Research

Humoral Immune Response in Behçet's Disease: Cross Sectional Study

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Abstract

Background: The etiology and pathogenesis of Behçet's Disease (BD) remain unknown. The primary aim of this study was to determine the relation between humoral immunity and BD. We especially evaluated humoral immunity in Behçet's patients with mucocutaneous involvement.

Material and Methods: The study population comprised of 50 patients with BD and 53 healthy controls. Plasma IgE, IgG, IgM, IgA, C-reactive protein (C-RP), anti-streptolysin O titers (ASO), and erythrocyte sedimentation rate (ESR) were measured for patients and control group.

Results: The mean ASO, C-RP and ESR levels were significantly higher in Behçet's patients than in control subjects. The mean IgA level in BD was significantly higher than in control subjects (268 ± 16.5 mg/dl vs 172,9 ± 16 mg/dl respectively p<0.001). The IgG and IgM levels were also significantly higher than that of control subject. There was no significant IgE level difference in study groups.

Conclusion: We have shown that humoral immune response has a role in patients with BD being highest in IgA, and being mild in Ig G and IgM levels. We thought that the high significance relation with IgA level in groups may be due to our patients clinical presentations because all of them had mucocuteneous involvement.

Introduction

Behçet's disease (BD) was first defined in 1937 by Hulusi Behçet as a triad of recurrent aphthous stomatitis, genital ulceration, and hypopyon iridocyclitis. Thereafter, arthritis, thrombophlebitis, central nervous system disease, positive pathergy test and gastrointestinal ulceration have been included in the clinical manifestation of the disease [1, 2].

The etiology and pathogenesis of BD remain unknown. The diagnosis is based on the clinical findings [1, 3]. Immunological investigations have demonstrated the presence of immune dysregulation among the patients with BD [4, 5].

Currently there are not defined laboratory markers that correlate well with clinical activity of BD. We wanted to eveluate the humoral immune response and inflammatory markers in Behçet's patients and to determine the possible association with activity of BD or BD itself. Accordingly, we aimed to assess and compare inflammatory markers and immunoglobulin (Ig) levels in patients with BD and control subjects.

Materials and Methods

The study was conducted on consecutive 50 patients with BD who followed at outpatient clinics of Dermatology and 53 healthy controls between July 2006 and July 2007 in Ankara Hospital, and Izzet Baysal Medical Faculty Hospital. All Behçet's patients were taking topical steroids and oral colchium. Control group was recruited from the hospital staff after having a questionnaire to rule out possible inflammatory or infectious diseases. The people who have parameters affecting inflammatory response were excluded from control group. All patients and controls provided informed consent to participate, in accordance with the ethical principles for human investigations, as outlined in the Second Helsinki Declaration. A complete medical history and a physical examination were recorded and performed for each patients and subjects. All the participants underwent routine biochemical and hematological measurements. The diagnosis of BD was based on the criteria of the International Study Group [**6**]. We used a standardized proforma [Behçet's Disease Current Activity Form (BDCAF)] to assess disease activity, based on history of clinical features [**7**]. We used BDCAF in patients to determine the disease activity. All venipunctures were carried out without interruption of venous flow and using a 19-gauge butterfly needle connected to a plastic syringe. All samples were transferred to the laboratory and, studied for the above-mentioned variables. Serum Immunoglobulin levels were measured by the radial immunodifusion technique in plates containing agar mixed with monospesific antiserum. Plasma IgE (normal, 0-87 IU/ml), IgG (normal, 7-16 mg/dl), IgM (normal, 0,4-2,3 mg/dl), IgA (normal, 0,7-4 mg/dl), C-reactive protein (C-RP) (0-3.19 mg/l), anti-streptolysin O titers (ASO), and erythrocyte sedimentation rate (ESR) were analyzed for each patients and control subjects.

Results of peripheral blood smear and microscopic stool examination were also analyzed, the latter performed at least three times for each patient having high IgE levels to rule out parasitic disease. Blood samples were withdrawn from large antecubital veins. Patients and controls having any accompanying disease, or otherwise having any condition that might lead to a rise in plasma IgE levels, having increased eosinophil count either by blood count or peripheral blood smear, or having any parasitic infection were excluded.

