Levels of Soluble Interleukin-2 Receptors in Serum of Patients with Behçet's Disease

Won Hur, M.D., Seung Hun Lee*, M.D., Dongsik Bang*, M.D.,
Sungnack Lee**, M.D., Jung Chul Kim***, and Tai Ho Chung***, M.D.

Department of Dermatology, Yonsei University Wonju College of Medicine, Wonju, Korea
Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea*
Department of Dermatology, Ajou University College of Medicine, Suwon, Korea*
*Department of Immunology, Kyungpook University***, Taegu, Korea*

Background: Interleukin-2 receptor (IL-2) is expressed and released predominantly activated T lymphocyte. Increased serum levels of soluble IL-2R have been noted in a variety of autoimmune diseases and in conditions associated with T lymphocyte activation.

Objective: We aimed to examine whether the T lymphocyte activation has any association with the pathogenesis of Behçet's disease.

Method: We have measured the serum level of soluble IL-2R in serum samples obtained from 67 patients with Behçet's disease and 30 healthy people as a control group, using a double-antibody sandwich enzyme-linked immunosorbent assay technique.

Results: Serum soluble IL-2R levels were found to be significantly elevated in the group of Behçet's disease as compared with the control group. No significant differences were found within clinical subtypes of Behçet's disease.

Conclusion: These findings suggest the presence of an ongoing T lymphocyte activation in this disease process. (Ann Dermatol 5:(1) 13–16, 1993)

Key Words: Behçet's disease, Soluble interleukin-2 receptor

Behect's disease (BD) is a multisystemic inflammatory disease of unknown etiology[1]. Although the definite pathogenetic mechanisms are not established, there is a great deal of evidence that an immunologic mechanism may be an important factor in the pathogenesis of BD[2-5]. During the past few years, there have been a number of studies to evaluate immunoregulatory functions in a facet of cell mediated immunity CMI of BD[3-8]. However the results are contradictory and no precise role of CMI on BD has clarified.

Recently, interleukin-2 receptors (IL-2R) with specific effects on immune regulation have been described[9]. Shortly after activation of the lymphocytes, IL-2R are expressed on their sufaces, and subsequently released in proportion to the state of activation. The soluble form of IL-2R (sIL-2R) can be detected in the blood and increased levels of this sIL-2R represent a very early sign of T cell activation[9-11]. The function of sIL-2R is uncertain, but an immunoregulatory role has been suggested in the binding of IL-2[10, 11]. Increased serum levels of sIL-2R have been found in autoimmune disorders such as rheumatoid arthritis and lupus erythematosus[12].

To evaluate the immunologic pathomechanism of BD, we measured the serum levels of sIL-2R in patients with this disease. In addition, we investigated whether clinical subtypes of BD were related to the serum levels of sIL-2R.

Received November January 13, 1993
Accepted for publication February 9, 1993

Reprint request to: Won Hur, M.D. Department of Dermatology Yonsei University Wonju College of Medicine, Ilsan-dong 162, Kangwon-do, Wonju, Korea.

MATERIALS AND METHODS

Subjects and controls This study included 67

Annals of Dermatology
Vol. 5, No. 1, January 1993

patients with complete, incomplete, suspected or possible types of Behect's disease who visited Behect's Disease Speciality Clinic of Severance Hospital, and 30 healthy controls, none of whom had a personal and/or family history of Behcet's disease.

Method After serum samples were collected from the patients and the controls, they were immediately frozen and stored at −70°C until testing. To assay soluble interleukein 2 receptor in the serum, sandwich enzyme immunoassay with two non-competing murin monoclonal antibodies to the a chain of the interleukin 2 receptor were used (T Cell Sciences, Cambridge, Mass, USA). To begin with, polystrene microtiter plates (Dynatech Laboratories, Alexandria, VA) were coated overnight with purified anti-Tac monoclonal antibodies. After the wells had been washed three times with phosphate buffered saline and 0.2% (vol/vol) polysorbate 20, the freshly thawed samples were added in duplicate and incubated for two hours at 37°C. Samples were then discarded, the plates washed, and an indicator antibody conjugated with horseradish peroxidase was added to each well. After another two hours at 37°C, plates were washed and incubated with O-phenylenediamine in citrate phosphate buffer containing 0.01% (vol/vol) hydrogen peroxide. After 30 minutes at room temperature in the dark, color was developed by the addition of 2N sulphuric acid (H_2SO_4) and the plates were read at 490nm with a Titertek ELISA reader. Units of soluble IL-2 receptor were calculated from a standard curve obtained with supernatant from peripheral blood mononuclear cells stimulated with phytohemagglutin (T Cell Science) designated 1000U/ml. Serum sIL-2R concentrations are expressed in units per milliliter (U/ml). 1000 U is described as the amounts of released or soluble IL-2R present in 1.0ml of culture supernatant from phytohemagglutinin-stimulated peripheral blood leukocytes.

