Streptococcal Infection in the Pathogenesis of Behçet's Disease and Clinical Effects of Minocycline on the Disease Symptoms

Fumio Kaneko, Noritaka Oyama, and Akiko Nishibu

Although the precise pathoetiology of Behçet's disease (BD) remains obscure, patients with BD have a high incidence of chronic infectious foci, indicating an enhanced susceptibility to chronic tonsillitis and dental caries. Sometimes, clinical symptoms appear after treatment of these foci in BD patients. It is believed that BD might be related to an allergic reaction to a bacterial infection in view of the many clinical symptoms, especially the presence of aphthous and genital ulcerations. An attempt to obtain cutaneous responses to bacterial antigens has been carried out using various vaccines developed from bacteria isolated from the ulcerative lesions and oral cavities of BD patients. BD patients often show intense hypersensitivity to various strains of streptococci, not only by their cutaneous reactions but also by in vitro testing. In this report, we describe our previous studies on the correlation between streptococcal antigens and the pathogenesis of BD and also discuss the recent reports of other authors. The intense hypersensitivity to streptococcal antigens acquired after streptococcal infection is thought to play an important role in the appearance of symptoms in BD patients since the production of pro-inflammatory cytokines by peripheral blood mononuclear cells (PBMC) was enhanced when stimulated with streptococcal antigen in a culture system. Minocycline, an antibiotic to which certain strains of streptococci are sensitive, reduced the frequency of clinical symptoms in BD patients as well as the production of pro-inflammatory cytokines by BD-PBMC stimulated with streptococcal antigen.

Key Words: Behçet's disease, cutaneous tests, streptococcal antigens, proinflammatory cytokines, minocycline

Behçet's disease (BD) is a chronic systemic inflammatory disorder whose main manifestations are muco-cutaneous and ocular lesions. However, the precise pathoetiology of this disease remains obscure. In Japan, 15,735 patients with BD were registered with the Ministry of Health and Welfare at the end of 1996. The number of patients increased and reached a peak in the 1980s, with a much higher rate than average in the northern part of Japan, especially on Hokkaido Island. It was suggested that patients with recurrent stomatitis (RS), which might be related to BD, were hypersensitive to streptococcal antigens based on the clinical evidence and in vitro studies (Graykowski et al. 1966; Donatosky and Bendixen, 1972). However, there were some arguments against a delayed type hypersensitivity reaction to streptococcal antigens in RS (Francis and Oppenheim, 1970).

We have attempted since the 1970s to determine whether the acquisition of streptococcal hypersensitivity after streptococcal infection represents a possible etiology of BD (Kaneko et al. 1977, 1978,
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1985b and 1991) and discuss our previous studies in this paper. We also discuss recent reports by other authors on the correlation of streptococcal allergy with BD pathogenesis as well as the therapeutic effects of minocycline hydrochloride (minocycline), an agent to which streptococcal bacteria are sensitive, on the major symptoms of BD (Kaneko, 1981).

MATERIALS AND METHODS

Streptococcal and other antigens

1) Intracutaneous tests were performed with various bacterial antigens to assess hypersensitivity against streptococcal strains in 84 BD patients, 15 RS patients, 9 patients with erythema nodosum (EN) (non-BD), 3 patients with erythema multiforme and 10 healthy controls. The bacterial antigens (bacterial vaccines, $1 \times 10^{9}$ org/ml, Hollister-Stier Lab., USA) used were as follows; *Streptococcus pyogenes* (β-haemolytics; Lancefield group A), *S. viridans* (α-haemolytics, consisting of 7 strains, Lancefield group D), *S. nonhaemolyticus* (Lancefield group D), *S. faecalis* (Lancefield group D), *Diplococcus pneumoniae* mix, *Staphylococcus* (Sta.) *aureus*, *Sta.* epidermidis, *Esherichia coli*, *Salmonella enteritidis*, *S. typhi*, *Shigella dysenteriae*, *Proteus* (Prot.) *vulgaris*, *Neisseria catarrhalis*, *Pseudomonas* (Pseud.) *aeruginosa* and *Haemophilus influenzae*. Subjects were injected with 0.1 ml of the bacterial vaccines containing $1 \times 10^{7}$ org/ml and the reactions were observed 48 hours (h) later.

