Down-Regulation of IFN-γ–Producing CD56⁺ T Cells after Combined Low-Dose Cyclosporine/Prednisone Treatment in Patients with Behçet's Uveitis

Jae Kyoun Ahn,[1] Jong-Mo Seo,[1] Jeesuk Yu,[2] Frederick Sehwoong Oh,[1] Hum Chung,[1] and Hyeong Gon Yu[1]

PURPOSE. To investigate the effects of combined low-dose cyclosporine and prednisone (Cs/Pd) treatment on circulating CD56⁺ T cells in patients with Behçet's uveitis.

METHODS. Ten patients with Behçet's uveitis and 10 healthy control subjects were prospectively recruited. The patients were treated with Cs/Pd for 2 months. Phenotypic and functional changes in circulating CD56⁺ T cells were assayed before and after treatment. CD56⁺ T-cell subsets were determined by flow cytometric analysis with monoclonal antibodies for CD3, CD4, CD8, CD56, pan γδ TCR, and Vα24. The absolute numbers of cells in the lymphocyte subsets were calculated. Cytokine (IFN-γ, IL-4, and IL-10) expressions were measured by ELISA and by intracellular cytokine staining.

RESULTS. The proportions of CD56⁺ T cells, specifically CD8^{high}CD56⁺ and CD56⁺γδ T-cell subsets, were significantly higher in active Behçet's uveitis but normalized after treatment, whereas the total T-lymphocyte count and the absolute numbers of CD56⁻ T cells were unaffected by treatment. The levels of IFN-γ and IL-4 were elevated in aqueous humor and serum in Behçet's uveitis ($P < 0.001$), whereas IL-10 was not detected. After treatment, serum IL-4 levels markedly increased ($P < 0.001$), and IFN-γ production by circulating CD56⁺ T cells was then suppressed. IL-4 and -10 production by CD56⁺ T cells was increased by treatment, but in contrast, minimal changes were found in CD56⁻ T cells.

CONCLUSIONS. The results imply that Cs/Pd treatment for Behçet's uveitis selectively affects the population of and the cytokine expression in CD56⁺ T cells, but without significant changes in CD56⁻ T cells, and that IFN-γ–producing CD56⁺ T cells are the central pathogenic immune cells in Behçet's uveitis. (*Invest Ophthalmol Vis Sci.* 2005;46:2458–2464) DOI: 10.1167/iovs.04-0792

Behçet's uveitis, a prototype of chronic recurrent uveitis with retinal vasculitis, is often accompanied by multisystemic manifestations in the mucocutaneous, arthritic, vascular, and central nervous systems.[1] Although various primary immune abnormalities involving genetic, infectious, and autoimmune components have been implicated in Behçet's disease, much remains uncertain about its pathogenesis.[2–4] Both innate and adaptive immune responses have been suggested to play a pathogenic role in Behçet's disease.[5,6] Aberrant cellular immunity, such as T-cell-mediated autoimmunity or an imbalance in the Th1/Th2 pathway, may be crucial in the pathogenesis of Behçet's disease.[7,8] Recently, CD56⁺ T cells were reported to be higher in the aqueous humor and peripheral blood of patients with Behçet's uveitis than were other endogenous uveitides.[9,10]

Surface CD56 (neuronal cell adhesion molecule), a receptor for natural killer (NK) cells, is found on heterogeneous T-cell subsets, such as CD4⁺, CD8⁺, γδ T, and Vα24⁺ T cells in humans.[11] CD56 is also induced on cytotoxic αβ or γδ T cells by TCR stimulation in a Th1-rich environment.[12] In a study of T-cell autoimmunity in diabetes mellitus, CD56⁺ T-cell subsets were found to be more autoaggressive effector cells than their CD56⁻ counterparts.[13] One striking property of these CD56⁺ T cells is their ability to produce both Th1 and Th2 cytokines (IFN-γ, TNF-α, IL-4, and IL-10) rapidly on TCR engagement at inflammatory sites.[14]

Cyclosporine (Cs) is widely used to treat immune-mediated ocular disorders and seems particularly useful for treating patients with bilateral, sight-threatening uveitis of a noninfectious origin.[15] Cs is thought to inhibit calcium-dependent T-cell activation therapeutically and to block the genetic expression of cytokines,[16] and is known to be effective for treating severe refractory bilateral uveitis, such as Behçet's disease, usually in combination with prednisone (Pd). Sugi-Ikai et al.[17] reported that effective Cs treatment in patients with Behçet's decreases the populations of IL-2- and IFN-γ–producing T cells. However, phenotypic and functional changes of CD56⁺ T cells in patients with Behçet's uveitis after immunosuppressive treatments, have not been prospectively monitored. In this study, we investigated the population changes in lymphocyte subsets and cytokine status (IFN-γ, IL-4, and IL-10) in patients with Behçet's uveitis after Cs/Pd treatment.

