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Increased vascular endothelial growth factor levels in aqueous humor and serum of patients with quiescent uveitis

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PURPOSE. *Vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) are angiogenic mediators that share a significant proinflammatory activity. Both substances have been suggested to play a key role in uveitis pathogenesis. The authors analyzed VEGF and IL-8 levels in the aqueous humor and serum of patients with different types of uveitis during a quiet phase of the disease.*
METHODS. *Thirteen patients with intermediate uveitis, uveitis associated with ankylosing spondylitis, Vogt-Koyanagi-Harada disease, Fuchs uveitis syndrome, idiopathic chronic anterior uveitis, or Behçet disease, as well as 10 normal matched subjects, were included in the study. VEGF and IL-8 concentrations were measured in aqueous humor and serum by enzyme-linked immunosorbent assay.*
RESULTS. *VEGF levels were significantly higher in both the aqueous humor and serum of patients with uveitis as compared with controls. IL-8 concentrations in aqueous humor were significantly higher in patients with uveitis with extraocular manifestations than in those with eye-limited disease.*
CONCLUSIONS. *These findings suggest that VEGF plays a role in uveitis pathogenesis even during inactive disease and that IL-8 levels are significantly influenced by the presence of uveitis-associated extraocular changes. (Eur J Ophthalmol 2007; 17: 938-42)*

KEY WORDS. *Uveitis, Vascular endothelial growth factor, Interleukin-8*

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INTRODUCTION

Uveitis is a spectrum of inflammatory eye diseases characterized by the intraocular infiltration of T lymphocytes, monocytes/macrophages, and neutrophils (1). The pathogenesis of the various forms of uveitis is far from being elucidated.
Vascular endothelial growth factor (VEGF) is a powerful angiogenic substance produced by endothelial cells, activated T lymphocytes, and macrophages (2). In addition to promoting angiogenesis, VEGF significantly increases vascular permeability and is associated with inflammation and immune-mediated pathology (3, 4).
VEGF is upregulated on retinal capillary endothelial cells and Müller glia in different ocular diseases, including diabetic retinopathy, age-related macular degeneration, and retinopathy of prematurity (5). Interestingly, VEGF is overexpressed in the retina of Lewis rats with experimental autoimmune uveitis (EAU) (6, 7). VEGF concentrations have also been reported to be significantly increased in the plasma of patients with Behçet disease (BD) (8, 9) and in the aqueous humor (AH) of patients with uveitis with associated cystoid macular edema (CME) (10).
Interleukin-8 (IL-8), a member of the CXC chemokine fam-

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ily, is produced by monocytes/macrophages, T cells, granulocytes, and endothelial cells. Similarly to VEGF, IL-8 not only promotes angiogenesis but also displays proinflammatory activity, which is mainly mediated by its chemoattractant properties (11). In a rabbit model of uveitis, IL-8 has been shown to play a key role in the pathogenesis of intraocular inflammation (12-14). In humans, high levels of IL-8 have been detected in the AH of patients with acute anterior uveitis (13) and in both the AH and plasma of patients with BD or Vogt-Koyanagi-Harada (VKH) disease (15, 16).

To better define the roles of VEGF and IL-8 in the pathogenesis of uveitis, we assayed both factors in the AH and serum of patients with various forms of uveitis in the presence or absence of extraocular manifestations. To obtain data possibly relevant in the actual clinical setting, we selected patients under treatment with topical and/or systemic steroids and, therefore, in a quiet phase of the disease.

MATERIALS AND METHODS

Patients and control subjects

Thirteen patients (6 male, 7 female) with uveitis undergoing cataract surgery were included in the study. The mean age was 56 years (range, 25 to 64 years). Of the 13 patients, 3 had intermediate uveitis (IU), 3 had VKH, 3 had Fuchs uveitis syndrome (FUS), 2 had idiopathic chronic anterior uveitis (ICAU), 1 had anterior segment (AS) uveitis, and 1 had BD. All patients included in this study had inactive uveitis. Four subjects were on both systemic and topical steroid treatment, whereas nine were on topical steroid treatment only. All patients received high doses of topical steroid for 3 days prior to surgery. Other complications associated with uveitis, including posterior synechia and CME, were not detected in any of the patients. Uveitis-associated extraocular manifestations were in a remission phase in all studied patients. The control group comprised 10 patients matched for gender and age undergoing elective cataract extraction with no prior history of uveitis or chronic inflammatory disease. All demographic and clinical characteristics of studied subjects are summarized in Table I. All patients voluntarily participated and gave written informed consent for the use of their AH and blood in this specific study. Institutional Review Board (IRB)/Ethics Committee approval was obtained.