Statistics

All data were analyzed using Statistical Package for the Social Sciences (SPSS) (version 13.0) for *Windows* (SPSS). Categorical variables were presented as percentage and continuous variables as mean±sd. Categorical variables and continuous variables were compared by chi-square test and unpaired t test and *Mann-Whitney* U test respecti-vely. Analysis of variance and T*ukey* post hoc test was performed to compare variables between control subjects, active and inactive patients with BD. A p value (two sided) <0.05 was considered to be statistically significant. Power analyse yielded a sample size of 40 with 90% power, at the alpha level of 0.05.

Results

The mean age and gender of the patients and control subjects are presented in **Table 1**. Thirty seven patients (74 %) were defined as in the active state of disease. Frequency of BD' manifestations were as follows in active group: aphthous ulcers in all patients (100 %), genital ulcers in 34 patients (92 %), erythema nodosum in 20 patients (54.1 %), patergy positivity in 19 patients (51.4 %), ocular findings in 10 patients (27 %), papulopustular lesions in 10 patients (27 %). Recurrent oral aphthous ulcers were the unique finding in inactive group. The mean ASO, C-RP and ESR levels were significantly higher in Behçet's patients than in control subjects, and these parameters did not show any significant differences in respect to active and inactive state of the disease (**Table 1**). The mean IgA level in BD was significantly higher than in control subjects (268 ± 16.5 mg/dl vs 172,9 ± 16 mg/dl respectively p<0.001). Additionally the mean IgA levels of the control subjects were significantly lower than that of both in the active and inactive state of the disease and there was no statistically significant difference between the active and inactive state of the disease (**Table 1**). The mean IgM level was 101.8 ± 8.7 mg/dl in control group and 126.4 ± 8.98 mg/dl in Behçet's patient group (p=0.048). The mean IgM level was 104.3 ± 17.52 mg/dl in inactive group and 134.19 ± 10.38 mg/dl in active group (p<0.056). The IgG levels of patients with BD was significantly higher than that of control subject (p=0.049). There were no significant IgE level difference between control, active and inactive Behçet's patients groups (P>0.05).

Discussion

There are several main findings in our study; first inflammatory markers such as ASO, CRP and immunoglobulins namely IgG, IgA levels were significantly higher in BD than in con-

Table 1. Baseline Characteristics, and Immunoglobulin Levels of Patients and Control Subjects

Active (n=37, 74%) Inactive (n=13, 26%)	Behçet's Disease (n=50)	Control Group (n=53)	P value^a
Age (years){Active}[Inactive]	33.5±9 {32±8} [38±10]	32±13	>0.05
Gender (Female){Active}[Inactive]	30 (60%) {22(59%)} [8 (61%)]	29 (58%)	>0.05
Gender (Male){Active}[Inactive]	20 (40%) {15 (75%)} [5 (25%)]		
ASO (IU/l){Active*}[Inactive]	177±99 {192±123§} [136±65§]	83±54	<0.001
CRP (mg/l){Active*}[Inactive]	5.2±10.1 { 5.7±11.3} [3.8±5.2]	2.5±5.4	0.025
ESR (mm/h){Active*}[Inactive]	26.9±19.7 {28.9±19§} [21.2±20]	12.8±7.3	0.001
IgM (mg/dl){Active}[Inactive]	126.4±9 {134±1.7*} [104±5]	101.8±1.2	0.048
IgG (mg/dl){Active}[Inactive]	1248±460 {1270±68} [1186±170]	1073±62	0.029
IgE (IU/ml){Active}[Inactive]	82.4±18 {35.8±6.5} [98.8±33]	57.7±7.6	0.163
IgA (mg/dl){Active}[Inactive]	268±16.5 {286±32§} [261±19§]	172.9±16	<0.001

§ vs control group p<0.01, *vs control group p=0.056, §,* analysis of variance, a unpaired t test or *Mann-Whitney* U test

trol subjects. Second, although inflammatory markers did not differ between the active and inactive state of disease, only IgA levels both at the active and inactive state of the BD was significantly higher than that of control subjects. Additionally IgG levels in Behçet group were also significantly higher than that of control subjects. IgM levels also tended to be higher than control subjects with a borderline statistical significance.