METHODS

Patient Selection

Patients with complete Behçet's disease (according to the criteria of the Japanese Behçet's disease research committee[18]) and active panuveitis were recruited from the uveitis clinic at Seoul National University Hospital from March 2002 to October 2003. Other etiologies were excluded after a complete ocular and systemic examination. Patients who had used corticosteroids (topical, oral, or intravenous) or immunosuppressive agents such as Cs within 1 month of the study's commencement were also excluded. All patients had an active intraocular inflammation (anterior chamber cells >2+). Aqueous and blood samples were obtained before the start of any topical or systemic treatment. All tenets of the Declaration of Helsinki were adhered to,

TABLE 1. Patient Characteristics

Sex/Age (y)	Ocular Features	Previous Treatments*	Doses of Cs/Pd (mg/kg)			Chamber Cells (Pre/Post)‡
			Initial	**1 mo**	**2 mo**	
M/43	B panuveitis	T + S + C†	3/0.4	4/0.4	5/0.3	3+/1+
M/39	B panuveitis	T + S + C	3/0.4	4/0.4	5/0.4	4+/3+
M/47	B panuveitis	T + S + C	3/0.4	4/0.4	5/0.3	2+/1+
F/30	B panuveitis	T + S + C	3/0.4	4/0.4	5/0.4	3+/2+
F/21	R panuveitis	T	3/0.4	3/0.3	3/0.2	3+/1+
M/31	R panuveitis	C	3/0.4	4/0.4	5/0.3	3+/1+
M/37	B panuveitis	C	3/0.4	4/0.4	5/0.3	4+/1+
F/40	B panuveitis	C	3/0.4	3/0.3	3/0.2	3+/0
M/38	B panuveitis	—	3/0.4	3/0.3	3/0.2	3+/0
M/33	B panuveitis	—	3/0.4	4/0.4	5/0.4	4+/2+

B, bilateral disease; R, right eye disease; T, topical steroids; S, systemic steroids; C, colchicines.

* All previous treatments had been stopped at least 1 month before combined Cs/Pd treatment.

† Anterior chamber cells were evaluated immediately before combined Cs/Pd treatment and 2 months later.

approval was received from the Investigational Review Board of the Seoul National University Clinical Research Institute, and informed consent was obtained from all patients and healthy control subjects.

Ten patients with Behçet's were recruited for the study (Table 1). Baseline immunologic studies on population changes of lymphocyte subsets and cytokine status (IFN-γ, IL-4, and IL-10) were performed before Cs (3 mg/kg per day) and Pd (0.4 mg/kg per day) treatment. Doses were adjusted according to clinical responses and side effects. Topical corticosteroid therapy was combined with systemic treatments in all patients, whereas periocular corticosteroid (triamcinolone, 40 mg) were used in only one patient after the baseline immunologic study. After 2 months of combined low-dose Cs/Pd treatment, immunologic measurements were repeated in each patient. Ten age- and sex-matched healthy control subjects were used for comparison purposes.

Phenotypic Analysis of Circulating CD56$^+$ T Lymphocytes

Ten milliliters of heparinized venous blood was obtained from each patient before and after treatment and also from healthy control subjects. Serum was stored at −70°C until used in cytokine ELISA. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation at 400*g* on a single-density gradient (Ficoll-Hypaque; Amersham Pharmacia Biotech, Piscataway, NJ). PBMCs were recovered from the buffy coat layer and washed twice to remove red blood cells. Surface phenotypes of cells were identified with monoclonal antibodies (mAbs) in conjunction with a three- or four-color staining method. Tube A contained isotype-matched control mAbs; tube B contained mAbs directed toward CD3 (FITC)/CD4 (APC)/CD8 (Cy-Chrome)/CD56 (PE); tube C contained CD3 (PerCP)/CD56 (FITC)/TCR-γδ (PE); and tube D contained CD3 (PerCP)/CD56 (FITC)/Vα24 (PE). Aliquots (100 μL) of cells were placed in round-bottomed polystyrene tubes, and the directly conjugated mAbs were added at predetermined optimal dilutions. After a 30-minute incubation, aliquots were washed twice in flow cytometry buffer (phosphate-buffered saline with 0.2% bovine serum albumin; FACS; BD Biosciences, San Jose, CA). All mAbs were purchased from BD Biosciences, and data was acquired with their flow cytometer and software (FACSCalibur and Cell Quest; BD Biosciences) and analyzed on computer. The absolute numbers of lymphocytes in each subset were calculated from complete blood counts and from relative proportions of flow cytometry data. Because CD56 expression levels may be divided into the CD56^{dim} and CD56^{bright} proportions, we also measured the mean fluorescence intensities (MFIs) of CD56 in CD8^{high}CD56$^+$ T cells and γδ$^+$CD56$^+$ T cells.

