

Insufficient expression of Fas antigen on helper T cells in Behçet's disease

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Abstract

Aims—To investigate the lack of equilibrium in the regulatory mechanism of the immune system in Behçet's disease (BD), the expression of Fas antigen, an apoptosis related antigen, on peripheral blood lymphocytes from BD patients was analysed.

Methods—Twenty one BD patients were the subjects in this study. Ten healthy adults were examined as controls. Cell surface antigens of lymphocytes were analysed with flow cytometry.

Results—There was a significant ($p < 0.01$) difference in the proportion of CD4 positive cells with CD25 between BD patients with active uveoretinitis (27.6% (SD 8.4%)) and the controls (14.7% (2.3%)), but no significant difference in the proportion of CD4 or CD45RO positive cells with Fas. On the other hand, the proportion of CD8 positive cells with Fas was significantly ($p < 0.01$) higher in BD patients with active uveoretinitis (45.6% (11.6%)) than in those with inactive uveoretinitis (23.8% (8.1%)) or in the controls (24.4% (2.5%)). The proportion of CD19 positive cells with Fas was also significantly ($p < 0.01$) higher in BD patients with active uveoretinitis (13.0% (5.0%)) than in the controls (5.1% (2.1%)).

Conclusion—The insufficient expression of Fas on activated CD4 positive T cells and its high expression on CD8 positive T cells seem to play an important role in the chronic inflammation in BD.

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A mechanism for maintaining the equilibrium between the rate of cell generation and cell death is required in multicellular organisms. Programmed cell death is considered to play a major role in this process, and is distinguished from accidental cell death, 'necrosis', caused by sudden injury or ischaemia. Apoptosis is a certain type of programmed cell death that is characterised by cell shrinkage, condensation of cytoplasmic and nuclear material, and DNA fragmentation.¹ Previous reports showed that it is also involved in negative and positive selection in thymus during the development of the immune system.²

Fas antigen (or CD95) is regarded as an apoptosis related antigen. It belongs to the tumour necrosis factor (TNF) – nerve growth factor (NGF) receptor family.³ Fas antigen is reported to be involved in the control of

lymphocyte proliferation not only in the development of the immune system but also in a mouse model of autoimmune disease.⁴

On the other hand, Behçet's disease (BD) is a chronic systemic inflammatory disease whose immunopathogenesis is still controversial. Although some studies have been made on the dysfunction of BD cellular immunity,⁵ the mechanism of activation for polymorphonuclear neutrophils and T lymphocytes in inflammatory sites is still unclear. We previously reported that in vitro tumour necrosis factor- α production from peripheral blood monocytes was significantly increased in BD patients with active uveoretinitis, and it was decreased significantly after treatment with immunosuppressive agents.⁶

Activated immunocompetent cells are thought to be deleted by apoptosis after the acute phase of inflammation in various diseases, but active inflammation persists in BD. Thus, there may be some inequity in the regulatory mechanism of the immune system in BD. In this work, we investigated the role of Fas antigen in the pathogenesis of BD with flow cytometric analysis.

Patients and methods

PATIENTS

Twenty one BD patients, who were seen in the Uveitis Survey Clinic of the Yokohama City University Hospital, Japan, were the subjects of this study. All of them had active uveoretinitis or a history of uveitis. The average age of the patients was 38 years. Five of them were diagnosed as the complete type and the rest of them as the incomplete type, according to the 1987 criteria of the Behçet's Disease Research Committee in Japan.⁵ Ten age matched healthy adults were also examined as controls. All procedures followed the tenets of the Declaration of Helsinki.

CLASSIFICATION OF ACTIVITY

The cases were divided into two groups according to the activity of uveitis. Group 1 were active cases; they suffered from an attack of uveoretinitis monthly or more. Eleven cases fitted in this group. Group 2 were inactive cases; they had had no attack for 6 months or more. Ten cases fitted in this group.

THERAPY

Four cases were treated with the immunosuppressive agent cyclosporin A, commencing at a

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Table 1 Monoclonal antibodies

Conjugation	Specificity
FITC	Fas*
PE	CD25 (IL-2R)† CD45RO (Leu 45RO)†
PerC-P	CD4 (Leu 3a)† CD8 (Leu 2A)† CD19 (Leu 12)†

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†Becton-Dickinson Immunocytometry Systems, CA, USA.
FITC=fluorescein isothiocyanate; PE=phycoerythrin;
PerC-P=peridinin chlorophyll.

