Serum Soluble CD4 and CD8 Levels in Patients with Behcet's Disease

BEHÇETLİ HASTALARDA SERUM SOLÜBL CD4 VE CD8 SEVİYELERİ

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Summary

Several immunological abnormalities have been described in Behcet's disease (BD). Serum levels of soluble CD4 (sCD4) and soluble CD8 (sCDS) can reflect in vivo T cell activation status. The objectives of this study are to investigate the serum sCD4 and sCDS levels, and to search for a relationship of sCD4 or sCD8 with clinical and laboratory disease activity in BD.

Forty-four patients with BD and 20 apparently healthy controls were included in the study All patients fulfilled the International Study Group criteria for the diagnosis oj'BD. The patients with BD consisted of 25 males and 19 females (mean age, 34.1 ± 8.6, range 16-53). The healthy controls consisted of 8 males and 12 females (mean age, 32.2 ± 9.6, range 22-49). Twenty-four patients with BD had active and 20 patients had inactive disease. An ELISA (T Cell Diagnostics, Cambridge, MA) wus used to measure the serum sCD4 and sCD8.

Although there was no statistically significant difference between mean sCD4 levels of patients with BD and controls (p > 0.05), serum sCD8 levels in the patients with active BD was significantly increased as compared to that in the controls and in the patients with inactive BD (p<0.05 and p<0.05, respectively). High serum sCD8 levels correlated well with clinical disease activity, but there was no correlation between the laboratory activity criteria (erythrocyte sedimentation rate, C-reactive protein) and sCD4 or sCD8 levels. We have concluded that high sCD8 levels may reflect an immune activation state of'CD8+ T lymphocytes in BD.

Key Words: sCD4, sCDS, Behcet's disease

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Özet

Behçet hastalığında bir çok immünolojik bozukluk tanımlanmıştır. Solübl CD4 (sCD4) ve solübl CD8 (sCD8) ' in serum seviyeleri in vivo T hücre aktivasyon durumunu yansıtabilir. Bu çalışmada Behçet hastalığında serum sCD4 ve sCD8 seviyelerinin tayini ve bunların Behçet hastalığının klinik ve laboratuvar aktivite biterleri ile karşılaştırılması amaçlandı.

Çalışmaya 44 Behçet hastası ve 20 sağlıklı kontrol dahil edildi. Bütün hastalar International Study Group (ISG) tanı kriterlerini dolduruyordu. Behçet hastalarının 25' i erkek ve 19' u kadındı (ortalama yaş, 34.1 ± 8.6, yaş aralığı 16-53). Kontrol grubu ise 8 erkek ve 12 kadından oluşuyordu (ortalama yaş, 32.2 ± 9.6, yaş aralığı 22-49). Behçet hastalarının 24' ü aktif ve 20' si inaktifti. Serum sCD4 ve sCDS seviyelerini ölçmek için ELISA (T Celi Diagnostics, Cambridge) tekniği kullanıldı.

Behçet hastaları ve kontrol grubu arasında ortalama sCD4 seviyeleri yönünden istatistiki bir fark olmamasına rağmen (p > 0.05), aktif Behçet hastalarındaki serum sCD8 seviyeleri hem inaktif hastalardan hem de kontrol grubundan anlamlı olarak yüksek bulundu (sırasıyla, p<0.05 ve p<0.05). Yüksek serum sCD8 seviyeleri klinik olarak hastalık aktivitesi ile iyi korele idi, ancak sCD4 veya sCDS seviyeleri ile laboratuvar aktivite kriterleri (sedimentasyon hızı, C-reaktif protein) arasında bir korelasyon bulunamadı.

Sonuç olarak çalışmada, Behçet hasta/ığındaki yüksek serum sCD8 seviyelerinin CD8+ T lenfositlerin aktivasyon durumunu yansıtabileceği tartışıldı.

Anahtar Kelimeler: sCD4, sCD8, Behçet hastalığı

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CD4 and CD8 are functionally important molecules expressed on helpcr/inducer and cytotoxic/supressor T lymphocytes, respectively. The CD8 molecule serves as a receptor for class T MHC and CD4 serves as a receptor for class II MHC molecules.

