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The Possible Role of T Cell and Macrophage in Development of Behçet's Disease-like Symptoms Induced by Herpes Simplex Virus in ICR Mice

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베체트병의 발병원인의 하나로 단순포진바이러스(herpes simplex virus:HSV)가 관여한다는 것은 알려져 왔으나, 바이러스 자체만으로는 발병 기전을 모두 설명하기에는 한계가 있다. 베체트병의 병인론에서 잘못된 면역조절기전(misimmunoregulation)으로 T cell과 macrophage의 역할에 대한 연구의 결과가 많이 발표된 바 있다. 이 연구에서는 ICR mice에서 HSV접종에 의한 Behçet's disease-like symptom의 발현에 T cell과 macrophage의 inactivation이 어떤 영향을 미치는가에 대하여 실험하였다. 4회의 anti-mac1 또는 anti-mac2 monoclonal antibody의 복강주사로 macrophage를 depletion 또는 inactivation시키거나, 6일간의 cyclosporin A 처리 또는 4회의 anti-CD4, anti-CD8 monoclonal antibody를 주사하여 T cell inactivation 또는 depletion 후 HSV inoculation으로 Behçet's disease-like symptom을 유발한 경우와 HSV inoculation 만으로 증상을 유발한 경우를 서로 비교하였다. 그 결과 anti-mac1, anti-CD4, anti-CD8 antibody, cyclosporin A를 HSV inoculation과 병행한 군에서 HSV만 inoculation한 대조군보다 낮은 발현율을 나타내었다. 이 결과로써 HSV접종에 의한 Behçet's disease-like symptom의 발현에 HSV가 감염되기 이전의 host의 면역체계, 즉 T cell과 macrophage의 상태가 중요한 역할을 함을 알 수 있었다.

Key words : Behçet's disease
Mouse
T cell and macrophage
Incidence, Etiopathogenesis

INTRODUCTION

Although the etiology of Behçet's disease is unclear, viral infection has long been postulated as being one of the factors. We already reported the presence of herpes simplex virus (HSV) DNA in saliva[1], intestinal mucosa[2], and genital mucosa[3] from patients with Behçet's disease using polymerase chain reaction (PCR). We have also been

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able to induce Behçet's disease-like symptoms in ICR mice by inoculation of HSV[4]. However, HSV alone is not sufficient to explain the pathogenesis of Behçet's disease.

There are some evidences to suggest that immunological abnormalities are important with its pathogenesis. A disturbance in circulating T cell function was found initially in a small decrease in CD4 cells[5-7] and a defect in suppressor function[8]. The mucocutaneous lesions of Behçet's disease were initially infiltrated with CD4, CD8 cells, macrophages and dendritic cells followed by neutrophils. However, more recently, attention has focused on TH1 and TH2 cytokines generated by T cells. A mixed pattern of TH1 and TH2 was observed, probably with a significant contribution from macrophages[9].

To study the possible relationship between immune regulation and induction of Behçet's disease-like symptoms, inactivation or activation of T lymphocytes or macrophages before and/or after inoculation of HSV was necessary. It has been reported that long-term treatment with silica or anti-mac1 or anti-mac2 monoclonal antibody may cause macrophage depletion or inactivation. Also treatment with anti-CD4 or anti-CD8 monoclonal antibody may cause T cell inactivation or depletion[10,11]. As an initial step of inactivation experiment, we have used several monoclonal antibodies against macrophages and T lymphocytes to inactivate T cells and macrophages followed by HSV inoculation in ICR mice to see whether they might play a certain role in the pathogenesis of Behçet's disease.

