THE aim of this study was to quantify soluble Fas/APO-1 (sFas/APO-1) protein in the serum of patients with Behçet's disease (BD) in active and inactive stages, compared with patients with systemic lupus erythematosus (SLE) and patients with rheumatoid arthritis (RA). Soluble Fas/APO-1 was quantified using a sandwich enzyme-linked immunosorbent assay. Increased serum sFas/APO-1 levels were observed in active BD, compared with inactive BD, RA patients and SLE patients. Increased serum sFas/APO-1 levels were correlated with the presence of neurologic manifestations or pulmonary involvement in active BD. In conclusion, increased levels of sFas/APO-1 occurred frequently and exclusively in active BD patients. Preliminary evidence suggested that elevated levels of sFas/APO-1 are associated with the clinical stage and clinical manifestations in BD.

Key words: Behçet's disease, Soluble Fas molecule, Vasculitis

Levels of soluble Fas/APO-1 in patients with Behçet's disease

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Introduction

Apoptosis or programmed cell death is an important mechanism that maintains cellular homeostasis.^{1,2} The programmed cell death involves the interaction between surface and soluble molecules: bcl-2, Fas and Fas ligand.³ Fas/APO-1/CD95 is a membrane receptor that signals apoptosis in activated mature lymphocytes.⁴ Recent studies have demonstrated the importance of this receptor in triggering activation-induced T cell death by apoptosis.⁵

Fas/APO-1 receptor molecule which lacked the transmembrane segment, resulted in a soluble form of Fas/Apo-1 (sFas/Apo-1).⁶ These studies suggested that elevated serum levels of sFas/Apo-1 receptor might provide a mechanism for downregulating programmed cell death, which could be important in the pathogenesis of several diseases: autoimmune and viral diseases.

Behçet's disease (BD) is characterized by a number of T lymphocyte abnormalities,⁷ overproduction of IgM and IgG immunoglobulins,⁸ production of autoantibodies and presence of immature CD4-CD8-T cells.⁹ In BD and in patients suffering from immunovascularitis, sarcoidosis, multiple sclerosis, SLE, increased numbers of activated immunocytes may serve as parameters in the evaluation of the clinical activity of autoimmunity.¹⁰ We have recently observed abundant expression of bcl-2 protein in T lymphocytes from peripheral blood and inflammatory sites: bronchoalveolar lavage (BAL) and cerebrospinal fluid (CSF) of active BD patients,¹¹ which may represent an attempt to main-

tain an activated lymphocyte population against the aetiological antigen or a primary abnormality of an autoimmune process. For these reasons, much interest has focused on the possible role of a defective Fas/APO-1 pathway in BD. To examine the role of sFas/APO-1 in BD, we quantified this protein in the serum of patients with active and inactive BD.

Materials and Methods

Patients

Serum was obtained from normal controls ($n = 20$) and from patients with BD ($n = 50$). Thirty patients were in active stage of BD and 20 patients were in inactive stage (mean age 46 years, range 27–42 years). They were characterized according to the criteria of the International Study Group of Behçet's Disease (ISG).¹² They had recurrent oral and genital ulcerations, and uveitis, associated to one or more other clinical manifestations. Ten of them were suffering from pulmonary manifestations (a chronic cough associated to interstitial shadows on the chest roentgenogram, pulmonary aneurysm). Another five BD patients had neurologic manifestations (meningoencephalitis, seizures, papilloedema, cranial nerve palsy).¹³ Serum was obtained from 23 randomly selected patients with systemic lupus erythematosus (SLE), and from 20 patients with rheumatoid arthritis (RA) (according to the criteria of the American Rheumatism Association for RA, and the ACR revised criteria for SLE).^{14,15}

Measurement of sFas/APO-1 levels

Soluble Fas/APO-1 levels were measured utilizing a sandwich enzyme-linked immunosorbent assay.¹⁶ Microtitre wells were coated with 1 µg/ml anti-Fas/APO-1 Mo Ab (rabbit polyclonal anti-Fas/APO-1 antibody) and blocked with 2% bovine serum albumin in PBS. Test sera were added neat and at serial dilutions and incubated for 3 h. The wells were washed and sequentially incubated with rabbit anti-Fas/APO-1 IgG followed by peroxidase-conjugated mouse anti-rabbit IgG (absorbed for cross-reactivity against human IgG; Dianova, Hamburg, Germany) and substrate solution (0.1% OPD in 0.05 M citric acid, 0.1 M Na₂HPO₄). The reaction was terminated at 10 min and the optical density at 492 nm read in an ELISA reader (Sanofi Pasteur, France). The detection limit of the assay was 2 pg/ml, the interassay variation was < 5% and intra-assay variation was < 25%.¹⁶

Statistics

Statistical significance was assessed by the Mann-Whitney *U* test for non-parametric data. Differences were considered significant if $P \leq 0.05$.

Results

The ELISA values were expressed as mean $\pm$ SD, in 20 BD patients in inactive stage, in 30 active BD patients, in 23 SLE patients and in 20 RA patients (Fig. 1). Soluble Fas/APO-1 obtained from the 20 normal controls were (2.13 $\pm$ 0.93 ng/ml). Patients with active BD exhibited increased sFas/APO-1 (4.04 $\pm$ 1.3 ng/ml). Values more than 2SD above the normal control mean (corresponding to 4 ng/ml) were considered positive. By this criterion, the most important values of sFas/APO-1 were observed in 13 active BD patients characterized by the presence of vasculitis (5.60 $\pm$ 0.94 ng/ml). Inactive BD had lower values of sFas/APO-1 (2.76 $\pm$ 0.89 ng/ml) than patients in active stage ($P < 0.01$). SLE patients and RA patients exhibited increased sFas/APO-1 respectively in 1/23 patients and in 2/20 patients. The values of sFas/APO-1 were in the same levels in SLE patients (2.12 $\pm$ 0.98 ng/ml) and RA patients (2.47 $\pm$ 1.16 ng/ml).