Soluble forms of these molecules (sCD4 and sCD8) are produced upon activation of corresponding lymphocytes (1-5). Cell surface molecules can be shed by activated T lymphocytes and measured in serum to assess in vivo T cell activation. The

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level of sCD8 has been shown to correlate with CD8+ T cell activation both in vivo and in vitro (6).

Increased serum sCD4 and/or sCD8 have been reported in several diseases in which immunological mechanisms have been implicated in the pathogenesis (7-13). Delineating the relationship between these serological markers and the state of in vivo T cell activation has been complicated.

The functional roles of these soluble molecules are not entirely understood. The sCD8 could interfere with normal activation of lymphocytes by inliibiting the interaction of M H C class I molecules with membrane-bound CD8 and interfere with interactions of CD8+ CTL with their targets. Soluble CD8 molecule release is reported to occur by alternative splicing of mRNA resulting in a secretory protein lacking a transmembrane domain (14,15). The mechanism of CD4 release is not yet clear but it is likely that release occurs through proteolytic cleavage at the cell surface (16).

Immunophenotyping and immunohistology of T cell subsets can give valuable information about the numbers and distribution in pathological tissues but they do not provide functional information regarding the subsets.

Behcet's disease (BD) is a multisystem inflammatory disease with a yet unknown etiology. Although no microorganism has been consistently isolated from patients with BD, some investigators have been suggested the possible involvement of infectious agents including viruses and some bacteria (17-19). Changes in the numbers and proportions of T cell subsets have been reported in BD (20,21). CD4 T cell counts are generally reported to be slightly low but some uncertainty has been suggested about any changes in the proportion of CD8 cells (17). Subtyping of T lymphocytes in patients with BD has yielded conflicting results. Normal, elevated or decreased levels of T lymphocytes have been found in different studies (22-25). Lim et al. (24) reported significantly lower levels of helper T cells and a concomitant increase in supressor T cells. Victorino et al. (21) have found decreased levels of helper T but normal CD8 levels. Hamzaoui et al. (25) have found a decreased ratio of CD4/ CD8 cells. Perhaps the cells, although quantitatively different, may be functioning at different levels of activity.

The aim of this study is to measure serum sCD4 and sCD8 levels in active and inactive BD patients, compare the results with those of healthy controls and to search for a relationship with clinical and laboratory markers of disease activity.

Patients and Methods

Forty-four patients with BD, attending the Department of Immunology and multidisciplinary Behcet's Disease Center of hospital between February-October 1995 and 20 apparently healthy controls were included in the study. A ll patients fulfilled the International Study Group Criteria for the diagnosis of BD (26). The patients with BD consisted of 25 males and 19 females (mean age (± SD) 34.1 ± 8.6, range 16-53). The healthy controls consisted of 8 males and 12 females (mean age 32.2 ± 9.6, range 21-49). At the time of blood withdrawal, 24 patients (15 males,9 females; mean age 34 ± 9.4, range 16-53) with at least two of the following were considered as having active disease: oral ulcer, genital ulcer, eye lesions determined by an ophthalmologist, skin lesions, arthritis, pulmonary involvement, central nervous system involvement, gastrointestinal system involvement and vascular lesions. Twenty patients (10 males, 10 females; mean age 34.1 *± 7.6, range 19-49) showing no symptoms related with BD for at least 1 month prior to blood withdrawal were considered as having inactive disease. The clinical features of patients with active BD are shown in Table 1. The mean disease duration, defined as the interval in months between the diagnosis of BD and the time serum was collected was 39.6 ± 32.8 month (range 2-104).in the whole patient group, 40.3 ± 34.7 month (range 2-104) in active and 38.9 ± 30.9 month (range 4-103) in inactive BD patients. A ll patients with active disease were on. colchicine therapy (500-1500 mg/day). Moreover, 6 patients were taking corticosteroid at 40-60 mg/day dose and one patient was taking cyclosporin-A at 200 mg/day dose in active BD group. Eleven inactive patients were on colchicine therapy (500 mg/day) and 9 inactive patients did not take any drug.

Sera were stored at -20° C until use.

