Behçet’s Disease and Related Diseases
-Immune Reactions to Oral streptococci in Their Pathogenesis

Fumio Kaneko, Ari Togashi, Erika Nomura, Koichiro Nakamura

Behçet’s disease (BD) is a systemic disorder characterized by the recurrent involvement in the muco-cutaneous, ocular, intestinal, vascular, and/or nervous system organs. The clinical muco-cutaneous manifestations including recurrent aphthous stomatitis (RAS), erythema nodosum (EN)-like eruption, genital ulceration, etc. of patients with BD were reviewed in their pathogenesis comparing with the similar symptoms seen in patients without BD (non-BD). Most of BD patients tend to have hypersensitivity against streptococci which might be acquired in the oral cavity through the innate immune mechanism. Generally, BD patients have the systemic symptoms following RAS symptom as an immune reaction. Then, the characteristics of hypersensitivity to oral streptococci may be utilized in order to make a diagnosis for BD. The skin prick with self-saliva including oral streptococci was much more sensitive than “Pathergy test” conventionally used for BD diagnosis. HLA-B51-restricted CD8+ T cell response is suspected to catch the target tissues expressing major histocompatibility complex class 1 chain-related gene A (MICA) by stress in active BD patients. Bes-1 gene and 65kD of heat shock protein (HSP-65) derived from Streptococcus sanguinis (S. sanguinis) are detectable in the lesions. The peptides of Bes-1 gene are highly homologous with the retinal protein Bm3b which might be connected with the eye involvement in BD patients. Also, the peptides seem to be homologous with HSP-65 in association with the human HSP-60 which reactivity appeared in serum of the patients involved by S. sanguinis. Then, the pathogenesis of BD was conclusively discussed on the relationship between RAS and the systemic symptoms by the vascular reaction due to immune responses against antigens derived from S. sanguinis. Non-BD patients with RAS and/or Lipschutz genital ulceration were weekly sensitized by oral streptococci, except for patients with EN.

Key words: Behçet’s disease; Bes-1 DNA; Heat shock protein (HSP); Recurrent aphthous stomatitis (RAS); Salivary prick test

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Kaneko F, Togashi A, Nomura E, Nakamura K. Behçet’s Disease and Related Diseases. Journal of Dermatological Research 2016; 1(3): 41-10 Available from: URL: http://www.ghrnet.org/index.php/jdr/article/view/1679
in the oral bacterial flora of BD patients in comparison with those of healthy controls. *S. sanguinis* from BD patients was identified as uncommon serotype KTH-1 (so-called BD113-20) by the bacterial and enzymatic properties. Most of BD patients tend to acquire hypersensitivity against *streptococci* in their oral flora, as previously demonstrated that the cutaneous reactions by the injection and/or prick with bacteria antigens of *streptococci* and enterococci were much stronger than the reaction by “Pathergy test”. The histology from the cutaneous streptococcal response of a BD patient is similar to the vascular reaction seen in EN-like eruption. The cutaneous reactions to streptococcal antigens induced the clinical symptoms in some BD patients.

Non-BD patients with RAS (non-BD RAS) were also considered to react with streptococcal antigen, although several environmental factors are also to be a trigger of aphthous ulceration. In vitro, inflammatory cytokines, interleukin (IL)-6 and interferon (IFN)-γ were produced from peripheral blood mononuclear cells (PBMCs) of BD patients by stimulation with streptococcal antigen. The titers of serum-antibody against *streptococci* were also elevated in BD patients. The 65kDa of heat shock protein (HSP-65) derived from *S. sanguinis*, can be detected along with counterpart human HSP-60 which reactively appears in the sera and lesions of BD patients. The peptides of HSP-65 show considerable homology with those of the human HSP-60.

Epidemiology surveys suggest that the prevalence of BD is highly distributed from the Mediterranean countries to Japan via China and South Korea, along so-called “old Silk Route”. The prevalence rates of 1990s were 8 to 37 per 100,000 in adult population of Turkey and 11-13 per 100,000 population in Ningxia and Heilongjiang of China. In Japan, the prevalence was suggested to be 13 per 100,000 as well as in Korea in the 1970s but its rate has decreased lately, because the environmental conditions, such as oral health behaviors, etc., are changed.

Then, we have attempted to review about the new diagnostic ways for BD in comparison to the related diseases showing the similar symptoms due to immunological reactions.