2) α-haemolytic *S. salivarius*, isolated from an aphthous ulceration (AU) of a patient with BD using Mitis-Salivarius agar and a broth agar containing sheep red cells, were grown in GAM broth agar. The cell walls of *S. salivarius* (CWSS) were extracted using a modification of a method of Cummins and Harris (Araki et al. 1972) and were adjusted to $10^{5}$ ng/ml of saline. This was used as the source of streptococcal antigen in both in vitro and in vivo studies as described previously (Kaneko et al. 1977, 1985b and 1991).

3) For the cutaneous prick tests, we used several bacterial cell wall antigens, *S. pyogenes* (ATcc 19618), *S. sanguis* (ST3), *S. sanguis* (B220), *S. sanguis* (SSH-3), *S. salivarius* (HHT), *S. faecalis* (478), *Sta. aureus* Smith, *Sta.* epidermidis (Se-360), *E. coli* (0~55, *Klebsiella pneumoniae* (K-9-13) (capsular type 2), *Acinetobactor calcoaceticus* (Acineto), and *Propionibacterium acnes* (ATcc 11827), all of which were supplied by the Behçet's Disease Research Committee organized by the Ministry of Health and Welfare of Japan (Mizushima, 1988; Mizushima et al. 1989).

Patients

The group studied consisted of 13 patients with active incomplete type BD (male and female, average age, 36 years) who had been diagnosed according to Japanese diagnostic criteria (1987) and referenced with the International Study Group for Behçet's Disease (1990). Their clinical symptoms were initially observed during treatment with non-steroid antiinflammatory agents which extended over more than 3 months prior to the use of antibiotics. Following this, the patients were prescribed 100 mg of minocycline per day to be taken by capsule (Ledari Pharmaceutical Co., Tokyo, Japan) and continued for at least 3 months. All of the patients were required to keep a daily diary monitoring their clinical symptoms, AU, genital ulcerations (GU), EN-like eruptions (EN-LE) and perioligoculitis (PE).

The clinical symptoms observed were compared before and after administration of minocycline. AU and EN-LE samples of 5 patients were biopsied and examined histologically and immunohistochemically using anti-human immunoglobulins (Hoechst-Behring, Germany), anti-streptococcus group D antibodies (Ab) (bacto-FA Streptococcus group D, Difco Lab., USA) and anti-T, B cell and macrophage Abs (Leu series; Becton Dickinson Inc., USA and OKM-1; Ortho Pharmaceutical Co., USA) to detect immune complex deposits containing streptococcus-related antigen and to analyze inflammatory cell infiltrations.

Preparation of peripheral blood mononuclear cells (PBMC)

Hepatinized peripheral blood was taken from 13 patients with BD and 14 healthy subjects (average
age, 33 years) as controls. PBMC were prepared by gradient centrifugation using Lymphoprep (Nycomed Pharm Assoc. Oslo, Norway), washed in MEPS buffer (154 mM NaCl, 10mM EDTA, 9mM KH$_2$PO$_4$ and 0.047 methyl cellulose) to remove platelets and adjusted to contain 5 x 10$^5$ cells/ml in RPMI 1640 (Grand Island Biological Co., NY USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO). These were incubated with CWSS at a concentration of 10$^{-3}$ ng/ml with or without minocycline at a final concentration of 10$^{-5}$ - 10$^{-4}$M for 24-72 hat 37°C in a 5% CO$_2$ incubator. The supernatants (Sup) were harvested at 0, 6, 24, 48 and 72 h after incubation and were assayed for cytokines. The cell pellets were used for detection of mRNA expression of each cytokine by the reverse transcription polymerase chain reaction (RT-PCR).

**Determination of chemoattractants and cytokines**

The chemotactic activity of the PBMC Sup incubated with or without CWSS was assayed using a 48-well micro-Boyden chamber (Neuroprobe, Academica Japan) with a polycarbonate membrane filter (Nuclepore Co., USA). Neutrophil-rich cells from a healthy volunteer were prepared by the Lymphoprep gradient method and adjusted to contain 1.6 x 10$^5$ cells/ml in 10% FBS-PBS (0.001 M phosphate buffered saline, pH 7.2). A 50 μl aliquot of the neutrophil-rich solution was placed on the side of the membrane facing the culture Sup in each well of the chamber and was incubated for 30 min at room-temperature. After incubation, the membrane was stained with Giemsa-solution and the neutrophils which had penetrated the membrane were counted under a microscope.