Intracellular Cytokine Staining

Cytokine (IFN-γ, IL-4, and IL-10) production by circulating CD3$^+$, CD8^{high}, and γδ T cells (with versus without CD56) was determined by intracellular cytokine staining according to previously described procedures.[19] Briefly, PBMCs (10^6/well) were stimulated in a 96-well round-bottomed plate containing phorbol myristate acetate (PMA, 50 ng/mL; Sigma-Aldrich, St. Louis, MO) and calcium ionomycin (1 μM; Sigma) for 6 hours at 37°C. Intracellular cytokine detection was optimized by activating cells in the presence of 1 μM brefeldin A (BD Biosciences), thereby preventing cytokine secretion and allowing intracytoplasmic accumulation. Unstimulated control wells were incubated with brefeldin A alone. Cells were fixed with 4% paraformaldehyde at the end of a 6-hour incubation period. They were also permeabilized with a wash solution containing saponin (Perm/Wash; BD Biosciences) to allow intracellular access of PE-labeled anti-cytokine mAbs. For negative control experiments, cells were stained with PE-labeled isotype-matched control mAbs. For phenotype staining, cells were incubated for 30 minutes at 4°C with mAbs directed toward CD3 (PerCP), CD8 (cytochrome), TCR-γδ (APCs), and CD56 (FITC) in flow cytometry buffer (FACS; BD Biosciences). They were immediately analyzed on the flow cytometer and the data were processed by the accompanying software (CellQuest; BD Biosciences). In both unstimulated and stimulated wells, analysis gates were set for lymphocytes using forward- and side-scatter properties, and the frequencies of cytokine producing cells were calculated by phenotype gating using anti-CD3, anti-CD8, anti-TCR-γδ, and anti-CD56. A gating histogram was determined for each cytokine using isotype controls and unstimulated control wells (Fig. 1).

Cytokine ELISA in Aqueous Humor and Serum

Aqueous humor was sampled from all patients before treatment and from 10 healthy control subjects during cataract surgery. Aqueous humor (200–300 μL) was immediately aliquoted into three microfuge tubes precoated with EDTA to prevent cell clumping. Aqueous samples were then centrifuged at 400*g* for 5 minutes at 4°C. Supernatants were stored at −0°C until required for cytokine ELISA. Serum samples were thawed only once immediately before cytokine assay. To measure IFN-γ, IL-4, and IL-10, we used sandwich ELISAs specific for each human cytokine. The minimum detectable concentration of each cytokine was as follows: IFN-γ, 1.5 pg/mL; IL-4, 1.5 pg/mL; and IL-10, 1.5 pg/mL. OD$_{450nm}$ of diluent controls were subtracted to construct standard curves.

Statistics

Differences in the phenotypic expressions of lymphocytes and cytokine levels between 10 patients with Behçet's uveitis and 10 healthy
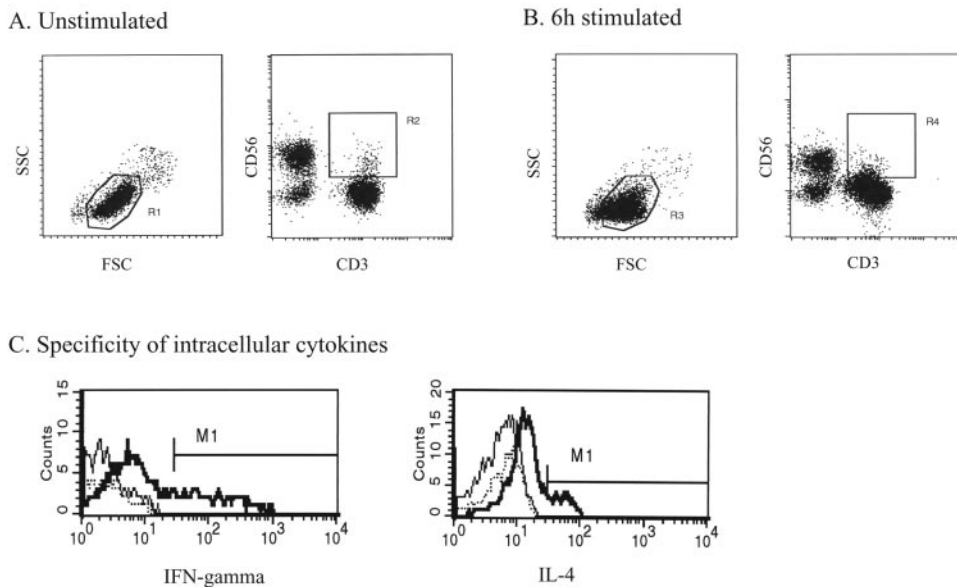
A. Unstimulated

B. 6h stimulated

C. Specificity of intracellular cytokines

FIGURE 1. Analysis gates and histograms for cytokine producing cells. (**A**) Unstimulated cells were gated on lymphocytes preincubated with anti-CD3 and anti-CD56. (**B**) After a 6-hour stimulation with phorbol myristate acetate (50 ng/mL) and calcium ionomycin (1 μM), stimulated cells were gated as described for unstimulated cells. (**C**) Histograms illustrating intracellular cytokine staining obtained without stimulation (*thin trace*) gated on R1 $\times$ R2, and after stimulation (*thick trace*) gated on R3 $\times$ R4, and mouse IgG1-negative isotype control (*broken trace*). Cells of patients with Behçet's uveitis stained for IFN-γ, whereas cells of patients with atopic dermatitis stained for IL-4.

control subjects were compared by the nonparametric Mann-Whitney test. In addition, the Wilcoxon matched-pair signed-rank test was used to identify differences between values obtained before and after treatment in the 10 patients. $P < 0.05$ was considered significant.

