Bratisl Lek Listy 2008; 109 (12)
551 – 554

CLINICAL STUDY

Cytokine profile in Behçet uveitis

Selim Nalbant[1], Burak Sahan[1], Murat Durna[2], Dilaver Ersanli[2], Mustafa Kaplan[1], Ozlem Karabudak[3], Melih Unal[2]

Department of Internal Medicine, GATA Haydarpasa Training Hospital, Istanbul, Turkey.
nalbantselim@hotmail.com

Abstract: *Objectives:* Investigate the role of cytokines in Behçet uveitis.
Background: Cytokines may take part in this pathogenetic mechanism. Elevated and/or altered levels of specific cytokines have been found in patients with Behçet's disease in many studies.
Methods: Twenty patients with Behçet uveitis and 20 patients with Behçet's disease without uveitis were included to the study in compliance with International Study Group Criteria. Twenty non-Behçet uveitis patients were included in the study. In this group, active uveitis was found in 30 % and arthritis in 35 % of patients. Serum levels of cytokines (Interleukin-2-4-6-8, IL-2-4-6-8), tumor necrosis factor-alpha, TNF-α) and local growth factor (vascular endothelial growth factor, VEGF) were measured.
Results: Levels of all cytokines were highest in Behçet patients without uveitis except for IL-2 and IL-6. These cytokines were also highest in patients with non-Behçet uveitis too. Despite the tendency of this distribution of cytokines, only three cytokines, TNF-α, IL-8 and IL-4 were significantly different from the controls. The levels of TNF-α, IL-8 and IL-4 were significantly lower in patients with Behçet uveitis than in Behçet patients without uveitis. Interestingly, the levels of IL-2 and IL-6 were statistically similar in all groups. VEGF showed no significant difference between the studied and control groups. However, in contrast to cytokines, only the levels of VEGF were correlated with the activity of uveitis ($p<0.05$).
Conclusion: Consistent with previous reports of different immune effectors in Behçet uveitis, it may be suggested that its underlying immunopathogenesis may be much different from other causes of endogenous uveitis *(Tab. 3, Fig. 1, Ref. 20)*. Full Text (Free, PDF) *www.bmj.sk*.
Key words: Behçet's disease, uveitis, cytokines.

Behçet's disease is a chronic, relapsing, inflammatory disease characterized by recurrent oral aphthae and any of several systemic manifestations including genital aphthae, ocular disease, skin lesions, neurologic disease, vascular disease, or arthritis. The underlying cause of Behçet's disease is unknown. As with other autoimmune diseases, the disorder may represent aberrant immune activity triggered by exposure to an agent, perhaps infectious, in patients with a genetic predisposition to develop the disease (1, 2). Cytokines may take part in this pathogenetic mechanism. Elevated and/or altered levels of specific cytokines have been found in patients with Behçet's disease in many studies (3–5).

However, Behçet uveitis, a prototype of chronic persistent uveitis with necrotizing retinal vasculitis, is accompanied by multisystemic manifestations. Recent reports have demonstrated that the immune effectors in Behçet uveitis were distinguished from other causes of endogenous uveitis (1–3). However, the specific factors that affect the differences in infiltrating immune cells in Behçet uveitis remain unknown. Cytokines control the recruitment, homing, activation, and differentiation of specific types of immune effectors in the retinal inflammatory sites. Because cytokines can play cross-regulatory roles in inflammation, selective defects or imbalances of cytokine production are responsible for several immunopathologic conditions in uveitis. Improved understanding of the role of cytokines may provide new clues to immunopathogenesis and effective treatment of Behçet uveitis (1, 6–8).

Therefore, this study has been aimed at investigating the profiles of cytokine (Interleukin-2-4-6-8 (IL-2-4-6-8), tumor necrosis factor alpha (TNF-α) and local growth factor (vascular endothelial growth factor (VEGF)) in Behçet uveitis by comparing their levels in patients with Behçet's disease without eye involvement and patients with primer uveitis.