Collection of AH and serum

AH (100 to 200 µL) was aspirated from each patient or control subject by means of limbic paracentesis under a surgical microscope immediately before the microincision procedure. Blood samples were collected from a peripheral vein in all subjects, and serum was obtained by centrifugation. All samples were snap frozen and maintained at –70 °C until use.

VEGF and IL-8 assays

VEGF and IL-8 concentrations were measured by enzyme-linked immunosorbent assay (ELISA; Quantikine Human VEGF or IL-8 Immunoassays; R&D Systems Europe Ltd., Abingdon, UK). The procedure was carried out in accordance with the manufacturer's guidelines. The lowest detection limits were 5.0 pg/mL for VEGF and 1.5 pg/mL for IL-8.

Statistical analysis

Statistical analysis was performed using SPSS 11.01 software (SPSS Inc., Chicago, IL). Data were analyzed using the nonparametric Mann-Whitney test, the Wilcoxon matched pair test, and the Spearman rank correlation test. Results were expressed as median, 25th percentile, and 75th percentile (interquartile range). The differences were considered significant if the p value was <0.05.

RESULT

VEGF and IL-8 concentrations

VEGF concentrations (median and interquartile range) in AH and serum of patients with uveitis were 118.2 pg/mL (77.8–668.1 pg/mL) and 321.4 pg/mL (269.7–607 pg/mL), respectively (AH vs serum: $p < 0.05$). VEGF concentrations in AH and serum of control subjects were 83.3 pg/mL (36.3–93.9 pg/mL) and 163.8 pg/mL (134.6–250 pg/mL), respectively (not significant). VEGF concentrations in both AH and serum were significantly higher in uveitis patients than in controls ($p < 0.05$ and $p < 0.01$, respectively).

IL-8 concentrations in the same patients were 718.0 pg/mL (409–5206 pg/mL) and 18.2 pg/mL (5–64.46 pg/mL) in the AH and serum, respectively (AH vs serum: p

TABLE I - VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND INTERLEUKIN-8 (IL-8) LEVELS IN PATIENTS WITH UVEITIS AND CONTROL SUBJECTS

Subject no.	Age (yr)	Sex	Uveitis	Extraocular manifestations	Treatment	AH IL-8 VEGF (pg/mL)	Serum VEGF (pg/mL)	AH IL-8 (pg/mL)	Serum IL-8 (pg/mL)
Pt.1	57	M	IU	—	TS	51	824	480	5
Pt.2	62	M	IU	—	TS	55	184	43	5
Pt.3	25	F	IU	—	TS	70	338	718	31
Pt.4	59	M	FUS	—	TS	195	259	1763	5
Pt.5	62	F	FUS	—	TS	118	304	416	5
Pt.6	60	F	FUS	—	TS	85	180	5.0	38.4
Pt.7	57	M	VKH	Auditory disturbances, alopecia	TS + SS	102	1121	5720	5
Pt.8	52	F	VKH	Vitiligo, poliosis	TS + SS	86	668	4832	107
Pt.9	64	F	VKH	Meningismus	TS + SS	165	280	5580	288
Pt.10	61	F	ICAU	—	TS	146	434	402	0
Pt.11	55	M	ICAU	—	TS	158	304	622	76
Pt.12	60	M	AS-uveitis	Ankylosing spondylitis	TS	434	546	768	52
Pt.13	66	F	BD	Arthritis, skin lesions	TS + SS	668	ND	6040	ND
Ctrl.1	54	M	NA	NA	NA	75	117	880	5
Ctrl.2	48	M	NA	NA	NA	40	163	2424	13
Ctrl.3	61	M	NA	NA	NA	97	787	558	5
Ctrl.4	59	F	NA	NA	NA	94	239	690	37
Ctrl.5	55	F	NA	NA	NA	86	227	7360	32
Ctrl.6	47	M	NA	NA	NA	80	261	5020	76
Ctrl.7	62	F	NA	NA	NA	28	164	256	20
Ctrl.8	54	M	NA	NA	NA	93	152	2536	56
Ctrl.9	44	F	NA	NA	NA	32	158	8460	94
Ctrl.10	66	F	NA	NA	NA	88	45	5	48