RESULTS

Normalization of CD56$^+$ T-Cell Populations in Patients with Behçet's Uveitis by Cs/Pd Treatment

The populations of CD56$^+$ T cells, especially in the subsets CD8^{high}CD56$^+$ and CD56$^+\gamma\delta$ T cells, were significantly increased in the peripheral blood of patients with active Behçet's uveitis versus control subjects (Table 2). After Cs/Pd treatment, the populations of CD56$^+$ T-cell subsets normalized (Table 2; Fig. 2). In patients and healthy control subjects, CD56$^+$ T cells were separated on the basis of CD56 expression, and the CD56^{bright} cells dominated CD8^{high}CD56$^+$ T cells, whereas CD56^{dim} cells dominated CD56$^+$ $\gamma\delta$ T cells. Furthermore, the MFIs of CD56 on CD56$^+$ T-cell subsets were also significantly

higher at the pretreatment stage than in healthy control subjects and returned to normal levels after treatment (MFI: for CD8^{high}, 427 $\pm$ 49 vs. 308 $\pm$ 43 and 334 $\pm$ 48; for $\gamma\delta$, 59 $\pm$ 5 vs. 47 $\pm$ 3 and 51 $\pm$ 2, respectively). In contrast, total T-lymphocyte counts and the absolute numbers of CD3$^+$CD56$^-$ cells were no different before and after treatment in patients with Behçet's. The population of CD4$^+$CD56$^-$ cells was depressed before treatment, but recovered to a normal level after treatment. The number of CD8^{high}CD56$^-$ cells and Vα24$^+$CD56$^-$ cells was similar in patients and healthy control subjects and did not change after treatment (Table 2). However, CD56$^-\gamma\delta$ T cells were significantly increased in the peripheral blood of patients with active Behçet's uveitis versus control subjects and then decreased after Cs/Pd treatment (Table 2). Given that topical steroids may inhibit the activation of lymphocytes by downregulating ocular inflammation, we measured the proportions of lymphocytes subsets in the peripheral blood of 10 patients with idiopathic anterior uveitis before and after 4 weeks of topical steroid treatment. We found that the proportions of lymphocyte subsets such as

TABLE 2. The Phenotypic Changes of Lymphocyte Subsets in Patients with Behçet's Uveitis before and after 2 Months of Low-Dose Cs/Pd Treatment

		Patients	
	Healthy Controls (*n* = 10)	**Pretreatment (*n* = 10)**	**Posttreatment (*n* = 10)**
CD3+CD56$^-$	71.1 $\pm$ 5.1 (1568 $\pm$ 289)	59.9 $\pm$ 8.2 (1441 $\pm$ 310)	68.7 $\pm$ 11.6 (1602 $\pm$ 228)
CD4$^+$	44.4 $\pm$ 6.9 (986 $\pm$ 259)	31.6 $\pm$ 6.2 (762 $\pm$ 208)*	39.3 $\pm$ 7.2 (914 $\pm$ 121)†
CD8^{high}	23.0 $\pm$ 4.7 (499 $\pm$ 102)	24.5 $\pm$ 5.3 (587 $\pm$ 147)	25.4 $\pm$ 7.3 (597 $\pm$ 181)
$\alpha\beta$ TCR$^+$	69.4 $\pm$ 7.3 (1488 $\pm$ 145)	54.9 $\pm$ 6.3 (1325 $\pm$ 121)*	64.7 $\pm$ 7.3 (1527 $\pm$ 141)†
$\gamma\delta$ TCR$^+$	2.9 $\pm$ 2.5 (65 $\pm$ 58)	5.2 $\pm$ 2.0 (125 $\pm$ 52)*	3.3 $\pm$ 1.6 (75 $\pm$ 36)†
Vα24$^+$	0.8 $\pm$ 0.2 (12 $\pm$ 5)	1.0 $\pm$ 0.4 (15 $\pm$ 7)	0.9 $\pm$ 0.2 (14 $\pm$ 4)
CD3$^+$CD56$^+$	4.0 $\pm$ 1.7 (80 $\pm$ 32)	12.7 $\pm$ 5.8 (296 $\pm$ 114)*	4.3 $\pm$ 0.9 (100 $\pm$ 24)
CD4$^+$	0.4 $\pm$ 0.3 (9 $\pm$ 7)	0.7 $\pm$ 0.3 (16 $\pm$ 6)*	0.3 $\pm$ 0.1 (7 $\pm$ 4)†
CD8^{high}	2.0 $\pm$ 1.0 (40 $\pm$ 22)	6.9 $\pm$ 3.4 (160 $\pm$ 71)*	2.1 $\pm$ 0.6 (49 $\pm$ 14)†
$\alpha\beta$ TCR$^+$	2.3 $\pm$ 1.1 (48 $\pm$ 23)	7.2 $\pm$ 3.1 (171 $\pm$ 63)*	2.5 $\pm$ 1.0 (54 $\pm$ 20)†
$\gamma\delta$ TCR$^+$	1.8 $\pm$ 1.1 (36 $\pm$ 22)	5.8 $\pm$ 4.0 (136 $\pm$ 85)*	2.0 $\pm$ 1.1 (46 $\pm$ 24)†
Vα24$^+$	0.1 $\pm$ 0.1 (2 $\pm$ 1)	0.2 $\pm$ 0.2 (3 $\pm$ 3)	0.1 $\pm$ 0.1 (2 $\pm$ 2)