Cytokines produced in the PBMC Sup were measured by an enzyme-linked immunosorbert assay (ELISA) using the following monoclonal Ab kits; an interferon (IFN)-γ kit, interleukin (IL)-1 α and IL-1 β kits, (Otsuka Assay Co., Japan), an IL-6 kit (Toray Co., Japan) and an IL-8 kit (Research and Diagnostics Systems, Minneapolis, Mn, U.S.A.). The assays were performed as follows; a microtiter plate with 96 polystyrene wells was coated with each murine monoclonal Ab and the Sup were suitably diluted and added to the wells of each microtiter plate. After incubation, the wells were gently aspirated, washed 3 times, and processed following the instructions supplied with the kit. Finally, after color development, the plated were read at the wavelength indicated in the instruction manual using a ELISA analyser (EAR400, SLT Lab instruments, Austria).

**Detection of mRNA expression by RT-PCR**

Levels of IL-1, IL-6, IL-8, and IFN-γ mRNA expression of the PBMC prepared as above were examined by RT-PCR, as described previously (Wandworanum and Strober, 1993). In brief, each mRNA was isolated from PBMC using a Micro-Fast mRNA isolation kit (Invitrogen Co., CA, USA) and was then reverse-transcribed with a cDNA cycle kit (Invitrogen). The volume of recovered cDNA was measured and 1 μg of each cDNA was subjected to 30 amplification cycles with the pairs of each primer. The PCR products were further analyzed by electrophoresis in a 2% agarose gel and detected by ethidium bromide staining.

**RESULTS**

**Skin tests by bacterial antigens**

Intracutaneous tests: The cutaneous tests using bacterial vaccines revealed that all streptococcal preparations caused strongly flared and erythematous pustular reactions and that Prot. vulgaris, Pseud. aeruginosa and E. coli resulted in mildly-flared reactions in BD patients, as previously reported (Kaneko et al. 1977 and 1978) (Table 1).

Skin prick tests with bacterial antigens: Nonlesional forearms of 6 BD patients and 6 healthy controls were pricked using 22-gauge needles treated with bacterial antigens and cutaneous reactions were observed after 48 h. All 6 BD patients showed small pustules with erythematous halos in response to the streptococcal antigens, but showed no reaction to Sta. aureus, Sta. epidermidis, saline used as control (Fig. 1) The results, including ours, were reported by the Behçet's Disease Research Committee (Mizushima et al. 1989).

Mucocutaneous reaction to CWSS: Before employing CWSS as a streptococcal antigen in the
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Table Intradermal reactions in patients with Behçet’s disease (n=84) and normal healthy controls (n=10)

<table>
<thead>
<tr>
<th>Bacterial vaccines</th>
<th>Behçet’s syndrome 15min</th>
<th>Behçet’s syndrome 48hr</th>
<th>Normal controls 15min</th>
<th>Normal controls 48hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes</td>
<td>7±8</td>
<td>41±15</td>
<td>11±11</td>
<td>8±6</td>
</tr>
<tr>
<td>S. viridans</td>
<td>8±7</td>
<td>46±11</td>
<td>4±4</td>
<td>2±3</td>
</tr>
<tr>
<td>S. non-hemolyticus</td>
<td>7±7</td>
<td>35±14</td>
<td>5±5</td>
<td>3±4</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>10±8</td>
<td>40±19</td>
<td>12±11</td>
<td>0</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>16±10</td>
<td>32±14</td>
<td>8±9</td>
<td>3±4</td>
</tr>
<tr>
<td>E. coli</td>
<td>6±7</td>
<td>16±11</td>
<td>2±1</td>
<td>1±2</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>11±10</td>
<td>15±15</td>
<td>9±13</td>
<td>11±12</td>
</tr>
<tr>
<td>Stu. aureus</td>
<td>8±7</td>
<td>12±13</td>
<td>3±7</td>
<td>0±1</td>
</tr>
<tr>
<td>Stu. epidermidis</td>
<td>5±7</td>
<td>6±7</td>
<td>2±7</td>
<td>1±2</td>
</tr>
<tr>
<td>Prot. vulgaris</td>
<td>10±16</td>
<td>28±13</td>
<td>6±6</td>
<td>17±4</td>
</tr>
<tr>
<td>Pseud. aeruginosa</td>
<td>1±1</td>
<td>22±11</td>
<td>2±3</td>
<td>5±0</td>
</tr>
<tr>
<td>SK-SD(50 U/ml)</td>
<td></td>
<td>9±12</td>
<td>20±9</td>
<td></td>
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<tr>
<td>Saline</td>
<td></td>
<td>2±4</td>
<td>0±1</td>
<td></td>
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</table>