Methods

Patients and controls

This is a prospective experimental case-control study of cytokines serum levels from patients with uveitis due to Behçet's disease (study group) and two control groups (patients with non-

[1]Department of Internal Medicine, GATA Haydarpasa Training Hospital, Istanbul, Turkey, [2]Department of Ophthalmology, GATA Haydarpasa Training Hospital, Istanbul, Turkey, and [3]Department of Dermatology, GATA Haydarpasa Training Hospital, Istanbul, Turkey

Address for correspondence: Selim Nalbant, MD, GATA Haydarpasa Egitim Hastanesi Ic Hst. Srv. Selimiye Mah. Tibbiye Cad. 34668-Kadikoy, Istanbul, Turkey.
Phone: +90.216.5422020-3214, Fax: +90.216.3472114

Tab. 1. Demographic and clinical features of the patients.

	Behcet uveitis (n=20)	Behcet patients without uveitis (n=20)	Non-Behcet uveitis (n=20)
Age (years)	32.90±3.09	31.95±3.45	38.21±3.21
Gender (M/F)	12/8	10/10	11/9
Active Uveitis	8 (40 %)	–	6 (30 %)
Erythema nodosum	–	3 (15 %)	–
Vascular involvement	–	6 (30 %)	–
Arthritis	1 (5 %)	2 (10 %)	7 (35 %)
CNS involvement	–	1 (5 %)	–
Genital ulcers	6 (30 %)	8 (40 %)	–
Pathergy positivity	14 (70 %)	10 (50 %)	–

Tab. 2. Serum cytokine levels according to groups.

	Behcet uveitis (n=20)	Behcet patients without uveitis (n=20)	Non-Behcet uveitis (n=20)
IL-2 (pg/mL)	14.28±5.58	13.19±4.97	17.03±9.35
IL-4 (pg/mL)	63.58±25.80	81.44±115.86	57.54±38.74
IL-6 (pg/mL)	11.02±8.99	11.63±9.20	13.46±8.22
IL-8 (pg/mL)	137.31±74.28	169.22±67.50	108.25±19.62
TNF-α (pg/mL)	19.73±12.69	66.21±25.15	38.80±27.67
VGF (pg/mL)	61.13±15.85	76.93±35.78	53.85±12.85

Tab. 3. Comparison of the levels of the serum cytokines levels among the groups.

		Behcet patients without uveitis	Uveitis patients without Behcet disease
Behcet patients with uveitis	IL-2	0.8	0.6
	IL-4	0.03	0.01
	IL-6	0.4	0.1
	IL-8	0.01	0.04
	TNF-α	0.001	0.02
	VEGF	0.2	0.02

Behçet uveitis and patients with Behçet's disease without uveitis). Twenty patients with Behçet uveitis (M/F: 12/8, mean age: 32.90±3.09) and 20 patients with Behçet's disease without uveitis (M/F:10/10, mean age:31.95±3.45) were recruited in compliance with International Study Group Criteria (9) from GATA Haydarpa§a Military Medical Training Hospital from November 2004 to March 2006. All patients with Behçet's disease had oral ulcers. In the group of Behçet patients with uveitis, active uveitis was present in 40 %, arthritis in 1 %, genital ulcers in 30 %, positive pathergy reaction in 70 %. In the group of Behçet patients without uveitis, erythema nodosum was found in 15 %, vascular involment in 30 %, arthritis in 10 %, CNS involvement in 5 %, genital ulcers in 40 %, pathergy positivity in 50 %.

Twenty non-Behçet uveitis patients (M/F:11/9, mean age: 38.21±3.21) were included in the study as long as their cellular and protein inflammatory activity exceeded the value of 3 in the anterior chamber according to the criteria of the Standardization of Uveitis Nomenclature Working Group grading scheme (10). The patients of the latter group were diagnosed as follows: 1 psoriasis, 6 ankylosing spondylitis, 13 primer uveitis. In this group, active uveitis was found in 30 % and arthritis in 35 %.

The inflammatory activities in the anterior chamber and visual acuities were similar in both groups of patients with uveitis. Demographic and clinical features in enrolled patients are listed in Table 1. Helsinki and local approval was received from the GATA Military Medical School of Medicine Ethic Committee. Informed consent was obtained from all patients and controls subjects after the explanation of research purposes.