Statistically, the Student's T-test was used to compare the levels of sIL-2R in the patients with BD and the controls.

RESULTS

The mean level sIL-2R in Behcet's disease compared with the controls are shown in Table 1 and Fig. 1. The mean level of the patients was calculated as 629.1 U/ml. This value expressed was elevated than the normal control (373.9 U/ml), and the elevation was statistically significant ($p<0.01$).

According to Shimizu's classification[13], the concentrations of sIL-2R in patients with the complete type, incomplete type, suspected type, and possible type were 797.5, 621.1, 525.2, and 591.6 U/ml (Table 1, Fig. 2). All the values were increased compared for normal control (373.9 U/ml) ($p<0.01$). But there was no statistically significance between each type.

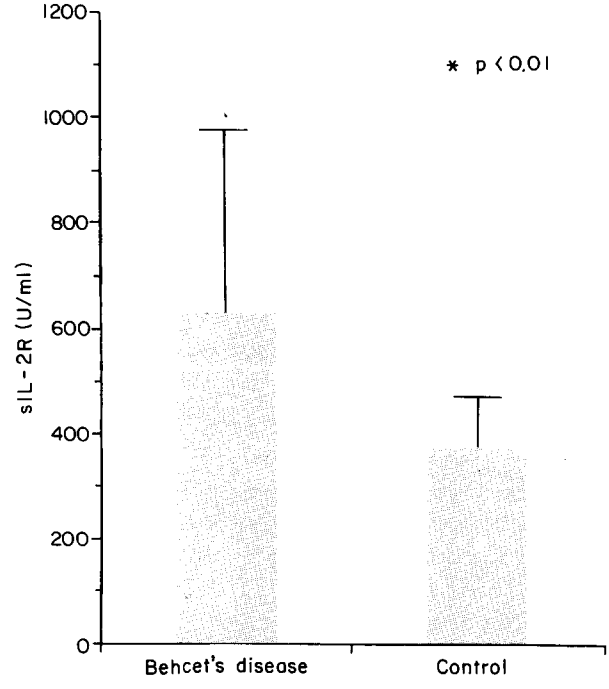

Fig. 1. Levels of serum sIL-2R in patients with Behcet's disease.

DISCUSSION

T lymphocytes play a central role in cell mediated immunity. An interaction of antigens or mitogens with specific T-cell receptors can initi-

Table 1. Serum levels of soluble interleukin 2 receptors (sIL-2R) in patients with Behcet disease according to classification by Shimizu et al (1974) and in healthy controls

Type	Number	sIL-2R (Unit/ml, Mean ±SD)
Complete	14	797.5* ±587.2
Incomplete	21	621.1* ±295.8
Suspected	15	525.2* ±213.8
Possible	17	591.6* ±157.5
Total	67	629.1* ±345
Control	30	373.9 ±96.8