Data are expressed as the percentage of total lymphocytes, with the absolute number of cells in parentheses (mean $\pm$ SD cells/mm^2). Significant difference from healthy controls (*) or pretreatment values (†).
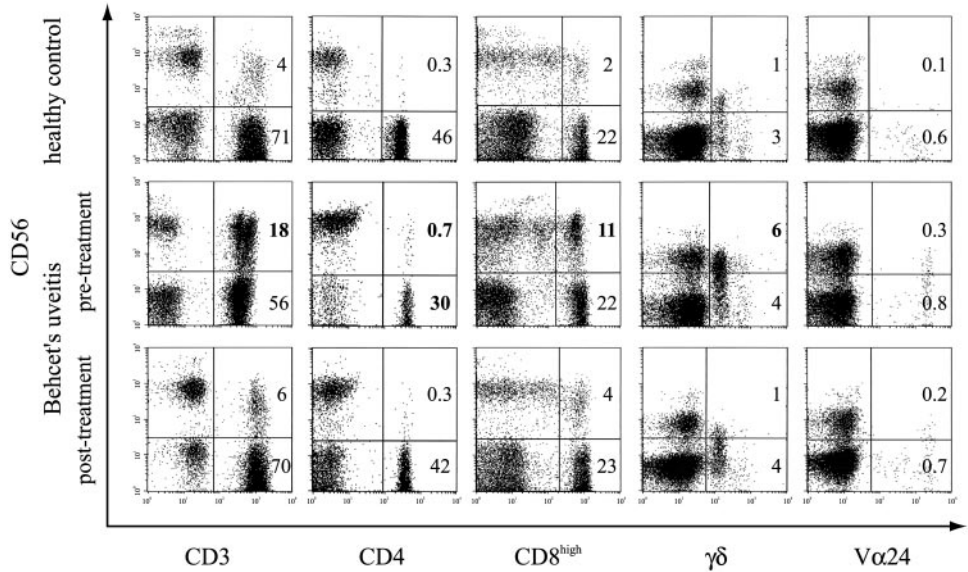
Figure 2. Representative flow cytometry showing the downregulation of CD56 expression on CD3$^+$ T cells in patients with Behçet's uveitis in response to combined cyclosporine and prednisone treatment. Four- or three-color staining of peripheral blood mononuclear cells with anti-CD3, CD4, CD8, CD56, γδ TCR, and Vα24 mAbs was performed in healthy control subjects and in patients with Behçet's. Pretreatment, upregulated CD56$^+$ T-cell subsets (*bold numbers*) including CD3$^+$CD56$^+$, CD4$^+$CD56$^+$, CD8^{high}CD56$^+$, and CD56$^+$γδ T cells were downregulated after treatment.

CD56$^+$ T cells were similar before and after treatment in these patients (data not shown).

Suppression of IFN-γ Production by Circulating CD56$^+$ T Cells by Cs/Pd Treatment

The number of circulatory CD56$^+$ T cells producing IFN-γ was higher before treatment in patients with Behçet's than in healthy control subjects. In contrast, the percentage of IL-4– and -10–producing cells was lower (Table 3; Fig. 3). After Cs/Pd treatment, the percentage of IFN-γ–producing CD56$^+$ T cells was significantly reduced (47% ± 13% vs. 29% ± 21%), whereas the percentage of IL-4– and -10–producing CD56$^+$ T cells was significantly increased (0.4 ± 0.2% versus 3.4 ± 1.5% for IL-4; 0.7% ± 0.3% versus 3.0% ± 2.4% for IL-10; Table 3; Fig. 3). Similar findings were observed in both CD8^{high}CD56$^+$ and CD56$^+$γδ T-cell subsets. In contrast, the frequencies of IFN-γ–, IL-4-, and IL-10–producing cells did not change markedly in CD56$^-$ T-cell subsets after treatment (Ta-

ble 3). As shown in Table 1, three patients showed only slight clinical improvement (anterior chamber cells > 1+) after 2 months of Cs/Pd treatment. In these patients, the level of IFN-γ–producing CD56$^+$ T cells did not change significantly, though the population of CD56$^+$ T cells decreased significantly after Cs/Pd treatment.

