Serum Tumor Necrosis Factor, Interleukin-1β and Interleukin-6 Levels in Behçet's Disease

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Background: **Although the precise pathogenesis of the Behçet's disease is not yet undertween the severity of Behçet's disease and the serum cytokine level.**
development of cytokine research has made it possible to find out if there is an association between the severity of Behçet's syndrome and the serum cytokine level.

Objective: **Our purpose was to elucidate whether the immunopathological mechanism is associated with the serum tumor necrosis factor (TNF) and interleukin-1β (IL-1β) which are predominantly produced by monocytes/macrophages, and interleukin-6 (IL-6).**

Method: **Sixty seven patients of Behçet's disease and ten healthy adults as a control group were studied. Serum TNF and IL-6 levels were detected by enzyme immumoassay and serum IL-1β levels by radioimmunoassay.**

Results: **There were no statistically significant differences in the serum levels of TNF, IL-1β, IL-6 compared with the control group.**

Conclusion: **These data suggest that the immunopathological reactions of the Behçet's disease are not associated with a monocyte/macrophage dependent mechanism, possibly due to other immunocompetent cells. (Ann Dermatol 5:(2) 69–73, 1993)**

Key Words: Behçet's disease, Cytokine, IL-1β, IL-6, TNF

Behçet's disease is a chronic inflammatory dermatosis commonly involving the oral cavity, genital mucosa, eyes and skin[1]. Severe cases, involving even the central nervous system, cardiovascular system and respiratory system have been reported[2,3,4].

The skin lesions of Behçet's disease are erythema nodosum, folliculitis, pyoderma, erythema multiforme and thrombophlebitis[5]. The most common histopathologic findings of Behçet's disease are vasculitis[5]. Recently cytokines have been reported to be involved in several diseases[6].

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Tumor necrosis factor (TNF) and interleukin (IL)-1β are known to be produced predominantly by activated monocytes and macrophages. Activated monocytes and macrophages are also well-known producers of IL-6. To elucidate whether the immunopatholgy of Behçet's disease are associated with monocyte/macrophage derived cytokines, we detected TNF, IL-1β and IL-6 in the patients' serum.

PATIENTS AND METHODS

Sixty seven patients of Behçet's disease (mean age; 33, M:F=38:29) and ten healthy adults as a control group (mean age; 37, M:F=6:4) were studied. The patients of Behçet's disease were composed of fourteen with complete type, twenty one with incomplete type, fifteen with possible type and seventeen with suspected type which

were classified by the Shimizu method[7]. The patients with typical clinical symptoms were selected and classified regardless of previous or current treatments. Instead they were classified by four major symptoms like oral ulcers, genital ulcers, skin manifestation and eye involvement. The patients showing all the above four major symptoms were classified into the complete type. The incomplete type requires three major symptoms or two major symptoms and eye involvement. The possible type requires two other major symptoms without eye involvement. The suspected type requires only one major symptom.

Blood samples were taken from the patients of Behçet's disease and the control group, then centrifuged at 4°C and the serum samples were stored at −70°C until testing.

Serum TNF concentration was detected by sandwich enzyme immunoassay (NEN, Boston, MA, USA) using two antibodies. In the polystyrene microtiter plates, which were coated with monoclonal antibody against TNF, serum samples and standard solutions were added at 4°C and incubated for twelve hours, then the wells were washed. The polyclonal rabbit anti-TNF antibody were added and incubated for one hour, then washed. The horseradish peroxidase conjugated goat anti-rabbit antibody were added and reacted for one hour at room temperature, then washed. The solution containing hydrogen peroxide and orthophenylenediamine were added. After one hour reaction at room temperature, the color reaction was stopped by addition of 2N H_2SO_4, and the absorbance was detected at 490nm and 690 nm within 30 minute by Titertek enzyme linked immunosorbent assay (ELISA) reader. A standard curve was drawn using recombinant TNF and the detection unit was pg/ml. The detection limit of this method was 34 pg/ml. Therefore, the sample values lower than 34 pg/ml were calculated to zero.

Serum IL-1β concentration was detected by radioimmunoassay (RIA: AMI, Cambridge, MA, USA). The serum samples (100μl) and rabbit anti IL-1β antibody (100μl) were reacted at 37°C for 2 hours in polypropylene tubes. Then ^{125}I labeled IL-1β (100μl) was added to the reaction to the reaction mixtures. The magnetic goat anti-rabbit IgG (500μl) was added to the mixtures. After centrifugation at 1000xg for 20 minutes, the supernatants were removed, and the pellets were counted for radioactivity by gamma-counter.