### MUCOCUTANEOUS INVOLVEMENTS

**RAS:** The oral aphthous ulceration punch-out shaped painfully occurs on the tongue, buccal mucosa, gingival and lip, continues around a week, though self-limited, and nearly 100% of BD patients will be associated as the initial signs (Figure 4 and 5). On the other hand, non-BD RAS is a very common disorder due to trauma, some viral and/or bacterial infections and other autoimmune diseases, because about 20% of the general population is thought to be affected in the world. The biopsy specimen from RAS lesion of a BD patient revealed the epithelial cells surrounded by neutrophils and T cells like the antibody dependent cell mediated cytotoxicity. The epithelial cells of the ulcer margin were stained with anti-human IgA, IgM, complement, streptococcal antibodies and HLA-DR monoclonal antibody. However, it is difficult to differentiate oral ulceration-lesions in patients with BD from non BD-RAS by the clinical and/or histological aspects.

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**Figure 1 a. Prick tests by bacterial antigens** (1 × 10⁸ org./mL) (Hollister-Stairs, USA). After 24-48 h, strong erythematous reactions appeared by *Streptococcus* (*S.*) *sanguinis*, *S.sarivarius*, *S.faecalis*, *S.pyogenes* and cell wall of *S.sanguinis* and *salivarius* antigens except *Staphilococcus* (*S.*) *aureus* antigen and saline (control). b. The cutaneous reaction by 0.01 mL injection of *S.viridans* and *Staphillococcus aureus* antigens after 48 hours. c. A biopsy specimen from the reactive site by *S.viridans* showed vascular phenomenon was similar to that of EN-like eruption of a BD patient.
Figure 2 a. EN-like eruption of a BD patient. b. A biopsy specimen showed vasculitis infiltrated by lymphoid cells and neutrophils in the dermis (HE stain, X 400). c. The vascular lesion was stained by anti-streptococcal antibody (Immunofluorescence, 400).

Figure 3 When saliva from a BD patient was incubated in Salivarius and Mitis (SM) agar, oral streptococci are limited grown in a few days. In a. area, streptococcal colonies from crude saliva were grown and in b area, no bacterial colonies was recognized from the saliva sterilized through the micro-filter with 0.2 μ pores.

Figure 4 A clinically typical and active case of 35 year-old female BD patient classified as “Incomplete type” by Japanese Classification (a). Although 2 mm erythema- reaction appeared by “Pathergy test” (b), more than 20mm diameter erythematous reaction was recognized 24-48 hours after self salivary prick (Salivary prick test). Also, by the sterilized saliva, more than 10mm erythema reaction was observed in this case (c).
Genital ulcer: The clinical features of genital ulceration are generally shaped as similar to oral aphthous ulceration of BD patients (Figure 5). A few cases of young female are suddenly attacked by genital ulceration as the initial BD symptom clinically like Lipschutz genital ulceration,[32] which is supposed to be due to Epstein-Barr viral (EBV) infection[33,34]. However, EBV was not detected from the lesion as our case listed in Table 1. About more than 50% of BD patients are found to be associated with genital ulceration (female: 55.5%, male: 58.7%), that is, ulcers occur on vulva (66.1%), vaginal mucosa (35.7%), anus (9.6%), cervix (4.1) and groin area (0.8%) in female and on the penis (46.5%), scrotum (38.5%), anus (9.2%) and groin area (5.0%) in male patients[35,36].

EN-like eruption of BD patients and non-BD-EN: More than 50% of BD patients are reported to be associated with EN-like eruption on the lower legs,[37,38] which looks smaller to EN of non-BD patients (Figure 2a). Generally, the histology is “vascular reaction” infiltrated by mainly lymphoid mononuclear cells, so-called “lymphocytic vasculitis”, and septal panniculitis in the subcutaneous fatty tissue. In acute and active phase of BD patients, however, vasculitis surrounded by neutrophils is able to be recognized in a few days after the occurrence. Although it is difficult to differentiate BD-EN like eruption from non-BD EN, features of venous thrombosis features are sometimes found in active BD-EN.[39] Immunofluorescence revealed deposits of IgA, IgM, C3 and streptococcal related materials by anti-streptococcal antibody in the vascular walls (Figure 2c,d).[12,17,31] On the other hand, the streptococcal related materials could not be detected in our cases with non-BD EN tissues. The findings suggest that streptococcal antigens might be playing an important role in the BD symptoms as the triggering extrinsic factor.[12,17,31] It is of interest that GroEL of S. sanguinis and human heterogenous nuclear ribonucleoprotein (hnRNP) A2/B1 were expressed on the vascular walls.[36,37] However, the causation of non-BD EN is also unknown, but the majority of EN patients have evidence of recent streptococcal infection or have no identifiable causes.[36,39]