Elevation of IL-4 Levels in Serum by Cs/Pd Treatment

Pretreatment levels of IFN-γ and IL-4 in both aqueous humor and serum were higher in patients than in healthy control subjects (Fig. 4). In addition, the levels of IFN-γ and IL-4 in aqueous humor were much higher than in serum from the same patients. Serum levels of IL-4 were significantly elevated by treatment (30 ± 17 pg/mL vs. 127 ± 38 pg/mL, *P* < 0.01). Levels of IL-10 in aqueous humor and serum were below the detection limit (<1.5 pg/mL) in all patients with Behçet's uveitis and remained low in serum after treatment.

TABLE 3. Intracellular Cytokine Staining of Lymphocyte Subsets in Patients with Behçet's Ureitis before and after 2 Months of Low-Dose Cs/Pd Treatment

	Healthy Controls (*n* = 10)	Patients	
		Pretreatment (*n* = 10)	Posttreatment (*n* = 10)
CD3+56$^-$ stimulated			
IFN-γ	14 ± 6	23 ± 7*	21 ± 19
IL-4	0.8 ± 0.8	0.5 ± 0.3	0.6 ± 0.7
IL-10	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
CD3$^+$CD56$^+$ stimulated			
IFN-γ	19 ± 10	47 ± 13*	29 ± 21†
IL-4	2.7 ± 1.3	0.4 ± 0.2*	3.4 ± 1.5†
IL-10	2.5 ± 0.9	0.7 ± 0.3*	3.0 ± 2.4†
γδ$^+$CD56$^+$ stimulated			
IFN-γ	18 ± 10	46 ± 17*	31 ± 12†
IL-4	3.0 ± 1.6	0.4 ± 0.5*	4.9 ± 1.2†
IL-10	1.0 ± 0.4	0.6 ± 0.5	1.2 ± 1.1
CD8^{high}CD56$^+$ stimulated			
IFN-γ	20 ± 6	52 ± 11*	28 ± 18†
IL-4	1.0 ± 0.5	0.5 ± 0.3*	3.4 ± 1.8†
IL-10	2.5 ± 1.4	0.9 ± 0.6*	3.2 ± 1.8†

Significant difference from healthy controls (*) or pre-treatment values (†).
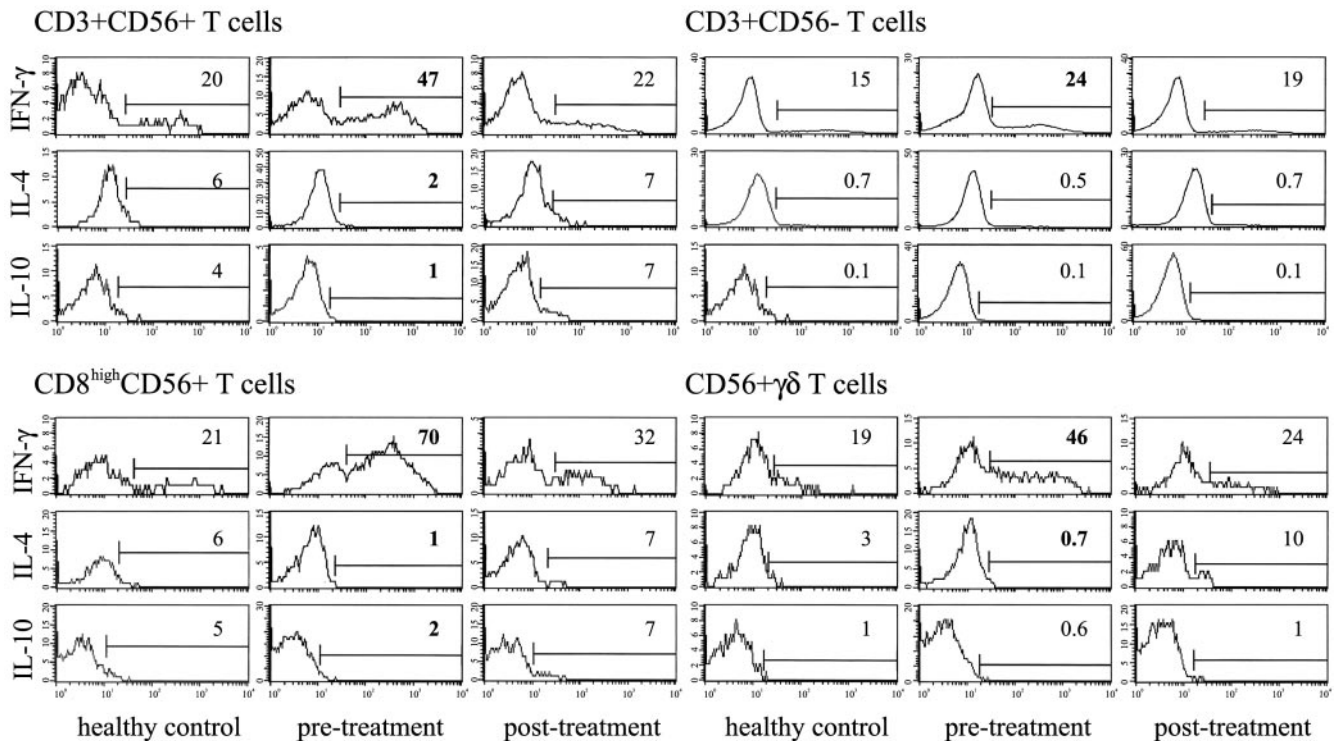
CD3+CD56+ T cells   CD3+CD56- T cells