The numbers denote mean±SD(mm) of length + width / 2 of erythemas. (Kaneko et al. 1977)

Fig. 1. Prick tests using bacterial antigens. Skin reactions to Sta. aureus, Sta. epidermidis, S. sanguis (ST3), S. sanguis (B220), S. sanguis (SSH-83), S. salivarius (HHT), and S. faecalis from left to right, on the L-arm of a 38 year-old female patient with incomplete Behçet’s disease (BD). The skin reaction was read at 48 hours (h) after pricking.

Fig. 2. Intracutaneous reactions to 100 ng/0.1 ml of the cell wall of S. salivarius (CWSS). The reactions were read at 48 h after injection.

M: medium only (control)
S: CWSS

the patient subjected to the oral prick test showed a AU-like reaction on the oral mucous membrane after 48 h (Fig. 3).

Immunohistological analysis

To detect the deposition of immune complexes containing streptococcus related antigen and to an-

experiments, 100 ng/0.1 ml of CWSS were intracutaneously administered to 3 patients with active BD and to 3 normal adult controls. We also attempted to gently prick the oral mucous membrane of one of the BD patients and a healthy control after obtaining their informed consent. All 3 BD patients showed positive skin reaction to CWSS (Fig. 2) and
analyze the infiltrated inflammatory cells in AU and EN-LE of 5 of the BD patients, an immunohisto-
logical examination was performed using anti-
human immunoglobulin and complement Abs, anti-
streptococcus group D Ab, and anti-lymphocyte and macrophage monoclonal Abs. Deposits of IgM and C3 were mainly detected on the vascular walls of the deep dermis of EN-LE and the ulcerated surface of AU. Streptococcal-realted antigen was also found at the same sites (Figs. 4 and 5a, b) (Kaneko et al.
1980, 1982 and 1985b) and was also seen in the cytoplasm of leukocytes adhering to the vascular walls. The infiltrated inflammatory cells around vessels in EN-LE consisted mainly of HLA-DR+, CD4+ T cells, as well as OKM-1+ monocytes in association with Leu 7+ natural killer (NK) cells (Kaneko et al. 1985b).

Chemoattractants and cytokines derived from PBMC

The chemotactic activity of BD-PBMC Sup in-
cubated with CWSS was significantly higher than with those of unstimulated BD-PBMC and normal controls (Fig. 6). Production of IL-1, IL-6 and IL-8 was significantly enhanced in Sup of CWSS-sti-
mulated BD-PBMC and the increases in cytokine
levels induced by CWSS were dose-dependent (Fig.
7) (Kikkawa et al. 1993; Oyama et al. 1997). The
rise in IFN-γ was detected in only 2 of 8 Sup of BD-PBMC stimulated with CWSS for 48 h.

Therapeutic trial using antibiotics

Clinical effects of minocycline: The average fre-
cuencies of clinical symptoms reported by 11 of 13 patients with active BD over a period of 3 months
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Fig. 5. a. Histology of vasculitis in an erythematous nodulosis-like eruption (EN-LE) (HE X200). b. Positive IF finding at the same site as the vasculitis in the EN-LE using anti-streptococcal group D Ab. Positive deposits of streptococcal-related antigen were found in the vascular wall and cytoplasm of infiltrates adhering to the endothelial cells.

![Image](image_url)

Fig. 6. Chemotactic activity of supernatants (Sup) of peripheral blood mononuclear cells (PBMC) determined using a micro-Boyden chamber. The Sup of BD-PBMC incubated with CWSS caused significant hyperactivity of neutrophils from a normal healthy volunteer.