The increased expression of sFas/APO-1 in active BD, was highly correlated to clinical manifestations (Fig. 2): patients with pulmonary manifestations and neurologic involvement.

Discussion

The aetiology of BD is unknown yet, and our aim is to understand the pathways of tissues injury. The sites of disease activity are characterized by prominent and

FIG. 1. Serum levels of soluble Fas/APO-1 (s-APO-1) in Behçet's disease with active and inactive stage. Soluble Fas/APO-1, expressed in ng/ml, was quantified by sandwich enzyme-linked immunosorbent assay using anti-Fas/APO-1 antibody for detection. Values more than 2SD above the mean in normal controls (shown by the horizontal line) were considered positive. Patients with active BD expressed high levels of sFas/APO-1, compared with patients with systemic lupus erythematosus (SLE) and patients with rheumatoid arthritis (RA). There was no significant difference between sFas/APO-1 levels in the control subjects and patients with SLE or RA.

persistent inflammatory infiltrates. We questioned the defect of apoptosis, enabling cytotoxic cells survival.

We investigated possible apoptotic abnormalities in patients with BD in peripheral blood lymphocytes, in sera and in inflammatory sites. We did observe high levels of bcl-2 protein on CD3⁺ T cells.¹¹ In this work, we looked for a possible dysregulation in Fas/APO-1 expression in patients with BD, depending upon the clinical stage. Levels of sFas/APO-1 from BD patients were compared with patients suffering from autoimmune disease: SLE or RA.

In our report 1/23 SLE patients and 2/20 RA patients exhibited an increased sFas/APO-1 level. Increased sFas/APO-1 expression was previously reported in 1/27 SLE patients and 3/10 JRA patients.^{16,17} High levels of sFas/APO-1 were also reported in patients with leukaemias and lymphomas.¹⁶ It has been suggested that increased levels of

FIG. 2. Serum levels of soluble Fas/APO-1 (sFas/APO-1) in active Behçet's disease (BD) according to the severity of the disease. BD patients with neurologic manifestations or with pulmonary involvement exhibited higher levels of sFas/APO-1 compared with patients free from these two manifestations (other active BD). Mean values are indicated by a line. Each dot represents one patient. White dots (□) represent patients with vasculitis, who expressed higher levels of sFas/APO-1.

sFas/APO-1 may be responsible in part for autoimmunity in SLE.⁶ Our report about SLE is in accordance with the results of Knipping;¹⁶ elevated levels of sFas/APO-1 were depicted only in a minority of SLE patients. We think that sFas/APO-1 is not an important primary event in the induction of human autoimmune disease.

In the present study elevated levels of sFas/APO-1 were detected in severe forms of BD. An explanation for the presence of sFas/APO-1 in BD serum could be proteolytic cleavage of the membrane form of Fas/APO-1. The detection of soluble form of Fas/APO-1 is an important finding which may explain the 'escape' of Fas/APO-1⁺ cells from detection by the Fas/APO-1 ligand.⁶ Soluble sFas/APO-1 can protect cells from undergoing apoptosis by preventing the binding of the Fas ligand to the target cells.

The increased expression of sFas/APO-1 in active BD (13/30 patients) with confirmed neurological and pulmonary manifestation, could be correlated to the severity of the disease. In eight BD patients with

pulmonary manifestations, and in five patients with neurologic involvement, vasculitis was confirmed by physicians.

Our laboratory results showed that BD patients with vasculitis, have particular biologic findings: accumulation of cytotoxic TCR $\gamma\delta^+$ cells at the sites of inflammation,⁷ high level of endothelin expression,¹³ high autoantibodies against endothelial cells (AECA),¹⁸ increased oxygen radical production,¹⁹ high levels of cICAM-1,²⁰ and CD11/CD18 bearing lymphocytes in cerebrospinal fluid.²¹ Most of these biological and immunological parameters have been described in various autoimmune and vasculitis conditions. The present report showed an additional finding: increased expression of sFas/APO-1. This result was specific to active BD with vasculitis, and not to an autoimmune disorder.

Apoptosis is a critical mechanism by which the immune system maintains tolerance to self-antigen. In BD, taking in account the ubiquitous increased levels of bcl-2 and sFas/APO-1 may be important in inhibition of apoptosis of resting cells, and sFas/APO-1 production by activated cells may lead to excessive immune responsiveness. The sFas/APO-1 is capable of inhibiting Fas-mediated apoptosis of reacting lymphocytes to self antigen *in vivo*. Soluble Fas/APO-1 can protect cells from undergoing apoptosis by preventing the binding of the Fas ligand to the target cells. Our finding in BD suggest a crucial role for this molecule. Therapies such as plasmapheresis that may remove soluble Fas/APO-1 from sera of severe BD may probably restore normal apoptosis and reduce an eventual aggression.^{22,23} In conclusion, our results suggest that sFas/APO-1 may be a useful marker in evaluating the extent of injury in BD vasculitis condition.

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