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LEVELS OF SOLUBLE IL-2 RECEPTORS IN SERA OF IRANIAN PATIENTS WITH BEHCET'S DISEASE

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ABSTRACT

It has been shown that activated T-cells produce and release both IL-2 and IL-2 receptors (IL-2R). The rate of IL-2 release is proportional to its cell surface expression and state of cell activation. To clarify the molecular basis of this immunological aberration, we analysed the amount of soluble IL-2R(SIL2-R) by an ELISA technique in 68 patients with Behcet's disease (BD), 28 patient controls (PC) and 31 normal controls (NC).

The data suggest that the amount of SIL-2R in BD is significantly higher ($P<0.005$) than that of NC. The same differences were seen between PC and NC($P<0.005$). However the level of SIL-2R in BD and PC were similar.

This study suggests that inhibition of an immunoregulatory cytokine by its soluble receptors might occur *in vivo*, as the reduction of SIL-2R levels approaching control values preceded clinical remission.
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INTRODUCTION

Behcet's disease (BD) is a systemic inflammatory disease of unknown etiology.[1,8] Viral, genetic and environmental factors have been implicated in the pathogenesis of this illness.[3] Furthermore, evidence suggest that at least some of the clinical aspects of BD may be due to an autoimmune response. These include elevated levels of immunoglobulins (Igs), immune complexes, immunoglobulins binding to oral mucosa and antibodies that react with fetal oral mucosal tissues.[5,6]

Patients suffering from BD have shown several T-cell abnormalities which may be relevant to the autoimmune origin of the disease. These include alterations in subpopulations of T-lymphocytes,[12] and deficient IL-2 levels which have been demonstrated in autoimmune diseases such as SLE. Some of the immunological aberrations observed in patients with BD could be explained by deficient IL-2 activity.[12] In BD patients it has been shown that activated T-cells produce and release both IL-2 and IL-2R.[12] The rate of IL-2R release is proportional to its cell surface expression and state of cell activation.[12]

To clarify the molecular basis of the immunological aberration, we analysed the amount of SIL receptors (Tac antigen or CD25).

MATERIAL AND METHODS

Patients

All immunological tests were done at the Department of Immunology, Tehran University of Medical Sciences. Samples from peripheral blood were obtained from 68 patients (mean age 26.1 years) with active BD based on clinical observation at the Dept. of Rheumatology, Shariati Hospital. Of 16 patients with recurrent aphthous stomatitis (RAS), 12 patients with uveitis were control patients (PC). Samples were also obtained from 31 healthy normal subjects as normal controls (NC, Table I).

Serum samples

Blood samples were kept at room temperature for 30

minutes and then sera were separated by centrifugation (2500 RPM, 20 min.) and stored at -20°C until use.

SIL-2R assay

We have used an ELISA Kit supplied by Immunotech, France.

T-cell subsets

To study the variations of the T-cell subsets in the peripheral blood of patients with active BD, PC and NC, we have used fluorescent labelled monoclonal antibodies (Behring) against T-cell surface markers CD3, and CD8.

Statistics

Data are expressed as the arithmetic mean±SD. A student t-test was used to compare the means.

Table I. Characteristics of patients

Patients	Male	Female	Total
BD*	33	35	68
RAS†	6	10	16
Uveitis	4	8	12
Total	43	53	96

*BD= Behcet's disease, †RAS= recurrent aphthous stomatitis

RESULTS

We have determined serum SIL-2R levels in patients with BD, control patients (PC) and normal controls (NC).

The 68 patients with BD showed a mean value of 9634.8±1986.6pg/mL for their serum SIL-2R whilecontrol patients had 10500±3819.9 pg/mL and NC had 6707.4±2459.52 pg/mL.

The data suggest that the amount of SIL-2R in BD is significantly higher (P<0.005) than that of NC. The same differences were observed between NC and PC. However the level of SIL-2R in BD and PC were similar. The results are shown in Tables II-IV.

The results of the T-cell subset variations in peripheral blood samples of the 68 patients with BD, 31 NC and 28 PC patients are shown in Table V.

Table II. SIL-2R level in BD in comparison to NC.

	BD(68)	NC(31)	Result
SIL-2R: Level	9634.8±1986.6pg/mL	6707.4±2459.52pg/mL	P<0.005

Table III. SIL-2R level in BD in comparison to PC.

	BD(68)	PC(28)	Result
SIL-2R: Level	9634.8±1986.6pg/mL	10500±3819.9 pg/mL	non-signif

Table IV. SIL-2R level in PC in comparison to NC.

	PC(28)	NC(31)	Result
SIL-2R: Level	10500±3819.9pg/mL	6707.4±2459.52pg/mL	P<0.005

Table V. The number of T-cell subsets in BD, PC and NC.

T-cell subsets	BD(68)	PC(28)	NC(31)
T(CD3+)	71.57±7.95	69.89±8.75	67.03±7.23
T(CD4+)	35.5±9.23	42.92±9.73	45.00±6.13
T(CD8+)	36.4±9.5	26.89±7.58	20.00±4.98
T(CD4)/T(CD8)	1.0669±0.45	1.762±0.68	2.36±0.78

Table VI. The statistical analysis of the T-cell subsets in BD in comparison to NC.