Prior to the use of minocycline were 5.3 AU, 0.7 GU, 2.3 EN-LE and 2.5 PF. After treating the patients daily with 100 mg of minocycline, symptoms improved within 3 months and frequencies were reduced to 4.8 AU (10% reduction), 0.46 EN-LE (80% reduction) and 0 PF (100%) which completely disappeared during treatment. No side effects were observed in any of the patients during the medication period except sunburn in one (Table 2). Cytokine production of BD-PBMC stimulated with CWSS in the presence of minocycline: To evaluate the clinical improvement seen with minocycline as well as the immunological efficacy of this agent, we further examined whether cytokine production by BD-PBMC stimulated with CWSS was influenced by minocycline in vitro. Minocycline reduced IL-1β and IL-6 overproduction of CWSS-stimulated BD-PBMC in a dose-dependent manner but did not alter IL-8 production (Fig. 8). RT-PCR analysis revealed that levels of IL-1β and IL-6 mRNA expression of CWSS-stimulated BD-PBMC were significantly reduced by minocycline in a dose-dependent manner, although IL-8 mRNA expression was unchanged (Fig. 9) (Oyama et al. 1997). However, detection of IFN-γ mRNA expression in these cells was poor (data not shown).

**DISCUSSION**

BD patients were reported to have certain immunological abnormalities in their backgrounds, such as aberrations in T-cell (Sakane, 1991) and NK-cell functions (Kaneko et al. 1985a; Onder et al. 1994) and an increase in numbers of T-cell receptor (TCR) γδ + T cells (Suzuki et al. 1992; Hanzouli et al. 1994), and they are highly associated with a positive HLA-B51 status (Mizuki et al. 1992). As BD patients have a tendency to exhibit non-specific cutane-
Fig. 7. Production of interleukin (IL)-1, IL-6 and IL-8 by BD-PBMC stimulated with or without CWSS. Production of these pro-inflammatory cytokines was significantly enhanced by CWSS and the increases were dose-dependent.

Fig. 8. Cytokine production by BD-PBMC stimulated with CWSS in the presence of minocycline. IL-1β and IL-6 production was reduced by minocycline in a dose-dependent manner, but IL-8 production was not affected.

ous hypersensitivity to even a minor injury, a positive reaction in the skin pathergy test is still considered a characteristic response in these patients and useful in the diagnosis of this disease (Dilsen et al. 1993; Gul et al. 1995). We previously found a positive pathergy rate of 36.9% among BD patients (Kaneko et al. 1985b). Pathergy, however, is presumed to be a reaction to certain bacterial organisms on the skin which penetrate with the needle prick, since this phenomenon can be minimized by adequate disinfection before puncture (Fresco et al. 1993). BD patients have a significantly higher incidence of chronic tonsillitis and dental caries, which seem to be affected by certain strains of streptococci before the onset of clinical symptoms (Kaneko et al. 1978; Mizushima et al. 1988). In addition, BD symptoms sometimes appear after treatment of dental caries or surgical treatment of chronic tonsillitis, as indicated by Mizushima et al. (1988). These chronic foci may contribute to sensitize these patients and may result in having intense hypersensitivity to streptococcal strains. We therefore attempted to define the correlation between the acquisition of hypersensitivity and the
Table 2. The effect of minocycline on the frequency of clinical symptoms in patients with Behçet's disease

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Frequency of the major clinical symptoms over 3 months</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>Apathous ulceration (AU)</td>
<td>5.3</td>
</tr>
<tr>
<td>Genital ulceration (GU)</td>
<td>0.7</td>
</tr>
<tr>
<td>Erythema nodosum-like eruption (EN-LE)</td>
<td>2.3</td>
</tr>
<tr>
<td>Perifolliculitis (PF)n</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Fig. 9. Effect of minocycline on cytokine mRNA expression of BD-PBMC determined by reverse transcription polymerase chain reaction (RT-PCR) analysis. BD-PBMC stimulated with CWSS were incubated with or without minocycline at concentrations of $10^{-8}$ and $10^{-6}$ M for 24 h. The RT-PCR analysis revealed that CWSS-induced over-expression of IL-1 and IL-6 mRNA was reduced by minocycline a dose-dependent manner.

pathogenesis of BD.