Serum sCD4 and sCD8 concentrations were determined by a sandwich ELISA (T Cell Diagnostics, Cambridge, MA) using two murine

Table 1. Clinical features of patients with active Behcet's disease.

Clinical features	n=24	%
Oral ulcer	22	91.7
Genital ulcer	14	58.3
Skin lesions	19	79.2
Eye lesions	8	33.3
Joint involvement	10	41.7
Thrombosis	3	12.5
Pulmonary involvement	2	8.3
CNS involvement	2	8.3
(SIS involvement	1	4.2

CNS .Central nervous system GIS: Gastrointestinal system

monoclonal anti-CD4 and anti-CD8 antibodies recognizing different epitopes of the CD4 and CDS molecules. The procedures suggested by the manufacturer were followed without any modification and the optical absorbance values were read on an ELISA reader at a wavelength of 490 nm. A11 samples were tested in duplicate and, sCD4 and sCD8 levels were calculated by comparing the mean absorbance of duplicate samples with that of the standard curve. Serum sCD4 and sCD8 levels are given as U/ml (one unit is defined by the manufacturer as the amount of CD4 or CDS found in 10^3 Jurkat T cells lysed with 1 % NP40). The mean sCD4 value of normal human sera determined by this kit is given as 8.1 U/ml (range 0-18 U/ml) with a kit sensitivity of 1.1 U/ml. The mean sCD8 value of normal human sera determined by this kit is given as 336 U/ml (range 138-533 U/ml) with a kit sensitivity of 50 U/ml.

Erythrocyte sedimentation rate (ESR) was determined by the Westergren method. C-reactive protein (CRP) was measured by nephelometry (Behring).

Statistical analyses were performed by student T test and Mann-Whitney U test.

Results

Serum sCD4 levels among BD patients were not significantly different than the normal controls (mean ± SD, 10.6 ± 4.5 vs 10.9 ± 3.3, p>0.05). The amount of serum sCD8 in the patients with active disease was significantly increased as compared to that in the controls (326.7 ±112.9 vs 226.3 ± 90.4, p<0.05) and in the patients with inactive disease (326.7 ± 112.9 ± 257.9 ± 92.2, p<0.05). Although there was statistically significant difference between mean ESR and CRP levels of patients active and inactive BD (pO.OOl and pO.OOl, respectively), serum sCD4 and sCD8 levels did not correlate with laboratory indices of inflammation such as ESR and CRP (p>0.05) (Table 2).

No clinical or laboratory features distinguished the patients with markedly elevated sCD8 levels.

Discussion

Delineating the relationship between these serological markers and the state of in vivo T cell activation has been complicated due to their natural fluctuations and the impact of various therapies. Various drugs taken by our patients may well influence the T cell activation, cell surface marker expression and clearance of soluble markers. The significance of any changes in marker levels can be rather speculative (27).

Low levels of sCD4 have been described in normal healthy individuals in previous studies (16)

Table **2.** Serum sCD4, sCD8, ESR and CRP levels in patients with Behcet's disease and healthy controls.

	Active BD (n=24) *Mean ± SD*	*Inactive BD (n=20)* *Mean ± SD*	*Controls (n=20)* *Mean ± SD*
sCD4 (U/ml)	(1 1.8 ± 5.4) [a,b]	(9.2 ± 2.7) [b]	(10.9 ±3.3)
sCD8 (U/ml)	(326.7 ± 112.9) [c,e]	(257.9 ±92.2)	(226.3 ± 90.4)
ESR (mm /h)	(64.4 ± 28.6)[e]	(11.1 ±6.3)	
CRP(mgAJI)	(31.8 ± 29.2)[e]	(2.5 ± 1.6)	

a : p>0.05 when compared with healthy controls, **b** : p>0.05 when compared with inactive BD.
c : p< 0.05 when compared with healthy controls, **d** : p< 0.05 when compared with inactive BD.
e : p<0.001 when compared with inactive BD.

and similar levels were found in our control population. At present little is known about the kinetics and magnitude of soluble molecule release after in vivo activation, the clearance of these molecules from the circulation, as well as the influence of therapy and disease activity on these properties (28). '

Further studies investigating in vitro sCD8 production by PHA-stimulated peripheral blood mononuclear cells derived from patients with BD arc essential to suggest that elevated sCD8 levels are due to increased CD8+ T cell activity.