A standard curve was drawn using recombinant IL-1β and the detection unit was ng/ml. The detection limit of this method was 0.2 ng/ml. Therefore, the sample values lower than 0.2 ng/ml were calculated to zero.

A serum IL-6 concentration was detected by Sandwich enzyme immunoassay (R & D systems, Minneapolis, MN, USA) using two antibodies. In the polystyrene microtiter plates which were coated with monoclonal antibody against IL-6, serum samples and standard solutions were added at room temperature for two hours, then the wells were washed. The horseradish peroxidase conjugated polyclonal antiobdy against IL-6 was added and incubated at room temperate for two hours, then washed. The solution containing hydrogen peroxide and tetramethyl benzidine was added. After 20 minutes reaction at room temperature, the color reaction was stopped by addition of 2N H_2SO_4, and the absorbance was detected at 490 nm and 690 nm within 30 minutes by Titertek ELISA reader. A standard curve was drawn using recombinant IL-6 and the detection unit was pg/ml. The detection limit of this method was 10 pg/ml. Therefore, the sample values lower than 10 pg/ml were calculated to zero.

For statistical analysis, Student's T-test was used to compare the differences of the patient and control groups of TNF, IL-1β and IL-6.

RESULTS

The number of serum samples above the detection limit among 10 control serums was 3 in TNF, 8 in IL-1β and 1 in IL-6. The number of serum samples above the detection limit among 14 patients of the complete type was 8 in TNF, 10 in IL-1β and 9 in IL-6. The number of serum samples above the detection limit among 21 patients of the incomplete type was 9 in TNF, 12 in IL-1β and 2 in IL-6. The number of serum samples above the detection limit among 17 patients of the possible type or 15 patients of the suspected type was 6 in IL-6 respectively.

Serum TNF levels of the thirty five patients of Behçet's disease (14 complete type; 21 incom-

plete type) were compared with the levels of ten healthy control serums. Each subtype was also compared with the control group. There were no statistically significant differences in the subtypes or total patients compared with the control group (Fig. 1).

Serum IL-1β levels of the thirty five patients of Behçet's disease (14 complete type; 21 incomplete type) were compared with the levels of ten healthy control serums. Each subtype was also compared with the control group. There were no statistically significant differences in the subtypes or total patients compared with the control group (Fig. 2).

Serum IL-6 levels of the sixty seven patients of Behçet's disease (14 complete type; 21 incomplete type; 17 possible type; 15 suspected type) were compared with the levels of ten healthy control serums. Each subtype was also compared with the control group. There were no statistically significant differences in the subtypes or total patients compared with the control group (Fig. 3).

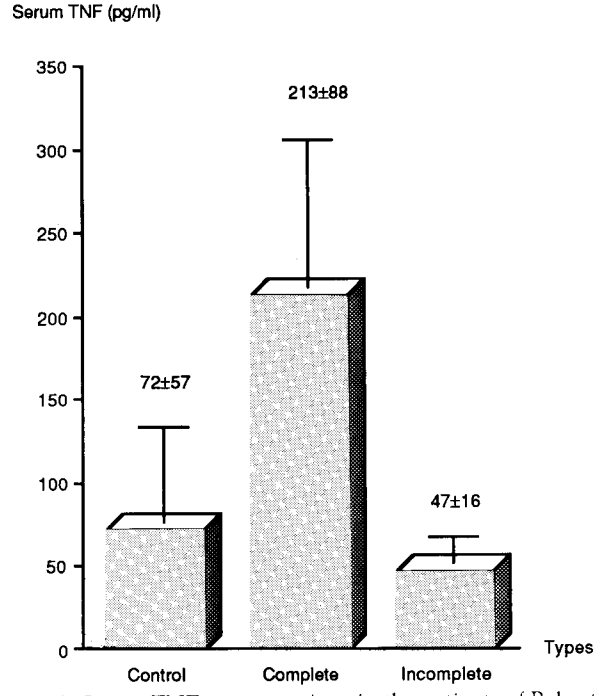

Fig. 1. Serum TNF concentrations in the patients of Behçet's disease (mean±SEM)

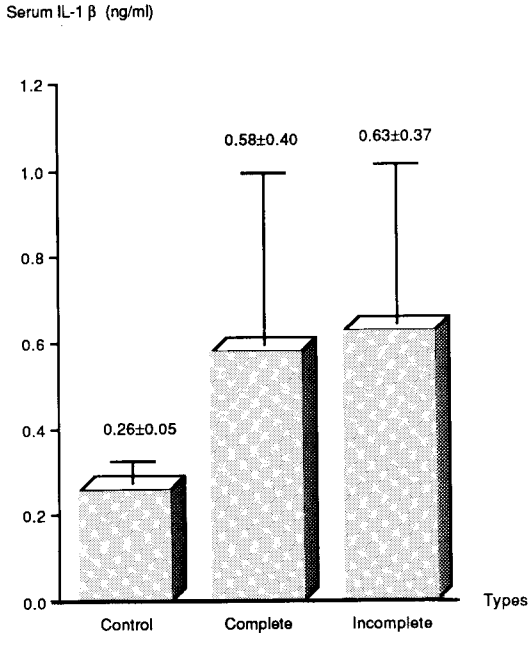

Fig. 2. Serum IL-1β concentrations in the patients of Behçet's disease (mean±SEM)