MATERIALS & METHODS

A total of 258 male, 4 to 5-week-old ICR mice were used for this study. Using the method of Hirata et al.[14], the ear-lobes of the mice were scratched with a needle, then inoculated with 1.0×10^6 plaque forming unit/ml of herpes simplex virus type 1 (F strain). Virus inoculation was performed twice with a 10 day interval, followed by 16 weeks of observation. As a control, mice were inoculated in the same site with a culture medium. And anti-mac1, anti-mac2, anti-CD4, anti-CD8, anti-mac1 + anti-mac2, and cyclosporin A were also intraperitoneally injected to mice for T cell or macrophage inactivation. These knockout methods followed Baek and Yoon's methods[10,11]. Mice were bred in temperature-and light-controlled conventional rooms (20-22℃, 12h light cycle starting at 8:00 a.m.). The mice had free access to food and water. During the experimental period, the animals were closely observed and photographed. Animals were handled in accordance with a protocol approved by our institutional animal care committee.

Antibody preparation

Hybridoma cell lines from ATCC were cultured as follows. ATCC TIB-166 (GK1.5), anti-CD4 antibody producing cell line was cultured in DMEM with 4.5 g/L glucose and 20% fetal bovine serum. ATCC TIB-105 (53-6.72), anti-CD8 antibody producing cell line was cultured in RPMI 1640 medium with 10% fetal bovine serum. ATCC TIB-128 (M1/70.15.11.5.HL), anti-mac1 antibody producing cell line was cultured in DMEM with 4.5 g/L glucose and 10% fetal bovine serum. ATCC TIB-166 (M3/38.1.2.8 HL2), anti-mac2 antibody producing cell line was cultured in RPMI 1640 medium with 20% fetal bovine serum. At the time of cell lysis without new medium exchange, the supernatant was collected and concentrated with Centriplus-50 (Amicon Inc., Beverly, MA, USA). The concentration of antibody was measured with Bio-Rad protein assay kit (Bio-Rad, Hercules, California, USA). The concentrated antibodies were used for T cell and macrophage knockout.

T cell inactivation

Mice were treated with 1mg/mouse/day of anti-CD4 or anti-CD8 monoclonal antibody intraperitoneally three times (on days -3, -2, -1) prior to viral infection and once (on day +1) after viral infection. Another group of mice was treated with 0.4 mg Cyclosporin A for 6 consecutive days prior to viral infection.

Macrophage inactivation

Mice were treated with 2mg/mouse/day of anti-Mac1 and/or anti-Mac2 monoclonal antibody intraperitoneally twice (on days −2 and −1) prior to virus infection and twice (on days +1 and +2) after viral infection.

Gross photography

Symptomatic mice were photographed with Nikon FM2 camera equipped with 105 mm microlens.

Histochemistry

The spleen tissues of mice were made into paraffin-embedded blocks and used for histochemistry. Primary antibodies (anti-mac1, CD4, CD8 antibody) were purchased from Boehringer Mannheim (Mannheim, Germany) and produced in our laboratory using hybridoma cell lines from ATCC (anti-CD4, ATCC TIB-166, GK1.5; anti-CD8, ATCC TIB-105, 53-6.72; anti-mac1, ATCC TIB-128, M1/70.15. 11.5.HL; anti-Mac-2, ATCC TIB-166, M3/38.1.2.8 HL2). Horseradish peroxidase-conjugated rabbit anti-rat Ig was used as a secondary antibody. The histochemical results were same using in commercial antibodies and produced antibodies from hybridoma cell line except anti-mac2 antibody. Anti-mac2 antibody was commercially not available.

RESULTS

The mice inoculated with HSV alone or combined with antibodies or drug manifested changes after the first virus inoculation. The signs that appeared in most groups were very similar, such as partial hair loss in the face region, erythema in the scratched earlobe, eye symptoms, and skin ulcerations of the earlobes, scruff, genitalia and other regions (Fig. 1a, b). The pattern and the incidence of manifestations were similar to our previous report in HSV injected mice. As we followed in previous paper, two or more symptoms in one mouse were considered to be an indication of Behçet's disease-like syndrome. As controls, the culture media inoculated, anti-mac1, mac2, CD4, CD8 antibody or Cyclosporin A injected mice had no Behçet's disease-like symptom.