Detection of serum cytokines and local growth factor concentrations

Peripheral blood (10-ml samples) was obtained from each patient and centrifuged at 3,500 rpm to get serum. Serum specimens were deposited in an Eppendorf tube at -70 °C for subsequent processing until assayed for cytokines by enzyme-linked immunosorbent assay (ELISA). Cytokines (Interleukin-2-4-6-8, IL-2-4-6-8), tumor necrosis factor alpha, TNF-α and local growth factor, vascular endothelial growth factor (VEGF) in the serum were measured with commercially available sandwich ELISA kits (NIBSC, Hertfordshire, United Kingdom and Biotrak Amersham Bioscience, USA). To reduce the effect of other proteins in the specimens that might be inhibitory, every sample was diluted five- to 20-fold, depending on the cytokine. The minimum detectable concentrations of each cytokine were as follows: IL-2-4-6-8, TNF-α 1.5 pg/ml. In all assays, OD450 nm values obtained from diluent controls were subtracted to construct Standard curves. Each experiment was performed in duplicate, and mean values of determinations were used for final results.

Statistical analysis

Statistical analyses were performed by SPSS 12.0 software version (SPSS Inc, Chicago, Illinois, USA). Levels of each cytokine were compared by means of the Wilcoxon Signed Ranks Test. Correlation of cytokine levels with uveitis activity in Behçet's disease was shown by Annova Test and Regression Curve. *P* values below 0.05 were considered significant.

Results

Distribution of serum cytokines concentrations in study groups

Levels of all cytokines were highest in Behçet patients without uveitis except for IL-2 (14.28±5.58 vs 13.19±4.97 and 17.03±9.35, $p>0.05$) and IL-6 (11.02±8.99 vs 11.63±9.20 and 13.46±8.22, $p>0.05$), they were both highest in patients with non-Behçet uveitis (Tab. 2 and 3). Despite the tendency of this distribution of cytokines, only three cytokines, namely TNF-α, IL-8 and IL-4 were significantly different from the controls. The levels of TNF-α (19.73±12 vs 66.21±25.15, $p<0.05$) and IL-8 (137.31±74.28 vs 169.22±67.50, $p<0.05$) and IL-4 (63.58±25.80 vs 81.44±11.58) were significantly lower in patients with Behçet uveitis than in Behçet patients without uveitis. In contrast to levels of TNF-α (66.21±25.15 vs 38.80±27.67, $p<0.05$) in Behçet uveitis, levels of IL-8 (169.22±67.50 vs 108.25±19.62, $p<0.05$) and IL-4 (63.58±25.80 vs 57.54±38.74, $p<0.05$) were significantly higher than in non-Behçet uveitis group. Interestingly, levels of IL-2 and IL-6 were very close in all groups despite some small differences.

VEGF showed no significant difference between the study and control groups (61.13±15.85 vs 76.93±35.78 and 53.85± 12.85, $p>0.05$).

Association of serum cytokines and VEGF concentrations with the activity of the uveitis

We could not find any correlation of serum cytokines levels with the activity of uveitis, even for TNF-α and IL-8, which showed significant differences between the groups, ($p>0.05$). Interestingly, VEGF levels showing no significant difference between the groups gave us a significant correlation with the activity of uveitis ($p<0.04$) (Fig. 1).

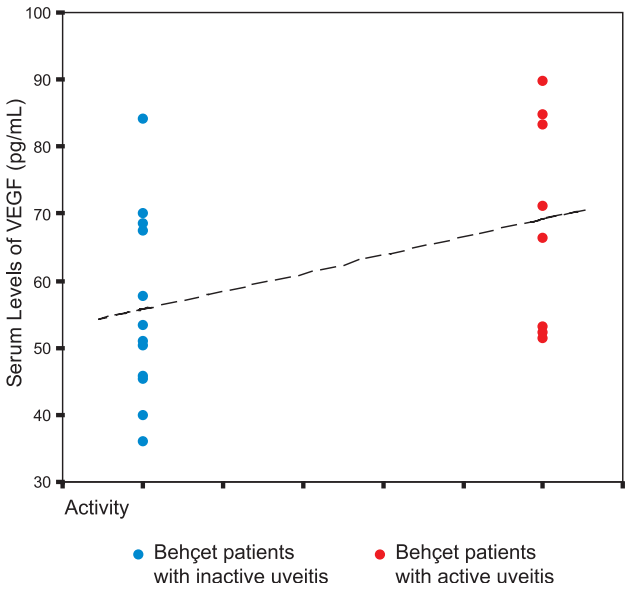

Fig. 1. Correlation of the serum VEGF levels and uveitis activity.