BD is a chronic multisystem disorder with unpredictable exacerbations and remissions. Although infectious agents, immune mechanism, and genetic factors are implicated, etiopathogenesis of the disease remains to be elucitaded. The pathology of the lesion consists of widespread vasculitis. Various manifestations of BD occuring unpredictably during the disease course and not linked to any previous risk factor may be associated with different organ-specific antigens or genetic predispositions [1].

Recent developments in the immunopathogenesis of BD reveal that both innate and adaptive immune systems are activated in BD, with a proinflamatory and Th1-type of cytokine profile. BD may be linked to a specific, primary immune abnormality with a genetic mutation affecting an adhesion molecule or a proinflamatory cytokine, which predisposes to early or more intense neutrophil and T cell responses [3].

BD is characterized by the presence of serum antibodies directed against oral and other mucosal epithelial cells. A humoral response against vascular tissues has also been implicated in the pathogenesis of BD. Antibodies to endothelial cell antigens (AECA) have been shown to correlate with disease activity in BD. These antibodies belong predominantly to the IgM and to a lesser extent IgG class of antibodies [8]. IgA, as the major class of antibody present in the mucosal secretion of most mammals, represents a key first line of defence against invasion by inhaled and ingested pathogens at the vulnerable mucasal surfaces. More IgA is produced in mucosal linings than all other types of antibody combined. IgA is also found at significant concentrations in the serum of many species, where it functions as a second line of defense mediating elimination of pathogens that have breached the mucosal surface. Because it is resistant to degradation by enzymes, secretory IgA can survive in harsh environments such as the digestive and respiratory tracts, to provide protection against microbes that multiply in body secretions [9,10].

One of the most striking finding in our study is that IgA level both in active and inactive state of the disease is higher than that of control subjects with the highest statistical significance compared to IgM and IgG differences. As a dermatology clinic we are following only Behçet's patients with mucocuteneous involvement. There was no other systemic involvement except ophtalmologic invasion in our patient group. The most conscipious IgA increase may probably specific to our patient group and there may be a relation between mucosal involvement in Behçet's patients and serum IgA response secondary to mucosal injury.

IgG is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids, constituting 75% of serum immunoglobulins in humans. IgG molecules are synthesised and secreted by plasma B cells. IgG antibodies are predominately involved in the secondary antibody response, (the main antibody involved in primary response is IgM) which occurs approximately one month following antigen recognition, thus the presence of specific IgG generally corresponds to maturation of the antibody response [**11**]. In our study we have found high levels of IgG and IgM in accordance with inflammatory response. On the other hand, absence of differences in respect to activity of the disease might be due to relatively low number of patients when divided into subgroups. IgE is a class of antibody that has only been found in mammals. It plays an important role in allergy, and is especially associated with type 1 hypersensitivity. IgE has also been implicated in immune system responses to most parasitic worms [**12**]. We found no relation between Behçet's disease and IgE response.

Suzuki et al evaluated B lymphocyte function in 23 patients with Behçet's disease at various stages. They revealed that the patients with active disease, but not those with inactive disease, were found to have elevated numbers of cells spontaneously secreting immunoglobulin Cowan 1. They concluded that B cell abnormalities, including some which are associated with disease activity, could be involved in the pathogenesis of Behçet's disease. It is postulated that the increased levels of immune comlexes results from polyclonal B cell activation are responsible from tissue damage [**13**].

Serum Ig levels in BD has been an important issue and significant IgA increase shown by *Scully* [**14**] et al. They found that IgA but not IgG, IgM, IgE were significantly raised in Behçet's disease compared with controls. However serum IgE concentrations but not IgA, IgG, or IgM were significantly greater in recurrent aphthous stomatitis than in controls.

To determine BD activity is very important issue and there are a lot of studies to determine the importance of different biological markers in BD. Although we have not any re-

lation with inflammatory markers and Behçet's disease activity, Adam and coll. found that CRP was significantly high in active Behçet's patients [**15**]. Additionally serum levels of IL- 2, IL- 6, nitric oxide concentrations and TNF alpha in patients with BD were found to be higher than those of controls [**16**].

We have shown that humoral immune response is increased in patients with BD being highest in IgA, and being mild in IgM levels especially in patients with mucocuteneous involvement. Levels of IgE antibody seem to be unaffected by neither BD itself nor the activity of the disease. We thought that mucocuteneous involvement may primarily or secondarily have an important role in increase of IgA.

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