Elevated Serum Levels of Neopterin in Patients with Behçet's Disease

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Background: **Neopterin is released from monocytes/macrophages specifically by stimulation with interferon-γ. Therefore the increase refers to the activation of T lymphocytes.**

Objective: **Our objective was to examine the potential role of neopterin in the cell mediated immune response to Behçet's disease.**

Methods: **We studied 67 patients of Behçet's disease classified by Shimizu and a control group of 30 normal healthy people. Serum neopterin was detected by radioimmunoassay technique.**

Results: **The serum neopterin concentration of the group with Behçet's disease was significantly elevated, compared with the control group (6.36 nmol/ml vs 3.63 nmol/ml). The increase of neopterin concentration was well correlated to the clinical severity of the diseases in increasing order.**

Conclusion: **Our data suggest that the T lymphocyte derived IFN-γ which stimulates the production of neopterin has some role in the pathogenesis and clinical severity of Behçet's disease.**
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Key Words: Behçet's disease, Interferon-gamma, Neopterin

About five decades have passed by since Hulusi Behçet[1] defined his syndrome and yet the diagnosis is based exclusively on clinical criteria. Behçet's disease is now recognized as a multisystemic disease with mucocutaneous, ocular, intestinal, articular, vascular, urogenital, and neurologic involvement[2-4]. No clear etiology has yet been established, but there is some evidence to suggest that immunologic abnormalities are important in its pathogenesis[5-14]. Recently attention has been paid to the cell mediated immune system in this disease[5-14]. However, the results reported were contradictory and no precise roles of cell mediated immune system on Behçet's disease have been clarified.

Neopterin is a metabolite of guanosine triphosphate in the synthetic pathway of biopterin, an essential cofactor in neurotransmitter synthesis, and coming from macrophage stimulated γ-interferon, which is in turn released from activated T lymphocytes[15-19]. The mode of action and the biological significance of neopterin is not yet known. Immune responses in vivo and in vitro are accompanied by increased neopterin levels, and considerable interest has centered on the measurement of neopterin as a marker of immunological activation in various diseases[20-33]. Usually increased neopterin production precedes the clinical manifestation and is correlated with the activity of the disease.

In view of the link between the cell mediated immunity and the neopterin, we were interested in determining whether a correlation existed between concentrations of neopterin and patients with Behçet's disease. Here we examined the concentrations of neopterin in Behçet's disease. The present study was carried out to investigate the

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status of cell-mediated immunity in Behçet's diseases by measuring the concentrations of neopterin and to determine its relationship to clinical disease activity.

MATERIALS AND METHODS

Subjects

Sixty-seven patients with Behçet's disease who visited Behçet's Disease Speciality Clinic of Severance Hospital (34 males, 33 females), and a control of 30 healthy people (20 males, 10 females) were included in this study. None of these controls had a personal and/or family history of Behçet's disease. Patients were aged 21-54 years (median 37.5 years). According to the Shimizu et al. classification, 17 patients were possible, 15 were suspected, 21 were incomplete and 14 were complete.

Methods

Serum samples of Behçet's disease were kept frozen until measurement of neopterin by liquid phase radioimmunoassay (Radio-immuno Special Vials, Starstedt, Numbrecht, F.R.G.) as previously described[22]. Serum (50ml) was incubated with 100ml neopterin antiserum for 1hr at room temperature. Then 100ml of ^{125}I-labeled tracer was added. After centrifugation at 2,000×g for 10 min, radioactivity was counted using the gamma counter (Packard). The detection limit was 1nm/L. Neopterin concentrations were compared with normal levels obtained from 30 healthy normal control subjects.

Statistical analysis

Results were analyzed by Student's t-test. Values presented are mean±one standard deviation. A value of $p<0.01$ was considered statistically significant.

RESULTS

Serum neopterin concentrations in the patients group were found to be significantly higher than that of the normal control group. The mean concentration of serum neopterin in each type was shown in Table 1. When comparing mean concentrations between the complete type and the other three groups of patients, patients with the complete type exhibited slightly higher mean concentration of serum neopterin than those with incomplete type; however, the differences were not significant (Table 1, Fig. 1). In contrast, the mean concentration of serum neopterin in the complete type was significantly higher than those with suspected and possible types.