PATHERGY TEST AND ORAL STREPTOCOCCI

It is not difficult to make a diagnosis for BD except for the atypical cases without the main muco-cutaneous symptoms including LAS. Pathergy reaction, which is a non-specific cutaneous hypersensitive response showing around 2 mm pustule 24-48 h after pricking with 20 G syringe needle, has been thought to be helpful to diagnose BD, because the phenomenon has been believed as a unique feature of BD described by International Study Group of BD.[40] Histology and immunohistology of the “Pathergy reaction” is similar to those of EN-like eruption from BD patients.[41,42] However, the reactivity by Pathergy test became chronologically lower to less than 5% (Figure 3b). It is of interest that the surgical cleaning of the forearm before needle prick reduced its reactivity,[43] suggesting that the “Pathergy reaction” might be a response to some bacteria living on the surface of the skin. Then, instead of “Pathergy test”, we tried to prick with self-saliva including oral streptococci (Salivary prick test) (Figure 3), to the forearm of BD patients using Lancetter with tiny stick (OY ALGO AB Espoo/ Esbo, Sweden), because the patients have hypersensitivity to streptococci. The results revealed more than 90% of 22 BD patients showed larger than 5 mm erythematous reaction with spot pustule by Salivary prick test (Figures 4 and 5, Tables 1 and 2). The histology of the positive site was basically similar to that of BD-EN-like lesions. Non-BD RAS and Lipschutz genital ulcer patients showed weaker reaction than those of BD patients, suggesting some possibilities to differentiate from each other and also correlation with streptococci in the pathogenesis. No reaction and/or tiny spot were seen by the prick with microfilter-sterilized saliva and saline in BD patients and the disease controls including patients with viral aphtha and non-BD EN and healthy persons (Table 1). Regarding non-BD RAS, the results also suggest that oral streptococci are playing an important role in the pathogenesis of RAS of BD patients, although there are many...
studies, still no clear causation is present[10,11]. The Salivary prick test is considerable to make a differentiation of BD from non-BD disorders with similar symptoms. The case with Lipschutz genital ulceration showed a weak skin reaction to self-saliva (Figure 6a, b, Table 1)[49].

**HLA GENOTYPING AND STREPTOCOCCAL INFECTION**

HLA-B51 is supposed to be a highly associated with BD patients as the genetic marker even in many different ethnic groups including European, Mediterranean and Asian people. BD has several unique epidemiologic features which seem to go from Southern Europe to Japan along “the old Silk Road”, as mentioned previously[5,7,8,52,53]. The appearance of BD lesions is not directly correlated with the genetic marker even in many different ethnic groups including European, Mediterranean and Asian people. BD has several unique epidemiologic features which seem to go from Southern Europe to Japan along “the old Silk Road”, as mentioned previously[5,7,8,52,53].

There are some reports that BD patients associated with HLA-B51 show much stronger reaction to “Pathergy test”. Although the patients with HLA-B51 showed relatively stronger skin reactions, the reactive severity seemed to be correlated with the disease severities. Five out of 15 patients (33.3%) were HLA-B51 possessor.

**Table 2** Self-salivary prick test in BD patients with or without HLAB51.

| Type of BD      | Patients(initials) | Prick test (mm) | HLA-B51 |
|-----------------|--------------------|-----------------|---------|
| Complete type   | M O               | S | SS | CS | (B51) |
| Intestinal type | F NH              | 20 | 7 | - | (B31,52) |
| Incomplete type | M H               | 10 | 8 | - | + (B51,01,01) |
| Complete type   | M A               | 30 | 4 | - | + (B31,40) |
| Complete type   | M A               | 7  | 4 | - | + (B31,46,B4,8) |
| Complete type   | M A               | 10 | 5 | - | + (B35,48) |
| Complete type   | Y K               | 10 | - | - | + (B44,03,01) |
| Complete type   | A T               | 22 | - | - | + (B40,48) |
| Complete type   | M A               | 10 | - | - | + (B15,38) |
| Complete type   | Y T               | 10 | - | - | + (B15,38) |
| Complete type   | Y T               | 4  | - | - | + (B40,44) |
| Complete type   | M A               | 4  | 3 | - | + (B35,44) |
| Complete type   | K F               | 7  | 2 | - | + (B46,54) |
| Complete type   | Y O               | 11 | - | - | + (B17,02,01) |

