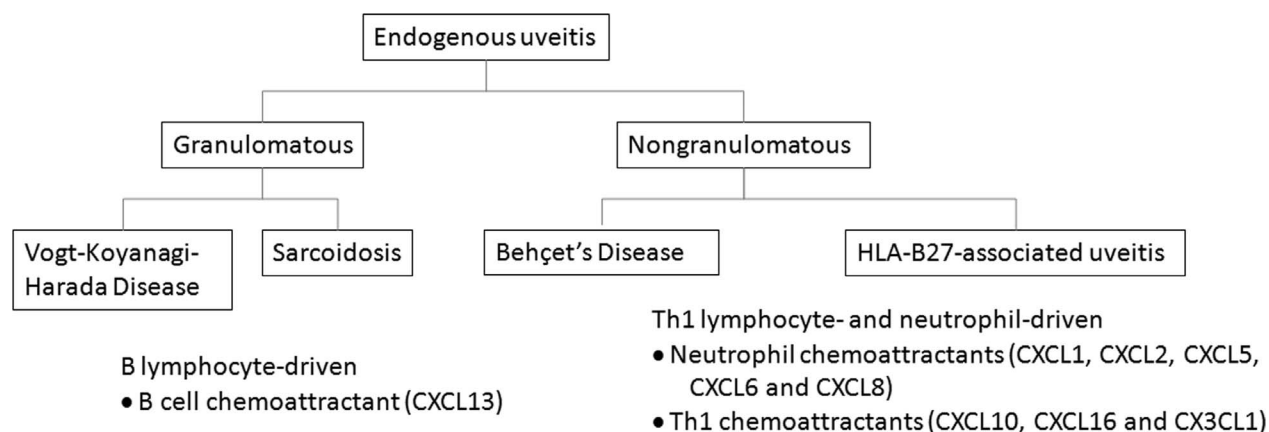
FIGURE 2. Suggested immunopathogenic mechanisms and specific uveitic entities.

inflammatory biomarker in patients with granulomatous uveitis associated with VKH disease and that B lymphocytes may be pathogenetically important in this uveitic entity. Therefore, treatment with rituximab, an antibody against CD20 leading to depletion of B lymphocytes, might be effective in patients with VKH disease.

The limitations of this study are the relatively small number of patients and that analysis of the AH samples was performed at one time point during the course of the disease. Animal models are needed to explore the expression of these chemokines at different time points. Nevertheless, with the use of a multiplex assay, we analyzed AH samples from patients with several specific clinical entities of endogenous uveitis in parallel allowing the measurement of many chemokines simultaneously in the same samples. Further studies with larger patient numbers are needed to determine the optimal cutoff values for chemokine levels that can be used to distinguish patients with nongranulomatous uveitis from patients with granulomatous uveitis. The identification of reliable biomarkers for these subsets would be useful as a tool to predict uveitis phenotypes and for the selection of therapy.

In conclusion, we demonstrated that the inflammatory chemoattractant profiles differ depending on the cause of uveitis. We identified novel biomarkers for different phenotypes of uveitis, some of which might serve as potential targets for selective therapy. Our findings suggest that inflammation mediated by neutrophils and Th1 cells appears to be more potent in nongranulomatous uveitis associated with BD and HLA-B27–related inflammation when compared with granulomatous uveitis associated with sarcoidosis and VKH disease. Our findings also suggest that B lymphocytes contribute to the inflammatory process in granulomatous uveitis, particularly in VKH disease (Fig. 2).

Acknowledgments

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