DISCUSSION

Various tests are currently used to evaluate the status of cell-mediated immunity (CMI) in a variety of conditions. However, many of these are *in vitro* tests which are too complicated for routine clinical use. In addition, *in vitro* tests do not necessarily reflect the *in vivo* situation. Quantification of circulating lymphokines is complicated by the limited their biological half-life, and the

Table 1. Levels of serum neopterin (nmol/l) in the patients with Behçet's disease.

Type[a]	Number of patients	Neopterin (nmol/l)[b]
Complete	14	7.68±2.80[c,d]
Incomplete	21	6.89±3.71[c,e]
Suspected	15	5.72±1.94[c]
Possible	17	5.20±1.33[c]
Total	67	6.36±2.80[c]

[a]The patients were classified by Shimizu's classification (Shimizu et al., 1974).
[b]Values presented are Mean ±SD.
[c]$p<0.01$, compared with control group (3.63±0.88)
[d]$p<0.01$, significantly different from suspected and possible groups
[e]Statistically not significant, compared with complete group

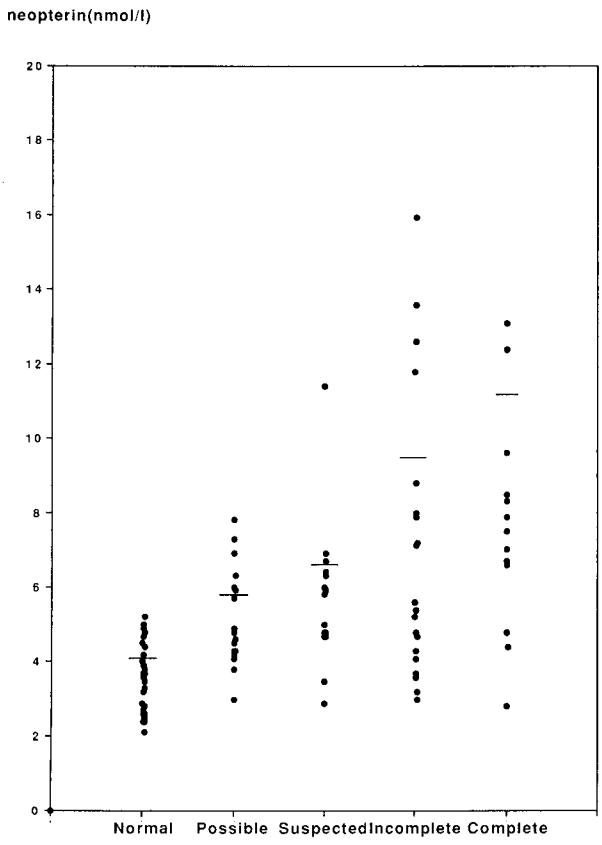

Fig. 1. Levels of serum neopterin in the patients of Behçet's disease and the control group.

circulating level may be decreased by binding to receptors. An alternative approach is needed to quantify the specific products of the cells of the immune system which are biologically stable and are sufficiently detectable in body fluids.

Neopterin is a metabolite of guanosine triphosphate coming from macrophages stimulated by IFN-γ, which is in turn released from activated T lymphocytes[15-19]. Human monocytes/macrophages were the specific source of neopterin when activated with IFN-γ or with at least a 1,000-fold higher dose of IFN-α[19]. Various clinical studies also imply that stimulation of human macrophages by IFN-γ is the single principal event in inducing neopterin synthesis and release[16, 19]

In vivo, increased neopterin concentrations in body fluids, for example, serum, urine and cerebrospinal fluid, constitute a sensitive indicator of cellular immune activation. High neopterin levels were found in cancer patients and patients suffering from viral infections, suggesting that high neopterin levels might reflect the host's response to tumor cells or virally transformed cells[20]. Fuchs et al revealed preactivation of CMI was associated with poor prognosis in cancer patients, and in human immunodeficiency virus (HIV) infection, neopterin levels increased in parallel with progressive disease, were inversely correlated with CD4+/CD8+ T-cell subset ratios and are of predictive significance[16, 21]. High neopterin levels have been found in acute viral infections such as hepatitis, rubella, several herpes infections[22, 23], allograft rejection[24], intracellular bacterial infection[25, 26], various malignancies[27, 29], and some autoimmune diseases such as systemic lupus erythematosus[30], rheumatoid arthritis[31] and celiac disease[32].

Behçet's disease is a systemic inflammatory disease of unknown etiology. Although the pathogenesis of Behçet's disease is still obscure, it is now accepted that cell-mediated immunity plays a significant role[5,8]. In view of the fact that neopterin represents a sensitive marker for activated CMI, it would be worthwhile to evaluate the level of neopterin in Behçet's disease.