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we have obtained interesting results that PBMCs from BD patients without HLA-B51 gene can be significantly stimulated by *S. sanguinis* antigen in the expression of IL-12p40 mRNA and that its protein level was also increased in connection with IL-12p70 (p35 and p40 subunits) rather than those of the patients with HLA-B51[59]. The antibacterial host response by T cell type immunity mediated by IL-12 is suggested to be much stronger in HLA-B51-negative BD patients in vitro experiment. In our cases, about 33% of the patients were associated with HLA-B51 (Table 2) and the severity of the Salivary prick test might be correlated with the disease activity in BD patients, though Pathergy test was reported to be stronger than those in BD patients in vitro experiment. In our cases, about 33% of the patients were associated with HLA-B51 (Table 2) and the severity of the Salivary prick test might be correlated with the disease activity in BD patients, though Pathergy test was reported to be stronger than those in BD patients[11,13,15,16].

**HYPERSENSITIVITY AGAINST S. SANGUINIS:**

Generally, the oral health is impaired in BD patients with the disease severity[11,13,15,16]. The antibodies against *S. sanguinis* showed cross reactivity with the synthetic peptides of HSP-65 derived from the...
bacteria\textsuperscript{[61,62]} and delayed type cutaneous hypersensitivity reactions against streptococcal antigens were also seen in BD patients. Actually BD symptoms were provoked by the antigens and aphthous ulceration can be also induced by a prick with streptococcal antigen on the oral mucous membrane of a BD patient\textsuperscript{[11,12,17,18]}, which is so-called "oral bacterial allergic reaction". Isogai \textit{et al}\textsuperscript{[63]} demonstrated that the symptoms mimicking BD appeared in germ-free mice when \textit{S. sanguinis} from BD patients was inoculated into their oral tissue damaged by heat shock and/or mechanical stress. This report suggests that the immunization with \textit{S. sanguinis} through the oral membrane route elicits BD-like symptoms in the animal model. We tried to find the presence of Bes-1 gene by polymerase chain reaction (PCR) in BD lesions using 2 distinct primer sets (peptides, 229-243 and 373-385) encoding \textit{S. sanguinis} (serotype KTH-1) prepared by Yoshikawa \textit{et al}\textsuperscript{[64]}. Bes-1 DNA was present in various muco-cutaneous lesions including oral and genital ulcerations and EN-like lesions. PCR-in situ hybridization revealed Bes-1 DNA expression in the cytoplasm of inflammatory infiltrated monocytes adhering the vascular walls in muco-cutaneous lesions (Figure 7)\textsuperscript{[65]}. In contrast, we failed to detect DNAs of herpes simplex virus (HSV)-1, HSV-2, cytomegalovirus, human herpes virus (HHV)-6 and HHV-7 in the lesions by PCR\textsuperscript{[66]}, although it is reported that the animal models infected by HSV mimicked BD-like symptoms\textsuperscript{[66]}.

Interestingly, the amino acid sequence of the peptides of Bes-1 (229-243 and 373-385) shows more than 60% similarity to the human intraocular ganglion peptide, Brn-3b which is a subfamily of POU (pit-Oct Unc) domain factors containing Brn-3a and Brn-3c\textsuperscript{[67]}. The peptide of Bes-1 (229-243) was also found to be correlated with the peptide of HSP-60 (336-351)\textsuperscript{[68]}. These results suggest that Bes-1 derived from oral \textit{S. sanguinis} might be an inducer for the possible retinal and neural involvement in BD patients.