T-cell subsets	BD(68)	NC(31)	P-value
T(CD3+)	71.57±7.95	67.30±7.23	P= 0.008
T(CD4+)	35.50±9.23	45.00±6.13	P<0.000001
T(CD8+)	36.40±9.50	20.00±4.98	P<0.000001
T(CD4)/T(CD8)	1.0669±0.45	2.36±0.78	P<0.000001

The results of T(CD4) and T(CD8) cells in the 41 patients with active BD which had higher levels of SIL-2R and the 27 patients with lower levels (than the upper limit in NC) are shown in Table VII.

Table VII. Results of T-cell subsets in high SIL-2R and low SIL-2R BD patients.

T-cell subsets	(41)BD high SIL-2R	(27)BD low SIL-2R	Result
T(CD4+)	35.12±8.8	36.44±9.6	non-signif.
T(CD8+)	35.90±9.06	37.10±10.28	non-signif.
T(CD4)/T(CD8)	1.05±0.4	1.116±0.5	non-signif.

Table VIII. The results of SIL-2R levels in patients of Table VII.

	(41)BD with high SIL-2R	(27)BD with low SIL-2R	Results
SIL-2R Level:	10594.92±1754.76pg/ml	8023.26±646.38	P<0.005

DISCUSSION

Activated T-cells produce both IL-2 as well as IL-2 receptors.[7] Human IL2-R are composed of α and β chains.[6] The α chain is also known as the Tac-protein or CD25. The activated T-cells also release a soluble form of this protein (SIL-2R).[9]
to its cell surface expression.[9]

Autoimmune diseases may also be associated with disorders in Tac-Ag expression. A proportion of the mononuclear cells in the involved tissues express the Tac-Ag, and the serum concentration of SIL-2R is elevated. Evidence suggest that T-cell activation and Tac-Ag expression disorders are present in patients with rheumatoid arthritis, SLE and sarcoidosis.[13] These disorders have been demonstrated in animal models of these diseases.[13]

In this study we found that serum SIL-2R levels in 68 patients with active BD were significantly higher (P<0.005) than that of NC. However in comparison to the patient controls (PC), the difference was not significant.

In another study by Symons,[4] it was shown that thereare higher SIL-2R levels in sera from patients with atopic eczema, psoriasis, BD and SLE. Tsuyoshi and Sakane reported that there was a marked reduction in the proportion of Tac-Ag-positive cells among activated T-cells from patients with early active disease.[10] They claimed that there were no changes in SIL-2R of the patients with chronic, active or inactive forms of BD in comparison to normal controls. These results indicate that unresponsiveness to IL-2 of the T-cells from patients with early active BD could be due to a substantial decrease in the number of cells bearing IL-2 receptors.[10]

In our study, 41 out of 68 patients with BD had higher levels of SIL-2R than the upper limit of the normal controls, which had the same ratio of T(CD4)/T(CD8) cells (Table VII). This means that the number of T(CD4) and T(CD8) cells among the two groups are not significantly different, but there is a significant increase (P<0.005) in
SIL-2R in the first group.
of T cell subsets in BD is not responsible for the increase in the level of SIL-2R.
may beam
in serum.

In this study we could not find any relationship between the specific clinical manifestations and higher levels of SIL-2R, but we did find that patients with simultaneous oral and genital aphthous and ocular lesions have
counts and high amounts of SIL-2R (10 out of 22) which may be evidence of higher activity of these cells in this group compared to other patients.

The role of T lymphocytes in the tissue pathology of BD has been demonstrated in immunohistopathological studies of the tissues from other affected sites. The identification of T(CD4) cells as the predominant cell type in the vascular lesions of the eye supports the view that cell-mediated immune response is responsible for the tissue damage seen in this condition. The absence of B cells and neutrophils indicate that humoral immune response does not play a major role in the ocular immunopathology.[2]

The higher levels of IL-2R in BD patients' sera may be due to an increase of IFN-γ
cells which express and release more Tac antigens.[16]
creased levels of IL-2R in serum might be a general phenomenon in diseases with an immune pathogenesis.
is the case, it could be said that SIL-2R levels reflect immunopathogenic activation.[4] It is also possible that increased levels of SIL-2R mediate an immunoregulatory effect that could contribute to the pathogenesis.[9]

Disease activity in RA was monitored based on patients' clinical symptoms and by laboratory measurements.
changes in serum IL-2R levels were seen whether clinical remission was associated with treatment or occurring without the use of a remission-inducing drug.[4] The reduction of SIL-2R levels approaching control values preceded clinical remission, suggesting that this was not a secondary event reflecting clinical improvement. It may have been related to the activation of immunopathogenic mechanisms that induce inflammation.[4]

Finally, this appears to be the first evidence suggesting that inhibition of an immunoregulatory cytokine by soluble receptors might occur *in vivo*. It should be noted that increased SIL-2R levels in BD patients may be a regulatory mechanism of decreasing levels of IL-2, which are produced by activated T-cells in inflamed tissues. This should be studied further.

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