In this study, the increase in mean neopterin concentration from the patients with Behçet's disease compared to the control value was statistically significant ($p<0.01$). Using the classification schemes by Shimizu et al[3], serum neopterin concentration was higher than that of control value in each type. In addition, the increased concentration of neopterin for each type seems to be related to the clinical activity of the disease. Clinical deterioration of Behçet's disease is associated with a further increase in neopterin levels, although there was no statistical significance between the complete and incomplete type.

In our investigation, we found that activation of CMI contributes to its pathogenesis of Behçet's disease. This is in sharp contrast to past studies that report either normal or decreased CMI[5-7], and opposite to our expection of some defect of expression or diminuation of neopterin concentrations. From the various immune studies reported in patients with Behçet's diseases in the literature, some alteration in the immunological function of T cells and T cell substs has been reported[6, 7, 9, 10]. These include a decrease in the

number of total T lymphocytes, the decrease of the number of helper T lymphocytes, and the reduced ratio of helper/suppressor T lymphocytes. Decreased T cell activity and a poor autologous mixed lymphocyte reaction were described by Sakane et al[6]. Lee et al. reported the total number of OKT4+ cells was reduced-declining in the sequence: suspect, incomplete, and complete types according to the Shimizu et al. classification. In the Lehner and Barnes classification, decline was in the sequence: mucocutaneous, arthritic, and ocular types[14]. Thus a significant inverse correlation exists between neopterin levels and CD4+/CD8+ T cell ratios and absolute CD4+T cell numbers.

It is interesting to note that in the complete type of Behçet's disease, low numbers of CD4+ cells and of total lymphocytes can induce the release of high neopterin concentrations. In children with primary severe combined immunodeficiency (SCID), neopterin levels were lower when free from infection, but even extremely low numbers of residual T cell in peripheral blood can give rise to high neopterin levels during infections[16]. Furthermore, neopterin autoinductive production can explain the amplification of neopterin release during activation of the cell mediated immune response, in spite of the decrease in the T helper cell subsets, which are the main IFN-γ products[33]. As can be concluded from these data, neopterin release is coupled with early events of activated CMI but does not depend on the function of cells of the immune system.

These findings suggest that general immune activation is an important aspect of Behçet's disease pathogenesis, although *in vitro* studies show a variety of suppressive effects on lymphocyte cell function. Moreover, recently there have been several reports of the effectiveness of cyclosporine in the treatment of Behçet's disease, presumably via its immunomodulatory effects[34, 35]. Cyclosporine is thought to work by blocking the synthesis and/or release of interleukin 1 from macrophage and interleukin 2 from T helper cells[34]. This resulted in marked reduction of neopterin concentration and resolution of clinical flare. In other words, T cell activation is evident in Behçet's disease. What is more, hematopoietic disturbances such as anemia are common in patients with Behçet's disease[36]. Recent studies on immune activation markers such as neopterin demonstrate that hematological abnormalities are associated with chronic immune activation[37]. Also, it has been suggested recently that continual activation of T cells may result in the progressive loss of memory T cell populations, eliminating the capability for the normal clonal expansion of cells in response to antigenic challenge[38]. Thus we may assume that continual activation of T cells by certain constantly existing antigens, such as a virus, bacterium, or environmental influences may suppress immune functions by making them unresponsive to antigens and other normal immune signals in Behçet's disease. Another possibility could be that the defective CMI may possibly be due to down-regulation of T cell responses in the peripheral blood by immunomodulatory mediators produced by hyperactivated immune cells in acute inflammatory state, and circulating mononuclear cells are exhaused after previous hyperactivation.

It is obvious that neopterin does not represent a specific marker for Behçet's disease. However, neopterin reflects T lymphocyte dependent activation of moncyte/macrophage, the key pathogenic mechanism in Behçet's disease, and that neopterin estimation has the potential to predict the clinical outcome. As mentioned above, the etiopathogenesis of Behçet's diseases remains unclear. Our data indicate that chronic immune stimulations is at least partly responsible.

In the present study, it was not possible to examine the neopterin concentration using assessment of clinical activity for the various reasons. Further investigations on neopterin and its application using a scoring method of clinical activity may be useful in defining the role of neopterin in the pathogenesis of Behçet's disease.

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