**HSP-65 DERIVED FROM MICROORGANISM AND HUMAN HSP-60**

HSPs, which scavenge denatured intracellular proteins, are supposed to be induced by microorganisms and mammalian tissues under a variety of stressful condition\textsuperscript{[69]} and they may be involved in the pathogenesis of some autoimmune diseases\textsuperscript{[70]}. The serum levels of IgA to mycobacterial HSP-65, which cross-reacts with selected pathogenesis of some autoimmune diseases\textsuperscript{[70]}. The variety of stressful condition to be induced by microorganisms and mammalian tissues under a certain condition. HSPs, which scavenge denatured intracellular proteins, are supposed to be induced by microorganisms and mammalian tissues under a variety of stressful condition\textsuperscript{[69]}. These results suggest that Bes-1 derived from oral \textit{S. sanguinis} might be an inducer for the possible retinal and neural involvement in BD patients.

**Aphthous ulceration and systemic symptoms in BD patients**

**Figure 8 A hypothesis showing the relationship between RAS and the systemic lesions in BD patients (97,98). a. The antigen presenting cells (APCs) (macrophages and/or dendritic cells) are immunized by streptococcal related antigens (Bes-1) including \textit{S. sanguinis}, though toll-like receptors (TLRs) expressing 2,4,6, and 9, as “innate immune mechanism”. The APCs expressing Bes-1 gene in the oral lesion might be carried through vascular ways to the peripheral regions. b. The APCs bond at the impaired and/or MICA (major histocompatibility complex class 1- related gene A) gene expressing endothelial cells of the peripheral vascular wall. Heat shock proteins (HSPs) 65/60 will be induced and the immunological reactions may be caused as BD lesions.**

A therapeutic trial by the peptide conjugated with rCTB was performed to BD patients with recurrent uveitis. The successful results were obtained to show that 5 of 8 patients had no relapse of uveitis, and that 2 of the remaining 3 patients had improved recurrent oral ulceration, folliculitis, EN-like eruptions and genital ulcers without any side-effects. In those patients with uveitis and extra-articular manifestations, a lack of the peptide-specific CD4\textsuperscript{+} T cell population, a decrease in expression of Th1 type cells (CCR5, CXCR3) and a reduction of IFN-γ, TNF-α and CCR7 T cells were observed in comparison to BD patients with relapse of disease\textsuperscript{[71]}. The HSPs presented by APCs can directly stimulate CD8\textsuperscript{+} T cells and CD69 T cells which play important roles in the oral mucosal immunity as the first defense against microorganisms. It is thought that Vγ9D2\textsuperscript{+} T cells, a major subset of γδ T cells, which recognize antigens in the innate and adaptive immune responses, were influenced by secreting IFN-γ. The γδ T cells expressing CD29 and CD69 produce IFN-γ and TNF-α from stimulation by HSP-65/60 in the lesions of BD patients with active disease\textsuperscript{[72,73]}. In the active stage of BD patients, IL-12 as a sign of Th1 type reaction, is also produced and advanced the symptoms. It is interest that the gene polymorphism in the promoter region regarding IL-12B, which is so-called "oral toleation" demonstrated by Stanford \textit{et al}\textsuperscript{[74]}. In order to understand the suppressive mechanisms of the cytokine production in PBMCs from active BD patients, we tried to find the binding sites of the peptides on monocytes by cDNA chips (Gene Chip; Human Genome) using NOMO-1 cells (human macrophage cell line)
activated by S. sanguinis antigen. Although the expression of IL-8, IL-16, IL-13R and IL-17R was decreased after incubation with LO1 and UK, respectively, LO2 did not decrease IL-8 production. CD58 (lymphocyte function-associated antigen-3) molecule and/or FK506 binding protein were highly expressed on the cell membrane after application of LO1 and UK[26,67].

TOLL-LIKE RECEPTOR (TLR) EXPRESSION

Regarding the recognition system for the microorganism antigens in humans, 10 numbers of TLR families are supposed to act as innate immune receptors by binding of particular structures present on bacteria, viruses, fungi, etc.[40]. TLR-3 [ds RNA] and TLR-6 [mycoplasma, staphylococci, etc.] are also reported to be enhanced in expression on neutrophils and monocytes of BD patients, when stimulated by HSP-60 and S. sanguinis antigen[41]. In RAS lesion of BD patients, expression of TLR-9 [unmethylated CpG DNA, bacteria and virus] has been also found[41]. These findings suggest that innate immune system contributes the acquisition of hypersensitivity against oral streptococci in the pathogenesis of BD as the extrinsic factor.