AH = Aqueous humor; IU = Intermediate uveitis; TS = Topical steroids; FUS = Fuchs uveitis syndrome; VKH = Vogt-Koyanagi-Harada disease; SS = Systemic steroids; ICAU = Idiopathic chronic anterior uveitis; AS-uveitis = Aankylosing spondylitis-associated uveitis; BD = Behçet disease; ND = Not done; NA = Not assessable

< 0.01), whereas IL-8 concentrations in controls were 1652 pg/mL (407–6190 pg/mL) and 34.8 pg/mL (9.2–247.1 pg/mL) in AH and serum, respectively ($p < 0.01$). IL-8 levels in both AH and serum did not differ between uveitis patients and control subjects.

An analysis of VEGF and IL-8 levels performed in different uveitis subgroups revealed that the highest AH IL-8 concentration was found in the three patients with VKH (4832–5720 pg/mL) and in the single patient with BD (6040 pg/mL). A comparison of uveitis patients with (VKH, AS uveitis, or BD) or without (IU, FUS, or ICAU) extraocular manifestations revealed that AH IL-8 concentrations were significantly higher in those with extraocular manifestations than those with eye-limited disease (median: 5580 pg/mL vs 425 pg/mL, $p < 0.05$). Differences in AH VEFG, serum VEGF, and serum IL-8 between patients with or without extraocular manifestations did not reach statistical significance (AH VEGF: 165 pg/mL vs 101 pg/mL; serum VEGF: 607 pg/mL vs 304.7 pg/mL; serum IL-8: 80 pg/mL vs 18.7 pg/mL). No significant correlation between IL-8 and VEGF levels in AH or serum in the studied population was found (not shown). Individual values of both VEGF and IL-8 of all studied subjects are represented in Table I.

DISCUSSION

In the present study, we found that VEGF levels were significantly higher in both the AH and serum of patients with uveitis as compared with control subjects. The finding that VEGF levels were significantly increased in the AH of patients with uveitis, even in the absence of CME, further underlines the role of this factor in the pathogenesis of human uveitis. We also found that the median of the AH VEGF levels varied greatly among the different types of uveitis. It was relatively low (<100 pg/mL) in all IU patients; higher (>100 pg/mL) in the majority of patients with FUS, VKH, or ICAU; and very high (>400 pg/mL in two patients, one with AS-associated uveitis and one with BD

(Tab. I). Therefore, the finding that the variations in VEGF levels among the different study groups were quite high suggests that the production of this cytokine strictly depends on both pathogenesis and primary anatomic manifestations of the uveitis. Further study involving a larger number of patients is warranted to clarify this important issue. Because uveitis is rarely associated with neovascularization and none of our patients had signs of neoangiogenesis, it is tempting to speculate that VEGF could play a major role in inducing and/or enhancing intraocular inflammation rather than acting as an angiogenic factor. Notably, VEGF overexpression observed in EAU occurs in the absence of neovascularization (7). It is of interest that all of our patients were under treatment with topical and/or systemic steroids and had quiet phase ocular disease, further emphasizing the possible role of VEGF in modulating the exacerbating/remitting phases commonly observed in intraocular inflammatory diseases.

The second relevant finding that emerged from our study is that IL-8 levels in both the AH and serum of patients with uveitis, although not significantly different from controls, were significantly higher in patients with extraocular involvement than those with eye-limited disease. This finding suggests that this chemokine might play a differential role in the pathogenesis of these two groups of uveitis.

In conclusion, we suggest that VEGF and IL-8 play a differential role in uveitis, depending on the type of uveitis considered. One limitation of our study is the small number of considered patients, and further investigation with more homogeneous study populations is warranted. Data obtained from a larger study might also indicate whether anti-VEGF drugs (17) and/or chemokine receptor antagonists (18) could be effectively used in the near future for the treatment of the different forms of uveitis, even in the inactive form of the disease.

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