The clinical and laboratory results obtained in our studies revealed the existence of an abnormal hypersensitivity to streptococcus-related antigens in patients with BD. BD-PBMC production of inflammatory cytokines, such as IFN-γ, IL-1, IL-6, IL-8, and tumor necrosis factor (TNF) α, was found to be enhanced when these cells were stimulated with streptococcal antigen, as has been reported by others (Hirohata et al. 1992). An increase in soluble IL-2 receptor (sIL-2R), which may induce proliferation of T cells bearing the receptor, and a rise in TNF α which contributes to the activation of inflammatory cells, have been demonstrated in the sera of patients with active BD (Sayinalp et al. 1996). It is possible that the acquisition of this immunological hypersensitivity to streptococcal antigens triggers various BD symptoms by means of abnormal immune responses which are manifested by a rise in TCR γδ + T cells, the induction of 65 kilodalton (kDa) heat shock protein (HSP) related to mycobacterium infection (Lehner et al. 1991), dysfunction of NK cells, hyperactivity of neutrophils (Kaneko et al. 1991; Inoue et al. 1994; Ozoran et al. 1996) as well as other clinical changes. HSP are highly conserved proteins which are synthesized when cells are exposed to stressful stimuli (Lindquist and Craig, 1988). It has been found that the mycobacterial 65 kDa HSP cross-reacts with strains of S. sanguis and is able to up-regulate the expression of TCR γδ + T cells (Lehner et al. 1991). On the other hand, anti-65 kDa HSP Abs (IgG and IgA) were suggested to be produced in large quantities by B cells whose sensitization mediated by T cells with the HSP epitope and these antibodies seem to regulate the HSP reaction in BD patients (Direskeneli et al. 1996). Several pro-inflammatory cytokines from monocytes, TNF α, IL-6 and IL-8, are also thought to be induced by 65 kDa HSP (Friedland et al. 1993). This hyperactivity of various inflammatory cells, including lymphocytes, macrophages and neutrophils, might be influenced by cytokines produced by inflammatory lymphocytes in BD lesions. The histology of cutaneous biopsy specimens from these lesions varies according to the lesional condition,
location and disease-stage. However, the perivascularly and interstitially infiltrating inflammatory cells mainly consist of monocytes and neutrophils (Kaneko et al. 1985b; Inoue et al. 1994; Jorizzo et al. 1995). Immunohistologically, deposits of IgM and complement are reported to be detected at the vascular walls of lesions as antiendothelial cell Ab (Ozoran et al. 1996). In our study, immune complexes containing IgM and streptococcus-related antigen were immunohistologically detected among AU epidermal cells and in the neutrophil-coated vascular walls of EN-LE of BD patients (Fig. 5). Tissue showing a positive pathergy reaction has been histologically observed to have cell infiltrations similar to those of BD lesions (Gul et al. 1995).

With respect to streptococcus strains in oral lesional membrane and/or dental plaque of BD patients, a characterized strain of S. sanguis has been isolated from patients with BD (Isogai et al. 1990; Yokota et al. 1995). However, the uncommon S. sanguis seems to be no different from the common S. oralis in terms of biochemical and serological properties (Narisawa et al. 1995). It is thought that the characteristics of S. oralis are variable and that it is not easily distinguished from other viridans streptococci (Hardie, 1986). These oral streptococci are thought to penetrate the oral membrane by means of their own enzymes, such as IgA1 protease and neuraminidase (Kilian and Holmgren, 1981). Streptococci isolated from the oral cavity of BD patients might penetrate the mucous membrane and contribute to the acquisition of hypersensitivity to streptococcal strains in these patients. In our previous study, Neisseria sp., Corynebacterium sp. and S. viridans were mainly isolated from oral aphthous lesions of BD patients and we attempted to determine the minimum inhibitory concentration (MIC) of antibiotics against these isolated bacteria using pencillin G (PCG) and minocycline (Kaneko et al. 1981). Although PCG exhibited a much higher MIC than did minocycline, its therapeutic efficacy was less. It was thought that minocycline accumulates in the skin and oral membrane much more readily than does PCG, and that it acts not only as a bacteriostatic agent but as an immunological regulator as well. We therefore studied the effects of minocycline on the cytokine production of BD-PBMC stimulated with streptococcal antigen and demonstrated at both the protein and mRNA level that minocycline regulates the inflammatory cytokines, IL-1 and IL-6 produced by BD-PBMC stimulated with CWSS. It was shown that tetracycline and its analogs inhibit neutrophil chemotaxis, production of superoxide anions (Gable and Creamer, 1991) as well as lymphocyte transformation and proliferation (Thong and Ferane, 1979). It has also been reported that minocycline has a clinical effect on the leukocytoclastic vasculitis associated with rheumatoid arthritis (Houck and Kauffman, 1997). It has also been reported that minocycline may be useful in obtaining mild clinical improvement in BD patients with recurrent mucocutaneous lesions.

In conclusion, the acquisition of hypersensitivity to streptococcal antigens plays an important role in the appearance of oral cavity and other lesions in patients with BD, although the mechanism by which this hypersensitivity is acquired remains obscure.

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