After antibody- or cyclosporin A-injected knockout or inactivation, the spleen tissues were used to confirm the existence of macrophage or T cell on immunohistochemistry. The spleen tissue of anti-mac1 antibody injected (i.p.) mice was not stained with anti-mac1 antibody (Fig. 2d). Anti-mac2 antibody treated spleen tissue of mice was also not stained with anti-mac2 antibody (Fig. 2c) compare to PBS injected control mouse (Fig. 2a; anti-mac2, b; anti-mac1). Anti-CD4 antibody treated mouse was stained with some anti-CD4 antibody (Fig. 3c) but more faint than PBS injected control mice (Fig. 3a; anti-CD4, b; anti-CD8).

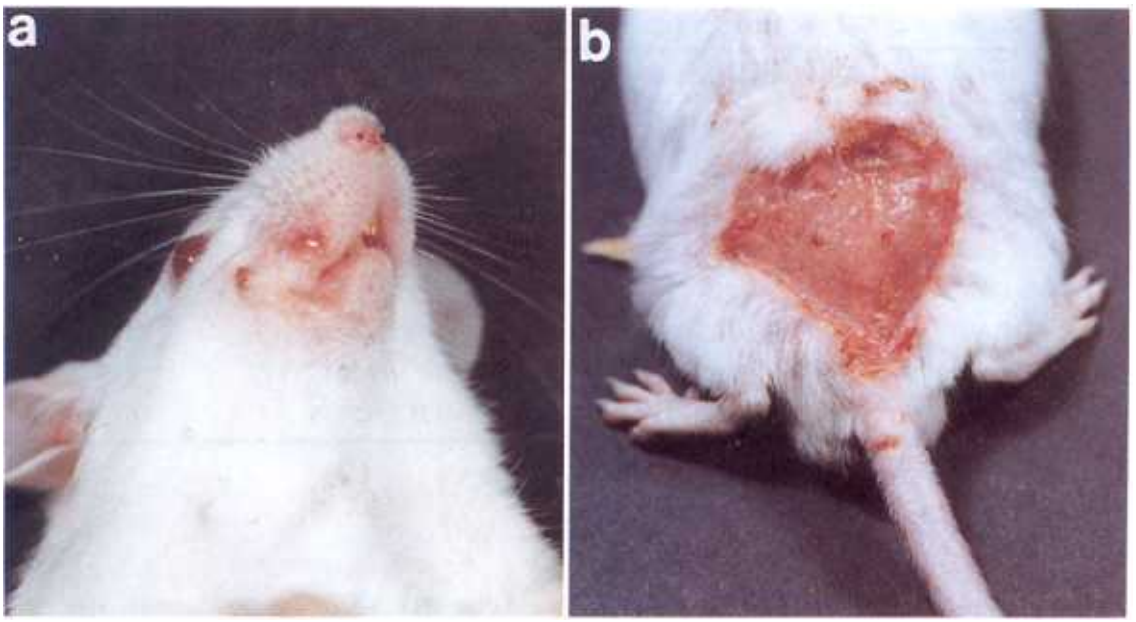

Fig. 1. Skin ulcerations of face and back after anti-mac1 antibody (a) or Cyclosporin A (b) treatment followed by HSV injection. The patterns of manifestations are simlilar to HSV only injection.