Discussion

The most prominent result of this study is that cytokine environment in the serum of patients with Behçet uveitis differs from that in the serum of patients suffering from either Behçet's disease without uveitis or uveitis based on other causes. Commonly, it is considered that uveitis is one of the severity criteria for Behçet's disease. Therefore, higher serum cytokine levels in patients with Behçet uveitis were expected according to the control groups. However, in contrast to this expectation, lower levels of IL-4-6-8, TNF-α and VEGF were found in patients with Behçet uveitis than in Behçet patients without uveitis, except for IL-2. However, only TNF-α and IL-8 were significantly lower in Behçet uveitis than in Behçet patients without uveitis. Therefore, these findings suggest that in addition to systemic inflammatory response there are additional immunopathogenic mechanisms that may play a role, such as local intraocular inflammatory mechanism in Behçet uveitis. These findings support the recent clinical evidence regarding the unfavourable effect of single anti-TNF therapy for Behçet uveitis (11–14).

In contrast to the levels of TNF-α, those of IL-8 were significantly higher in Behçet uveitis group than in non-Behçet uveitis group. IL-8 is a very strong chemoatractant cytokine that is secreted from neutrophils and macrophages, and believed to be involved in vascular part of inflammation (5). On the other hand, in the previous study, IL-8 level was detected as a more sensitive marker of disease activity, even more sensitive than erythrocyte sedimentation rate and C-reactive protein. High systemic levels of IL-8 may trigger some other intraocular inflammatory mechanisms. It may be speculated that Behçet's disease might be more systemic at IL-8 levels exceeding those in our study.

The immunoregulatory cytokine IL-4 has been reported to function as an important anti-inflammatory molecule in patients with uveitis (4, 15–18). Moreover, it may be suggested that intraocular IL-4 might influence the type and course of ocular inflammation in patients with uveitis (19). Our results gained from patients with Behçet uveitis showed lower levels of IL-4 when compared to Behçet patients without uveitis. This result may be in agreement with previous 4–6 clinical cases giving evidence that the therapeutic responses are positively correlated with the IL-4 positive T cells in patients with uveitis (11, 12). Moreover, it has been shown that depressed aqueous IL-10 levels (another anti-inflammatory cytokine) could in part contribute to persistent intraocular inflammation found in Behçet uveitis.

We could not find any differences between the studied and control groups for IL-2 and IL-6. This result is similar to previous

Behçet's studies. Ahn et all found out that aqueous humor in patients with non-Behçet uveitis showed levels of IL-2 to be higher than those in normal controls whereas the latter levels in patients with Behçet uveitis were the same as those in the control group. However, as reported before, levels of IL-2 and 6 may increase during the disease activity, the fact of which we have not studied (3).

Interestingly, the levels of VEGF correlated with uveitis activity despite being similar to those in the controls. VEGF is produced by macrophages, neutrophils and vascular endothelial cells and can alter the vascular permeability. Vascular endothelial dysfunction is one of the prominent features of the Behçet's syndrome. It has been previously demonstrated that proinflammatory cytokines (TNF-α, soluble interleukin-2 receptor, IL-6 and IL-8) play roles in the etiopathogenesis of Behçet's syndrome. Since VEGF expression is induced by these cytokines and VEGF itself is a potent stimulator of NO production with endothelial cell effects, it should have a very important role in the etiopathogenesis of the uveal involvement of Behçet's syndrome (20, 21).

In conclusion, Behçet uveitis leads to destructive ocular damage and is often resistant to conventional corticosteroid therapy.

Our results, may explain why uveal inflammation is often severe and intractable to medical therapy in patients with Behçet uveitis. Furthermore, consistent with previous reports of different immune effectors in Behçet uveitis (15, 16), it may be suggested that its underlying immunopathogenesis may be much different from other causes of endogenous uveitis. Therefore the latter detail as well as the fact that one-side cytokine blockage treatment could be insufficient, should be considered during the studies of anti-cytokine treatment of the Behçet's syndrome.

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Received August 10, 2008.
Accepted October 27, 2008.