COMPLEMENT SYSTEM

Deposits of complement C3 with immunoglobulins are frequently detectable at the vascular involvement by immunofluorescent techniques in BD patients[92,123,129] and the titers of serum complement is generally high in the inactive stage. However, the levels of mannose-binding lectin (MBL) pathway are generally decreased in the patients[25]. The MBL pathway is considered to play an important role in the innate immunity. Ficolin (FCN) is a soluble protein that binds to carbohydrate on the microbial cells and 3 different types of FCN are detected. FCN 1 and 2 genes are located in the chromosome 9q34 and FCN3 gene is assigned to chromosome 1. FCN 1 and 2 genes are located in the chromosome 9q34 and FCN3 gene is assigned to chromosome 1. FCN 2 binds to lipoteichoic acid on the cell wall constituent in all Gram-positive bacteria and activate immune cells to produce proinflammatory cytokines[90]. We have found that novel FCN 2 gene single nucleotide polymorphisms (SNPs) are identified in the promoter regions as well as in the exon regions. The MBL genetic polymorphisms might be involved in immune responses to streptococcus infections in BD patients, because the relationship between MBL gene mutations and microbiological factors were suspected in the lesional immune reaction of BD patients[39]. Although no significant difference was present in the genotype allele frequencies of MBL gene SNPs between BD patients and healthy controls, the allele frequencies of FCN2 gene SNPs were significantly recognized in the promoter regions (-557 and -64 sites) among HLA-B51 positive BD patients[39]. The findings suggest the possibility that FCN gene of the MBL pathway in complement system contributes to the innate immunity in BD patients.

RAS AND SYSTEMIC SYMPTOMS

BD symptoms are characterized by vascular involvements histologically showing swollen endothelial cells of the micro-veins infiltrated by inflammatory monocytes with a few neutrophils, so-called “vascular reaction” seen in EN-like eruption and other lesions.[117,42,76,79]. The strong hypersensitivity reaction against S. sanguinis agents carried by APCs can be suspected in the pathogenesis of BD which may be one of the extrinsic triggering factors.[30,123,174,60] Regarding the treatment, low dose administration of minocycline is clinically effective for BD patients, because minocycline is experimentally administered not only to decreases a growth of oral S. sanguinis but also works to suppress IL-1β and IL-6 production from T cells inflamed. Actually, we recognized clinically effective for RAS, acne-like eruption and EN-like lesion in BD patients[122]. Other studies also showed that combination therapy, colchicine and benzathine penicillin, was effective to suppress BD symptoms compared to colchicine monotherapy[90-91]. The oral infectious agents suggest the hypothesis that after Bes-1 gene derived from streptococci taken in the cytoplasm of APCs through the TLRs in RAS lesion of BD patients, the APCs carrying the streptococcal antigen produce HSP-65 in the peripheral vascular lesions. The APCs impair MICA expressed endothelium of the vessels in correlation with HSP-65/60, VEGF, adhesion molecules, etc. BD lesions will be induced by the “vascular reaction” and/or “lymphocytic vasculitis” as the immunological reactions due to the APCs expressing S. sanguinis antigen[97,98] (Figure 8).

THERAPY

In order to treat for BD patients, we should know about the clinical manifestations and pathogenesis, as described above. It is important to analyse clinical metabolic biomarkers of inflammation in the advanced systemic symptoms of BD including involvements of ocular, vascular, neurosystem and gastrointestinal organs. However, though the therapy for the muco-cutaneous symptoms such as RAS, genital ulceration and acne-like eruption is centered on the topical measures, low dose of minocycline capsule (50-100 mg/day) for long time treatment is effective not only for the clinical symptoms, but also inflammatory cytokine production from activated lymphoid cells, as described previously[12]. And administration of colchicine (0.5-1.0 mg) can also manage the inflammation of EN-like symptoms and the joint involvements[90]. As to the immune reduction, azathioprine, cyclosporine and corticosteroids are used in cases with severe resistant muco-cutaneous and articular manifestations of BD. To date, in the point of immunological mediators correlated to the systemic involvements, some biological antibodies, as infliximab, adalimumab, etc., are applied for BD patients[90].