Table 2 Proportion of CD4 positive cells with CD25*

	Active BD (n=11)	Inactive BD (n=10)	Controls
CD25+CD4+/CD4+	27.6 (8.4)†‡	18.7 (4.3)†	14.7 (2.3)‡

*Mean (SD) (%), †p<0.05; ‡p<0.01; Mann-Whitney test was used for statistical comparisons.
Cell surface antigens were analysed by flow cytometry with an Epics Elite flow cytometer (Coulter Electronics, Hialeah, FL, USA). More than 2×10⁴ viable lymphocytes were examined in each assay.
BD=Behçet's disease. Active BD=suffering an attack of uveoretinitis monthly or more. Inactive BD=suffering no attack 6 months or more.

dose of 2 to 5 mg/kg/day. The dosage of cyclosporin A was controlled to keep at a whole blood trough concentration of 50 to 200 ng/ml. Six cases were treated with FK-506, a new immunosuppressive agent, at a dose of 0.05 to 0.15 mg/kg/day. Five cases were treated with colchicine, one case was treated with methylprednisone, and the remaining five cases were given no drug.

CELL PREPARATION

Lymphocyte subsets were detected using monoclonal antibodies (mAbs) conjugated with fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll (Table 1). To stain cells, heparinised venous blood diluted with phosphate buffered saline was incubated with mAb for 30 minutes at 5°C. After red blood cells were lysed, the cells were fixed with formaldehyde and analysed by flow cytometry with an Epics Elite flow cytometer (Coulter Electronics, Hialeah, FL, USA). In each assay, 2×10⁴ viable lymphocytes were examined. Non-specific fluorescence of mAbs to lymphocytes, when isotype matched conjugated antibodies of irrelevant specificity was substituted for mAb, was less than 0.2%. The relative proportion of each subset was obtained as a percentage of the total lymphocytes.

The Mann-Whitney test was used for statistical comparisons.

Results

There was a significant difference in the

Table 3 Proportion of CD4 positive cells with Fas*

	Active BD (n=11)	Inactive BD (n=10)	Controls
Fas+CD4+/CD4+	49.9 (10.6)	49.5 (12.7)	41.9 (9.5)
Fas+CD45RO+/CD45RO+	64.4 (5.8)	59.7 (10.7)	55.1 (16.9)

*Mean (SD) (%).

Cell surface antigens were analysed by flow cytometry with an Epics Elite flow cytometer (Coulter Electronics, Hialeah, FL, USA). More than 2×10⁴ viable lymphocytes were examined in each assay.
BD=Behçet's disease. Active BD=suffering an attack of uveoretinitis monthly or more. Inactive BD=suffering no attack 6 months or more.

proportion of CD4 positive cells with CD25 (IL-2 receptor) between BD patients with active uveoretinitis (27.6% (SD 8.4%)) and those with inactive uveoretinitis (18.7% (4.3%)) (p<0.05) or the controls (14.7% (2.3%)) (p<0.01), whereas no significant difference was seen between BD patients with inactive uveoretinitis and the controls (Table 2). We found no significant difference between BD patients with active uveoretinitis (49.9% (10.6%)) and those with inactive uveoretinitis (49.5% (12.7%)) or the controls (41.9% (9.5%)) in the proportion of CD4 positive cells with Fas. There was also no significant difference in the proportion of CD45RO positive cells (a subclass of CD4 positive cells) with Fas between BD patients with active uveoretinitis (64.4% (5.8%)) and those with inactive uveoretinitis (59.7% (10.7%)) or the controls (55.1% (16.9%)) (Table 3). On the other hand, the proportion of CD8 positive cells with Fas was significantly (p<0.01) higher in those with active uveoretinitis (45.6% (11.6%)) than in BD patients with inactive uveoretinitis (23.8% (8.1%)) or in the controls (24.4% (2.5%)). CD19 positive cells with Fas were also present in significantly (p<0.01) higher proportion in BD patients with active uveoretinitis (13.0% (5.0%)) than in controls (5.1% (2.1%)) (Table 4).