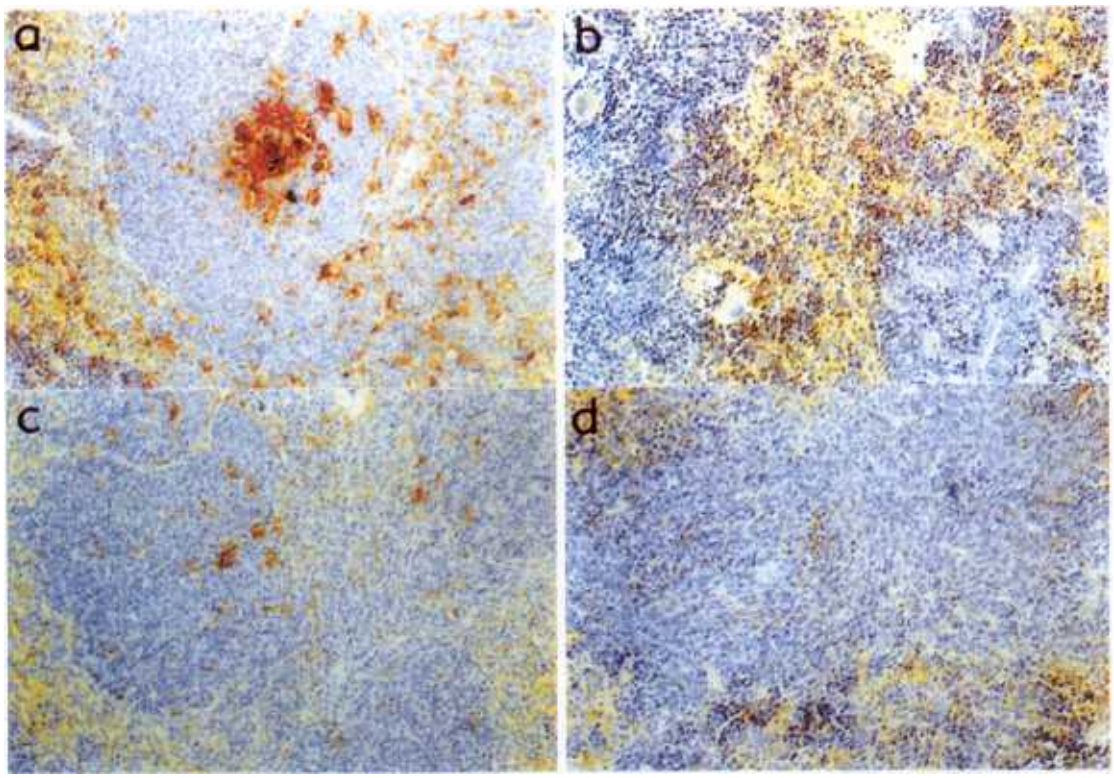

Fig. 2. Immunohistochemical results from spleen of normal mouse stained with anti-mac1 antibody (b) and anti-mac2 antibody (a). (d) is the anti-mac1 antibody treated and stained with anti-mac1 antibody, (c) is the anti-mac2 antibody treated and stained with anti-mac2 antibody.

Table 1. The number of ICR mice according to manifestation after HSV inoculation and/or immune cell inactivation.

treatment	single symptom/total number	BD-like symptom / total number	death / total number
culture media	0 / 32	0 / 32	0 / 32
HSV	6 / 30	8 / 30 (26.7%)	5 / 30
HSV+mac-1 Ab	7 / 10	2 / 10 (20%)	1 / 10
HSV+mac-2 Ab	6 / 10	3 / 10 (30%)	0 / 10
HSV+CD4 Ab	7 / 10	2 / 10 (20%)	1 / 10
HSV+CD8 Ab	9 / 10	1 / 10 (10%)	0 / 10
HSV+mac1+mac2 Ab	7 / 9	1 / 9 (11.1%)	0 / 9
HSV+Cyclosporin A	18 / 38	4 / 38 (10.5%)	0 / 38

Anti-CD8 antibody treated mouse was not stained with anti-CD8 antibody (Fig. 3d). The spleens of Cyclosporin A injected mice showed negative results when stained with anti-CD4 (Fig. 3e) or anti-CD8 antibody (Fig. 3f).

Table 1 lists the numbers/percentages of mice afflicted with HSV and/or antibodies or drug. The incidence of