ACKNOWLEDGMENTS

Our fundamental studies were done by the financial support of the Study Group of Behcet’s Disease organized by Japanese Ministry of Health, Labour and Welfare and we deeply thank valuable suggestions by Drs. Martin M, Black and Anne Kobza-Black and late Scientist Mr. Balbir Bhogal from St. John’s Institute of Dermatology, St. Thomas Hospital, London, UK.

CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

REFERENCES

1. Behcet H. Uber rezidivierende, aphthous durch ein Virus verursachte Geschwure am Mund, am Auge und an den Genitalien. Dermatol Wochenschr 1937; 105: 1152-7.
2. Sakane T, Takeno M, Suzuki N, Inaba G. Current concepts : Behcet’s disease. Dermatol Wochenschr 1995; 161: S107-11.
3. Suzuki- Krokawa M, Suzuki N. Behcet’s disease. N Eng J Med 1999; 341: 1284-91.
4. Bang D, Yoon KH, Chung HG, Choi EH, Lee ES, Lee S. Epidemiological and clinical features of Behcet’s disease in Korea. Yonsei Med J 1997; 38: 428-36.
5. Altenburg A, Papoutsis N, Orawa H, Martus P, Krause L, Zoubou-
Kaneko F et al. Immune reactions to oral streptococci in their pathogenesis.

lis CC. Epidemiology and clinical manifestations of Adamantiades-Behcet’s disease in Germany - Current pathogenic concepts and therapeutic possibilities. J Dtsch Dermat Ges 2006; 4: 49-64.

Alspoy E, Zouboulis CC, Ehrlich GE. Mucocutaneous lesions of Behcet’s disease. Yonsei Med J 2007; 48: 573-85.

Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behcet’s disease. Arch Ophthalmol 1982; 100: 1455-8.

Zouboulis CC, May T. Pathogenesis of Adamantiades-Behcet’s disease. Med Microbiol Immunol 2003; 192: 149-55.

Karaycian A, Zouboulis CC. An update on Behcet’s disease. J Eur Acad Dermat-Venereol 2007; 21: 1-10.

Krause I, Weinberger A. Behcet’s disease. Current Opin Rheum 2008; 20: 82-7.

Kaneko F, Kaneda T, Ohnishi O, Kishiyama K, Takashima I, Fujiwara Y, Kado Y. Virology infection in Behcet’s disease (1). Jpn J Allergol 1978; 27: 440-50.

Kaneko F, Ozawa K, Nishibh N. Streptococcal infection and Behcet’s disease. Infection 1997; 25: 43-8.

Isogai E, Ohno S, Araki Y, Oguma K. Antibody response to oral streptococcus sanguis isolated from patients with Behcet’s disease. Microbiol Immunol 1995; 39: 729-32.

Isogai E, Ohno S, Takashi K, Yoshikawa K, Turumizu T, Isogai H. Yokota Y, Hashimoto T, Shimizu M, Matsuda S, Fujii S, Yama-

Kaneko F, Takahashi Y, Muramatsu Y, Miura Y. Immunological studies on aphthous ulcer and erythema nodosum-like eruptions in Behcet’s disease. Br J Dermatol 1985; 113: 303-12.

Mizushima Y, Matsuda T, Hoshi K, Ohno S. Induction of Behcet’s disease symptoms after dental and streptococcal antigen skin test. J Rheumatol 1988; 15: 1029-30.

Graykowski EA, Baril MF, Boyd LM, Stanley HR. Recurrent aphthous stomatitis: Clinical therapeutic and histopathologic and hypersensitivity aspects. JAMA 1966; 196: 637-44.

Akitoye SO, Greenberg MS. Recurrent aphthous stomatitis. Dent Clin N Am 2014; 58: 281-97.

Hirohata S, Oka H, Mizushima Y. Streptococcal antigens stimulate production of IL-6 and interferon-γ by cells from patients with Behcet’s disease. Cell Immunol 1992; 140: 410-9.

Yokota K, Hayashi S, Araki Y, Isogai E, Kotake S, Yoshikawa K, Hashimoto T, Shimizu M, Matsuda S, Fujii S, Yama-

Kaneko F, Takahashi Y, Muramatsu Y, Miura Y. Immunological studies on aphthous ulcer and erythema nodosum-like eruptions in Behcet’s disease. Br J Dermatol 1985; 113: 303-12.