*Statistically significant

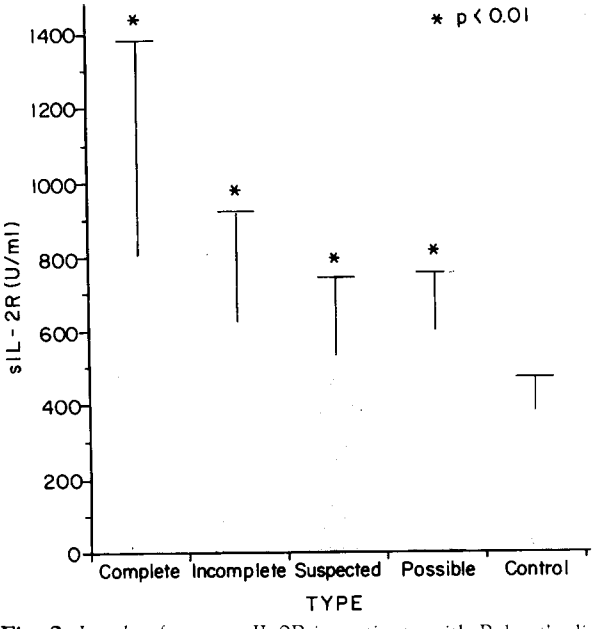

Fig. 2. Levels of serum sIL-2R in patients with Behcet's disease according to Shimizu classification.

ate a cascade of biochemical and morphological events in the T cells and one of these is the secretion of interleukin 2 (IL-2) and its receptor (IL-2R)[11, 14]. The lymphokine IL-2 has a major role in the activation of T cells, resulting in further lymphokine production and cellular proliferation. It also acts on other leukocytes including B cells, natural killer cells, and macrophages, cells which can also express IL-2R[14]. IL-2 carries out its biological effects by interacting with specific high-affinity receptors on the surface of activated T cells. In contrast to other hormone-mediated systems, cellular activation is a prerequisite for the induction of both ligand (IL-2) and its receptor (IL-2R), the latter being expressed rapidly at the cell surface in a time-dependent and heterogeneous manner[9]. The surface receptor complex is composed of three separate peptide chains[9]. The α chain of the receptor (Tac protein) is released from the cell surface and in its soluble form retains affinity for IL-2[9, 15]. This soluble form of IL-2R (sIL-2R) is released proportionally to its rate of synthesis by activated peripheral blood mononuclear cells and levels of sIL-2R is directly correlated with lymphocyte activation[10]. Thus, the elevated level of sIL-2R represent an early measure of T cell activation. Levels of sIL-2R have been shown to be increased in a number of pathological conditions accompanied by T cell activation including certain lymphomas and leukemias[16], allograft rejection[17] and viral infections such as acquired immune deficiency syndrome[18], and some dermatosis such as atopic dermatitis, psoriasis and lichen planus[19].

As well as being a useful marker of immune activity, by binding IL-2, sIL-2R may have an immunoregulatory role with inhibitory effects on T cell activation[10]. The release of sIL-2R by activated lymphocytes might serve an immunoregulatory role by competing with cellular IL-2R for the growth factor IL-2 and thus down-regulating the immune response. Recent studies clearly demonstrate that the released IL-2R molecule is capable of binding IL-2[10, 11, 18]. Because this sIL-2R molecule can bind its ligand, it could potentially affect IL-2 dependent immune responses and altered states of immune responsiveness, such as immunodeficiency diseases or autoimmune diseases which might be associated with increased or deficient production of sIL-2R, respectively.

In Behcet's disease (BD), Sakane et al found defects in responsiveness to IL-2 in the T cells of patients with this disease[3, 6]. This defect was present at all times regardless of the clinical state of the patients, but their cells could produce IL-2 normally after stimulation by mitogen[3, 8]. So, it was thought that the impairment of IL-2 responsiveness might contribute to the immunologic abnomalties and, in spite of normal IL-2 producing ability, the development of defective IL-2 responsiveness could be due to a decreased number of

cells bearing IL-2 receptors or a decrease in the density of IL-2 receptors on T cells. Thus, it seemed to be important to investigate the IL-2R in BD. In the results of this study, the sIL-2R concentration was significantly increased in the serum of patients with all types of BD when compared to the controls ($p<0.01$). These results showed that certain stimulations which may activate T cells exist constantly in BD and T cell activation may play an important role in the propagation of the inflammatory response in this disease. In BD, some authors believe that there is hyperactivation of CMI in the early active state, but after a longstanding inflammatory process, a CMI defect such as depletion of T helper cell may occur and finally, there is functional aberration of immunoregulation[4, 6]. We may assume that the defective CMI may possibly be due to down-regulation of T cell responses in the peripherhal blood by immunomodulatory mediators produced by hyperactivated immune cells in the acute inflammatory state. Another possibility that could be suggested is that circulating mononuclear cells are exhausted after previous hyperactivation. In the present study, we was not possible to demonstrate the impairment of IL-2 expression and these results were opposite to was expected, that is some defect of expression or diminution of sIL-2R concentration.