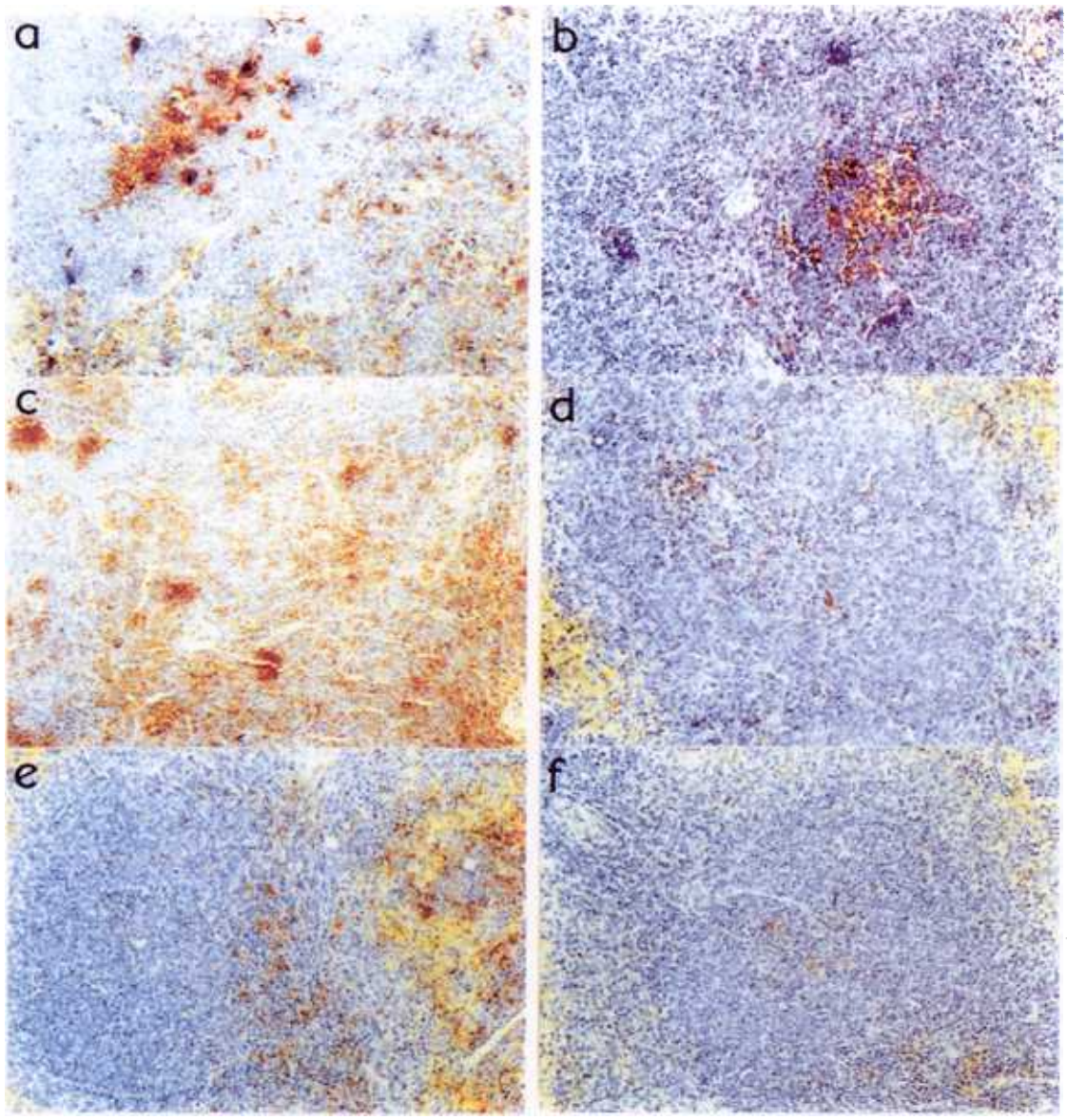

Fig. 3. Immunohistochemical results from spleen of normal mouse stained with anti-CD4 antibody (a) and anti-CD8 antibody (b), anti-CD4 antibody treated and stained(c), and anti-CD8 antibody treated and stained (d), cyclosporin A treated and stained with anti-CD4 antibody (e) and stained with anti-CD8 antibody (f).