CD8^high CD56+ T cells   CD56+γδ T cells

healthy control   pre-treatment   post-treatment   healthy control   pre-treatment   post-treatment

FIGURE 3. Histograms of the results of flow cytometric analysis of intracellular cytokines in CD3$^+$CD56$^+$, CD3$^+$CD56$^-$, CD8^{high}CD56$^+$, and CD8^{high}CD56$^-$ cells. PBMCs of healthy control subjects and patients with Behçet's uveitis were stimulated with phorbol myristate acetate and calcium ionomycin and stained for intracellular cytokines (IFN-γ, IL-4, and IL-10) and surface markers (CD3, CD8, and CD56). *Bold numbers*: significant difference from the control subjects or pretreatment levels ($P < 0.05$).

DISCUSSION

In this study, we found that the IFN-γ–producing CD56$^+$ T cells are elevated in patients with active Behçet's uveitis, illustrating that IFN-γ–producing CD56$^+$ T cells are a pathogenic T-cell subset in the disease. This increase in cell population mainly consisted of CD8^{high}CD56$^+$ and CD56$^+$γδ T cells rather than CD4$^+$CD56$^+$ and Vα24$^+$CD56$^+$ T cells. Moreover, we found that the elevated CD56$^+$ T-cell count in the pretreatment stage reduced to the normal level in response to Cs/Pd treatment, without significant changes in total T lymphocytes and CD56$^-$ T cells. Although several studies have reported that CD3$^+$, CD4$^+$, CD8^{low}, and γδ T cells coexpressing CD56 are increased in Behçet's disease,[9,10,20,21] few prospective studies have been conducted to investigate phenotypic changes in the same patients after Cs/Pd treatment.

Our results show that CD56 expression on circulating T cells may be a useful marker for monitoring the therapeutic effects of Cs/Pd treatment in patients with Behçet's uveitis. A recent study in a T-cell-mediated autoimmune disease suggested that CD56 expression may reflect the aggressiveness of autoreactive effector T cells.[13] Satoh et al.,[12] reported that cytotoxic αβ or γδ T cells coexpressing CD56 are induced from human peripheral blood lymphocytes in a Th1 dominant microenvironment. It has also been reported that IL-12, a possible Th1 cytokine that augments the cytotoxic functions of CD56$^+$ T cells, is increased in the aqueous humor and vitreous samples of patients with uveitis.[22] We also showed that the aqueous humor of patients with active Behçet's uveitis is rich in IFN-γ, a representative Th1 cytokine. Thus, the increased circulating CD56$^+$ T cells in our patients may harm ocular structures after infiltrating the eye. One may suspect that the CD56$^+$ T cells in our study were a subset of cytokine-induced killer cells. However, CD56$^+$ T cells may differ from cytokine-induced natural killer cells because of the presence of TCR and
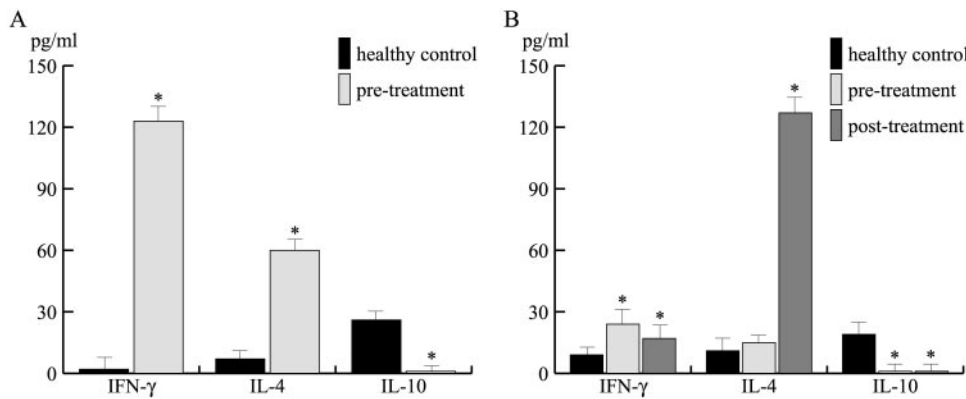

A

B

FIGURE 4. Cytokine levels in aqueous humor (**A**) and serum (**B**) in 10 healthy control subjects and in 10 patients with Behçet's. The aqueous humor and serum were drawn from the same subject. Patients were treated with Cs and Pd. *Significantly different from healthy control subjects.

high expression levels of CD8. They can be derived from the conventional CD56⁻ T cells by chronic antigenic stimulation and designated as a cytolytic effector T cells.[12,23,24] Moreover, NK receptors like CD56 are expressed on subsets of effector T cells, particularly those that have replicated extensively, and may act as costimulatory signals mediated through the T-cell antigen receptor.[25]

Our results regarding cytokine production in active Behçet's uveitis are in line with those in previous reports[8,17,26] and confirm that a strong Th1-polarized cellular immune response could play a critical role in disease pathogenesis. The frequency of IFN-γ–producing T cells increased in our patients with active Behçet's uveitis. IFN-γ, a representative Th1 cytokine, was reported to promote cytotoxic CD8⁺ T cell-mediated target cell destruction through the upregulation of MHC class I expression and to induce NO, which is cytotoxic to vascular endothelial cells.[27,28] We also found that levels of IFN-γ were elevated in both the aqueous humor and serum of patients with Behçet's uveitis. Furthermore, the levels of IFN-γ were much higher in aqueous humor than in serum, which indicates that it may be actively produced in eyes with Behçet's uveitis.