Discussion

Fas antigen is a cell surface protein that shows a similarity to TNF receptor, NGF receptor, and the human B cell antigen CD40, and is involved in the control of lymphocyte apoptosis.⁷ Mouse anti-Fas monoclonal antibody has a cytolytic activity on human cells that expresses the antigen.³ Miyawaki *et al*⁸ examined human peripheral blood lymphocyte subpopulations at various ages and found that Fas antigen was definitely detected on T and B cells, whereas its expression was absent on NK cells. They further stated that Fas antigen was expressed preferentially on CD45RO positive (memory or previously activated) cells, but not on CD45RO negative naive cells and suggested that expression of Fas antigen on T and B cells in the peripheral blood may reflect their *in vivo* antigen activated status. Amasaki *et al*⁹ reported that the expression of Fas on CD45RO positive cells of patients with systemic lupus erythematosus (SLE) was significantly higher than that of controls and

Table 4 Proportion of CD8 and CD19 positive cells with Fas*

	Active BD (n=11)	Inactive BD (n=10)	Controls
Fas+CD8+/CD8+	45.6 (11.6)†‡	23.8 (8.1)†	24.4 (2.5)‡
Fas+CD19+/CD19+	13.0 (5.0)§	9.2 (5.7)	5.1 (2.1)§

*Mean (SD) (%); †, ‡, § p<0.01; Mann-Whitney test was used for statistical comparisons.

Cell surface antigens were analysed by flow cytometry with an Epics Elite flow cytometer (Coulter Electronics, Hialeah, FL, USA). More than 2×10⁴ viable lymphocytes were examined in each assay.
BD=Behçet's disease. Active BD=suffering an attack of uveoretinitis monthly or more. Inactive BD=suffering no attack 6 months or more.

that the difference may reflect the activated cell response in SLE. Owen-Schaub *et al*¹⁰ demonstrated that, although both resting and IL-2 activated lymphocytes expressed Fas, the ability of anti-Fas antibody to mediate apoptotic cell death in lymphocytes was dependent on the status of cellular activation. These reports indicate that Fas antigen is a proper target for immunosuppressive mediations and that activated Fas positive lymphocytes may be led to apoptosis when stimulated with IL-2 or interferon- γ .

Behçet's disease is a chronic systemic inflammatory disease whose immunopathogenesis is still controversial. Some studies have been made on dysfunctions of cellular immunity in BD. In our study, Fas antigen was not upregulated remarkably in CD4 positive T cells in BD with active uveoretinitis, although CD25 antigen, which signified their activation, was highly expressed. On the other hand, Fas antigen was definitely observed in CD8 positive T cells and B cells in these patients. The expression of Fas antigen in them was significantly higher than in BD patients with inactive uveoretinitis or in the controls. Among active BD patients, there was no significant differences in Fas expression between those who had a severe attack of uveitis or those who did not at the time of examination and between those who received immunosuppressive therapy or not (data not shown).

It is suggested that, in BD patients with active uveoretinitis, activated CD4 positive T cells with insufficient expression of Fas antigen, that would not undergo apoptosis, must be responsible for the severe chronic inflammation. We have been analysing the apoptosis in these cells in response to anti-Fas antibody from clone CH-11 by the TdT mediated dUTP-biotin nick end labelling (TUNEL) method.¹¹ It is still unclear why the expression of Fas antigen remains inconsequent in active BD patients.

We reported previously the enhanced production of TNF- α from peripheral blood monocytes in BD and stated that the activation of polymorphonuclear neutrophils in BD might be caused by TNF- α , a monocyte derived inflammatory cytokine.⁶ Mangan *et al*¹² reported that cytokines derived from type 1 helper T cells (Th-1 cells) inhibit apoptosis in activated monocytes. It is possible that sustained activated helper T cells, which consecutively produce cytokines, and activate monocytes which produce TNF- α , provoke the severe chronic uveoretinitis in BD. We have observed the high proportion of Th-1 cells in CD4 positive cells from peripheral blood lymphocytes in BD (manuscript in preparation). Further detailed study must be

made on the relation between apoptosis and helper T cell subsets.

Watanabe *et al*⁴ reported that mice carrying the lymphoproliferation (lpr) mutation had defects in the Fas antigen gene. These mice develop a SLE-like autoimmune disease, including increased autoantibody production, glomerulonephritis, and development of lymphadenopathy. Steinberg¹³ suggested that failure of peripheral apoptosis of CD4 positive cells allows self reactive helper T cells to persist and drive autoantibody production in lpr mice. Suda *et al*¹⁴ detected Fas ligand on the cell surface of a cytotoxic T cell hybridoma, and suggested that Fas ligand was a type II transmembrane protein that belongs to the TNF family. They also found that Fas ligand was expressed in activated splenocytes and thymocytes, consistent with its involvement in T cell mediated cytotoxicity. It is plausible that some dysfunction in the Fas/Fas ligand system or its regulatory mechanism causes the prolonged inflammatory diseases such as BD.

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