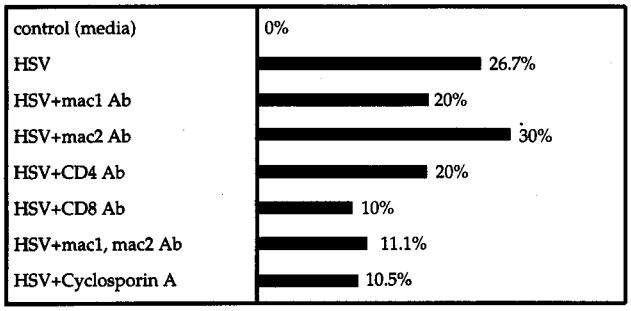

Fig. 4. Comparison of the rate of incidence in Behcet's disease-like symptom after HSV inoculation and/or immune cell inactivation

Behçet's disease-like symptoms was different in each of experimental groups. Except for the group of HSV combined with anti-mac1 antibody, all experimental groups showed lower incidence of Behçet's disease-like symptom than the group inoculated with HSV only.

DISCUSSION

Behçet's disease has been considered at times to be a viral infection or autoimmune disease; streptococcal-related antigens, specific alleles of the human major histocompatibility complex, and hazardous chemicals have also been proposed as etiologic factors[12,13,15-19]. The virus as a causative agent was first propounded by Turkish dermatologist, Hulûsi Behçet in 1937[20]. Since then, many studies have suggested a viral involvement in the disease. Eglin et al. showed by in situ DNA-RNA hybridization that at least part of the HSV-1 genome is transcribed in peripheral blood mononuclear cells of patients with Behçet's disease[21]. This was confirmed by Bonass et al. who detected HSV-1 DNA by dot blot DNA-DNA hybridization in patients with Behçet's disease[22]. Bergmann et al. found an association between intraoral ulcers and HSV among immunocompromised patients with hematologic malignancies. HSV-1 saliva cultures were positive in 9 of 16 patients with intraoral ulcers (56 percent) but negative in all 23 patients without such ulcerations[23]. In our previous study, 26 of 66 saliva specimens from patients showed an unequivocal amplified band for HSV DNA after PCR, suggesting some connection between Behçet's disease and the presence of HSV DNA. Nevertheless, there were factors to indicate that HSV was not the only pathogen involved in the patients. For example, treatment with acyclovir, which is of proven efficacy in the treatment of HSV infection, failed to alleviate the frequency and severity of orogenital ulceration or other clinical features of Behçet's disease[24]. Furthermore, investigations by Young et al. of the possible relationship of HSV-1 to the pathogenesis of the disease showed that HSV-1 stimulation of CD4+ cells of patients with Behçet's disease produced low proliferative responses[25]. This strongly implies that these patients' immune response to HSV is already impaired.

Macrophages play a central role in the immune response against immunologically active molecules[26]. The key roles of macrophages include the presentation of processed antigen to helper T lymphocytes in the context of MHC class II molecules present on the surface of macrophages. The helper T lymphocyte is activated only when it interacts with the antigen presented on the surface of a macrophage or other antigen-presenting cell. In contrast, the direct exposure of lymphocytes to an antigen in the absence of antigen-presenting cells has been shown to induce immunological tolerance to the specific antigen[27]. Therefore, it could be hypothesized that a decrease in macrophages and/or T lymphocytes may further hinder the immune process.

There is a hypothesis about the relationship between the pathophysiology of Behçet's disease and autoimmune responses present in the disease[28]. Antigens, such as bacteria, virus, heat shock protein, stimulate macrophages, and stimulated macrophages activate T cells, neutrophils, or induce tissue damage directly. Therefore, we hypothesized that inactivation of macrophage and/or T lymphocytes before HSV inoculation may decrease the expression of Behçet's disease-like symptoms.

In this study, antibody-or drug-treated knockout or inactivation of immune cells decreased the incidence of Behçet's disease-like symptoms in HSV inoculated mice, whereas the positivity of a single symptom was higher. These results suggest that the immunologic status of the host before HSV inoculation may play an important role in the induction of Behçet's disease-like symptoms. Further studies including activation of macrophages or T cells along with HSV inoculation will be necessary to confirm the relationship between immune regulation combined with viral etiology and the etiopathogenesis of Behçet's disease.

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