However, based on our findings, although the increase in serum sIL-2R is not specific to BD, T cell activation is evident in this disease. Whether T cell activation represents a primary event in the pathogenesis of BD is unclear at the present. Further investigations are necessary to determine which stimulti trigger the T cell response and which difference exist in the ability to express IL-2R by the stimulated T cells in vitro.

REFERENCES

1. Behcet H: *Uber rezidivierende, aphthose durch ein Virus verursachte Geschwure and Mund, am Auge, und and den Genitalen, Dermatologische Wochenschrift, 105:1152-1157, 1937.*
2. Lehner T: *Immunologic aspects of Behcet's syndrome. Proceedings of an international symposium on Behcet's disease, Excerpta Medica, Amsterdam-Oxford, Istanbul, 1977, pp 203-211.*
3. Sakane T, Kotani H, Takada S, Tsunematsu T: *Functional aberration of T cell subsets in patients with Behcet's disease, Arthritis and rheum, 25:1343-1351, 1982.*
4. Lim SD, Haw CR, Kim NI, Fusaro RM: *Abnormalities of T-Cell Subsets in Behcet's syndrome, Arch Dermatol, 119:307-310, 1983.*
5. Lee S: *Behcet' syndrome. Proceeding of the XVII World congree of Dermatology, 1987, pp 87-91.*
6. Sakane T, Suzuki N, Ueda Y et al: *Analysis of interleukin-2 activity Behcet's disease-ability to T cells to produce and respond to interleukin-2, Arthritis Rheum, 29:371-378, 1986.*
7. Lee BK, Choi IH, Shim WN et al: *Study on cell-mediated immunity in patient with Behcet's syndrome, Kor J Immunol, 10:118-128, 1988.*
8. Lee ES, Lee YD, Lee SH et al: *A study of interleukin-2 in Behcet's syndrome, Ann Dermatol, 2:5-11, 1991.*
9. Rubin LA, Kurman CC, Fritz ME et al: *Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro, J Immunol, 135:3172-3177, 1985.*
10. Rubin LA, Jay G, Nelson DL: *The released interleukin 2 receptor binds interleukin 2 efficiently, J Immunol, 137-3841, 1986.*
11. Wang HM, Smith KA: *The interleukin 2 receptor-functional consequences and its biomolecular structure, J Exp Med, 166:1055-1064, 1987.*
12. Symons JA, Wood NC, DiGiovine FS, Duff GW: *Soluble interleukin 2 receptor in rheumatic diseases: correlation with disease activity, interleukin 1 and interleukin 2 inhibition, J Immunol, 141:2612-2618, 1988.*
13. Shimizu T, Mishima S, Miyoshi K et al: Guide to diagnosis of Behcet's disease, Behcet's disease research committe of Japan, Jap J Ophthal, 18:291-294, 1974.
14. Robb RJ: *Interleukin 2-the molecule and its function, Immunology Today, 5:203-209, 1984.*
15. Uchiyama T, Broder S, Waldmann TA: *A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells, J Immunol, 126:1393-1397, 1981.*
16. Shirrmacher V, Josimovic O, Osaa H, Diamantstein T: *Determination of cell-free interleukin-2 receptor level in the serum of normal animals and of animals bearing IL-2R receptor positive tumours with high or low metastasis capacity, Br J Cancer, 55:583-588, 1987.*
17. Colvin RB, Fuller TC, Mackeen L: *Plasma interleukin-2 receptor levels in renal allograft recipients, J Clin Immunol Immunopathol, 43:273-276, 1987.*
18. Reddy MM, Grieco MH: *Elevated soluble interleukin-2 receptor levels in serum of human immunodeficiency virus infected population, AIDS Res Hum Retroviruses, 4:115, 1988.*
19. Kapp A, Piskorski A, Schopf E: *Elevated levels of interleukin 2 receptor in sera of patients with atopic dermatitis and psoriasis, Br J Dermatol, 119:707-710, 1988.*