In this study, our intracellular cytokine staining showed that IFN-γ production was increased in both CD56⁺ and CD56⁻ T cells compared with healthy control subjects, but augmented by CD56⁺ T-cell subsets such as CD8^high CD56⁺ and CD56⁺ γδ T cells more than CD56⁻ T cells. Cs/Pd treatment suppressed IFN-γ production in CD56⁺ T-cell subsets rather than CD56⁻ T cells. This suppression may be attributed to IL-2 gene expression inhibition by Cs, because cytokine production in CD56⁺ T cells has been reported to be more dependent on IL-2 than CD56⁻ T cells.[29] Moreover, inducible NK receptors appear on subsets of rapidly proliferating effector T cells, which are more sensitive to treatment.[25] However, the increased IFN-γ production did not return to normal levels. This may be explained in part by suggestions that T cells in Behçet's disease respond to specific or nonspecific stimulation in a hypersensitive manner because of intrinsic T-cell defects.[30] Thus, it is conceivable that some of the hypersensitivity may remain after treatment, because Cs/Pd treatment does not completely restore these underlying defects.

IL-4 and -10, two representative Th2 cytokines, were markedly lower in the CD56⁺ T cells of pretreated patients with Behçet's, though levels normalized after treatment. In contrast, CD56⁻ T cells did not reveal any significant difference in cytokine production, irrespective of inflammation. This implies that CD56⁺ T cells play a major role in the immunopathogenesis of and in recovery from Behçet's uveitis. IL-4 production by CD56⁺ T cells and the serum levels of IL-4 were significantly increased by Cs/Pd treatment, and the resultant levels were higher than those in the control subjects. These results suggest that Th2 immune responses may not be inhibited but are somewhat augmented by Cs/Pd treatment. Previous studies on cytokine profiles in patients with Behçet's revealed that IL-4–producing cells and serum concentrations were elevated irrespective of inflammatory activity.[31,32] This difference regarding IL-4 production and inflammatory activity may reflect different stages of the immunologic processes or the inclusion of patients undergoing ongoing immunosuppressive treatment. This study enrolled only patients with Behçet's who had severe panuveitis and had not received immunosuppressive treatment within 1 month of the study's commencement. Thus, our results show a relatively consistent distribution of lymphocyte subset percentages and cytokine profiles. It may be concluded that Cs/Pd treatment in Behçet's uveitis selectively suppresses Th1 immune response and aids the restoration of the underlying Th2 responses. However, further study is needed to determine whether the Th2 immune response plays a protective role in Behçet's disease.

We also found that attenuated populations of CD4⁺ cells in patients with Behçet's uveitis normalized after Cs/Pd treatment. Sakane et al.,[33] reported that the CD4⁺-CD8⁺ cell ratio was reduced in patients with Behçet's, as a result of a decrease in CD4⁺ cells and a concomitant increase in CD8⁺ cells. The CD4⁺-CD8⁺ ratio normalized during the inactive phase. The similar number of CD8^high CD56⁻ T cells between control subjects and patients, irrespective of treatment, indicates that increased populations of CD8^high T cells express CD56. In this study, γδ T cells with or without CD56 were increased in patients with active Behçet's uveitis. γδ T cells are well known to increase in the peripheral blood of patients with Behçet's with mucocutaneous manifestations and to correlate with disease activity.[34,35] Although functional differences between CD56⁺ and CD56⁻ γδ T cells in patients with Behçet's are not clear, CD56 expression may be associated with the potent cytotoxic activity, since it can be induced from γδ T cells in IL-2- and -12-rich microenvironments.[12] In our patients, CD56⁺ γδ T cells showed more dramatic changes in the population and cytokine production after treatment than did the CD56⁻ subset, thus implying that Cs/Pd treatment affects both αβ and γδ⁺ CD56⁺ T cells rather than CD56⁻ T cells. In addition, the finding that total lymphocyte count did not change after Cs/Pd treatment confirms that Cs use does not result in leukopenia.[36]

In summary, the populations and IFN-γ production by CD56⁺ T-cell subsets were increased in patients with active Behçet's uveitis, but normalized after combined low-dose Cs/Pd treatment without significant changes in total T lymphocyte counts and CD56⁻ T cells. These results imply that IFN-γ–producing CD56⁺ T cells are important pathogenic immune cells and that current Cs/Pd treatment for Behçet's disease selectively affects the CD56⁺ T-cell subset.

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