In the present study we did not investigate the numbers of CD4 and CD8 positive cells. Our study is a cross-sectional work and we did not make prospective sequential studies of individual patients.

Symons et al.(29) have found that sCD8 levels were high in patients with active rheumatoid arthritis. As patients' disease activity diminished, so serum sCD8 levels fell into the normal range. However, as patients passed into clinical remission the sCD8 levels exhibited a secondary rise that was maintained until discharge from hospital. In a second group of patients who, following initial clinical improvement, exhibited a subsequent clinical relapse, serum sCD8 levels again showed an initial increase as the patients improved. However, in these patients serum sCD8 began to fall and in each case a subsequent clinical exacerbation occurred. In both groups the changes in scrum sCD8 preceded the changes in clinical status suggesting that this was not a secondary event reflecting clinical disease activity but was more likely to be related to the activation of immunopathogenic mechanisms that produce inflammation. Although admission levels of sCD8 were high, in general a rising scrum sCD8 was associated with onset of clinical remission whereas falling levels was associated with the onset of clinical exacerbation (29). Tumor necrosis factor-alpha (TNF-oc) has been shown to potentiate CD8+ T cell function in vivo and in vitro (30,31).

An increased level of sCD8 has been detected in patients with measles, infectious mononucleosis and HIV-infected populations which suggests that high sCD8 levels might reflect virus infection (7,8,32). In measles sCD8 levels tended to increase only when the rash appeared and quickly subsided after the disappearance of this rash (8). On the contrary however, high levels of sCD8 persisted for years in HIV-infected populations (7). Increased levels of sCD8 levels in patients with Behcet's disease might be due to a chronic infection by an as yet unidentified virus.

High levels of sCD8 correlated well with disease activity in BD. Traditional laboratory indices of disease activity in BD have been some tests such as erythrocyte sedimentation rate, C-reactivc protein, complement components 3 and 4 all of which reflect acute phase response. These laboratory markers of inflammation do not shed any light on the immunopathogenic mechanisms of the underlying specific disorder. sCD4 and /or sCD8 measurement can help to assess the in vivo immune system activation in several inflammatory and immunological diseases along with a potential to provide new insights in to the etiopathogenesis of such disorders.

The biological and immunological functions of the sCD4 and sCD8 molecules are not yet well understood. sCD4 and sCD8 molecules have been demonstrated to retain the ability to bind their corresponding MHC molecules (33,34). These soluble molecules may stimulate or inhibit the interaction of CD8 T cells with their target cells and CD4 T cells with APCs leading to aberration of CD8 or CD4 T cell activation or function. However, it is shown in vitro that recombinant sCD4 protein cannot inhibit class Il-specific T cell interactions (35). These suggest that sCD4 is not likely to be an immunorcgulatory molecule. If sCD8 retains the ability to bind to class I MHC molecules, it may inhibit the interaction of CD8+ T cells with their antigen bearing cells leading to a down regulation of CD8+ T cell activation or function (29).

Conclusion

In conclusion, this study showed that in patients with BD, serum levels of T cell activation marker sCD8 but not sCD4 were increased. This may reflect an immune activation state of CD8+ T lymphocytes in BD. As sCD4 levels did not differ from controls, high sCD8 levels can not solely reflect a generalized immune system activation.

Further studies will help to delineate the immunological events in inflammatory diseases

that produce elevations of sCD4 and/or sCD8 levels.

The level of sCD4 and sCD8 in serum may be important in monitoring or characterizing disease processes and may provide insight in to the immunoregulation of cell growth and cell differentiation.

IL-2R, TNF-alpha and gamma-IFN levels were increased in patients with BD (36,37). These results suggest that the immunological abnormalities observed in patients with BD may be related to abnormal responses in T cells and monocyte/macrophages. Increased levels of sCD8 levels in patients with Behcet's disease might be due to a chronic infection by an as yet unidentified virus.

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