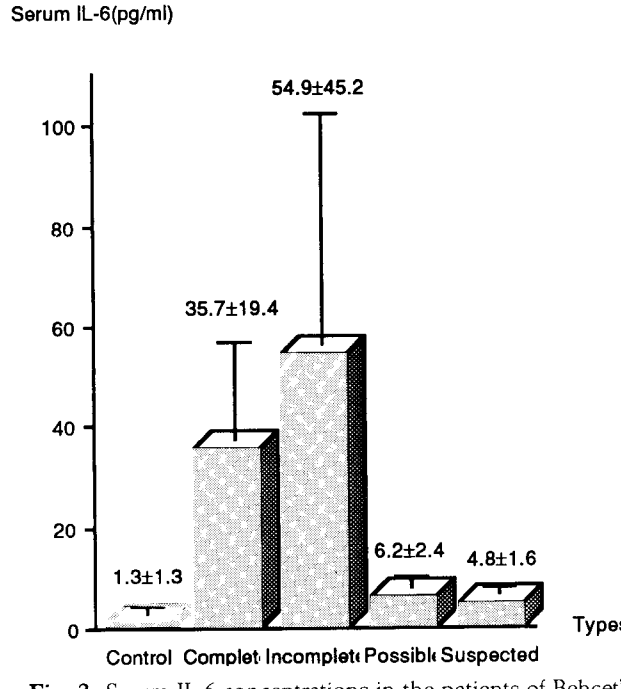

Fig. 3. Serum IL-6 concentrations in the patients of Behçet's disease (mean±SEM)

DISCUSSION

Current development of cytokine research has stimulated the studies of detecting cytokine changes in several human disease[6]. Marked elevation of serum TNF and IL-6 levels were found in cerebral malaria[6], purpura fulminans[6,8]. In gram negative septic shock, high levels of IL-6 were detected[9]. Elevated TNF, IL-1 and IL-6 had been reported in leprosy reaction of which histopathology shows erythema nodosum[6]. In the case of vasculitis, marked elevation of interferon (IFN) α and IL-2 has been reported[10].

The elevated serum levels of IL-6 were detected more frequently in the severe types of patients of Behçet's disease. Twenty on serums of elevated IL-6 levels out of 35 serums of the complete and the incomplete types compared to 12 elevated serums out of 32 patients of the possible and the suspected types were detected. However, the serum IL-6 levels were not significantly elevated in the patients compared to the control group.

There were neither elevated serum concentrations nor detection frequencies in the cases of TNF and IL-1β. The serum levels of TNF, IL-1β and IL-6 did not have any correlation to the elevation of each other. Our results on IL-6 and TNF agree with the previous reports[11,12]. The discrepancies in TNF levels reported by Erken et al may be due to either the differences in the detection method using immunoradiometric assay or ethnic group[13]. The discrepancies in IL-1 levels reported by Menisoglu et al may be due to the differences in ethnic group[14].

Considering that the common histopathology of Behçet's disease is vasculitis, elevation of serum IL-2 and IFNα like other types of vasculitis are speculated[10]. However, serum IL-2 activity in Behçet's disease was not elevated[15]. Recently, the statistically significant increase in the soluble IL-2 receptor levels in the patients of Behçet's disease, which also represent T-lymphocyte activation status, was reported and also observed by us[12]. Therefore we may predict that the detection of serum IL-2 was blocked by soluble IL-2 receptor, which may explain the negative result of the detection of IL-2 activity[12]. The detection of serum IL-2 by ELISA or RIA is necessary to prove the above possibility.

The histopathology of the Behçet's disease, which changes from leukocytoclastic vasculitis in the early stage, to lymphocytic vasculitis in late stage was addressed by Jorizzo[16]. Therefore a chemotactic factor like IL-8, which recruits both polymorphonuclear leukocytes and T-lymphocytes, seems to be a good candidate for a mediator of the immunopathology[17].

Our results suggest that the immunopathological reactions of Behçet's disease are not associated with a monocyte/macrophage dependent mechanism, possibly due to other immunocompetent cells.

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