Mizushima Y, Matsuda T, Hoshi K, Ohno S. Induction of Behcet’s disease symptoms after dental and streptococcal antigen skin test. J Rheumatol 1988; 15: 1029-30.

Graykowski EA, Baril MF, Boyd LM, Stanley HR. Recurrent aphthous stomatitis: Clinical therapeutic and histopathologic and hypersensitivity aspects. JAMA 1966; 196: 637-44.

Akitoye SO, Greenberg MS. Recurrent aphthous stomatitis. Dent Clin N Am 2014; 58: 281-97.

Hirohata S, Oka H, Mizushima Y. Streptococcal antigens stimulate production of IL-6 and interferon-γ by cells from patients with Behcet’s disease. Cell Immunol 1992; 140: 410-9.

Yokota K, Hayashi S, Fujii N, Yoshikawa K, Kotake S, Isogai E, Ohno S, Araki Y, Oguma K. Antibody response to oral strepto-

cocci in Behcet’s disease. Microbiol Immunol 1992; 36: 815-22.

Lehner T. The role of heat shock protein, microbial and auto-

immune agents in the etiology of Behcet’s disease. Intern Rev Im-

munol 1997; 14; 21-32.

Kaneko S, Suzuki N, Yamashita N, Nagafuchi H, Nakajima T, Wakisaka S, Yamamoto S, Sakane T. Characterization of T cells specific for an epitope of human 60-kD heat shock protein (hsp) in patients with Behcet’s disease (BD) in Japan. Clin Exp Immunol 1997; 108: 204-12.

Kibaroglu A, Eksigolu-Demiralp E, Akoglu T, Direskeleni H. T and NK cell subset changes with microbial extracts and human HSP60-derived peptides in Behcet’s disease. Clin Exp Rheumatol 2004; 22(Suppl. 34): S59-S63.

Saylan T, Mat C, Fresko I, Melikoblu M. Behcet’s disease in the middle east. Clinics in Dermatology 1999; 17: 209-223.

Behcet’s Syndrome, in Zhang N2 (ed): Clinical Rheumatology, 1998, Shanghai, Science and Technology Press, Shanghai (in Chinese),(English translated by Dr. Jian-zhong Zhang)

Ohno S, Isogai E, Kaneko F, Nambara K, Sato K, Kitaichi N. Why does Behcet’s disease decline in Japan?: possible association between economic development and decrease rise of Behcet’s disease. In 14th International Conference of Behcet’s Disease, 2010, London.

Ship JA. Recurrent aphthous stomatitis. An update. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod 1996; 81: 141-7.

Shastri A, Srivastava R. Erythaphagenesis, diagnosis and recent treatment modalities for recent aphthous stomatitis; A review. Int J Contn P Med Res 2015; 2: 1-5.

Kaneko F: Behcet’s disease. In A color Atlas of Dermato-Im-

munohistology edited by Hiroaki Ueki and Hideo Yatoita, Wolfe Medical Publication Ltd, London, 1989, 84-5.

Lipschutz B. Ulcer vulvae acutum. In: Handbuch der Haut und Geschl (Jadassohn,ed), Berlin: Springer 1977; 27: 392-414.

Cheng SX, Chapman MS, Margesson LJ, Birenbaum DA. Genital ulcer caused by Epstein-Barr virus. J Am Acad Dermatol 2004; 51: 824-6.

Huppert JS. Lipschtz ulcers: evaluation and management of acute genital ulcers in women. Dermatologic Therapy 2010; 23: 533-40.

35 Cebeci F, Onsan N, Ulusal HA, Iban B. The relationship between vein thrombosis and erythema nodosum in male patients with Behcet’s disease. Eur Per Med Pharmacol Sci 2014; 18: 3145-8.

18 Cho S, Zheng Z, Cho S, Ahn K, Choi M, Kim DY, Lee KH, Bang D: Both the sera of patients with Behcet’s disease and Streptococ-
sanguis stimulate membrane expression of hnrNP A2/B1 in endothelial cells. Scand J Rheumatol 2013; 42(3): 241-6.

Cho SB, Zheng Z, Ahn KJ, Cho S, Kim DY, Lee HS, Bang D: Serum IgA reactivity against GroEL of Streptococcus sanguinis and human heterogeneous nuclear ribonucleoprotein A2/B1 in patients with Behcet’s disease. Br J Dermatol 2013; 168: 977-83.

Cribier B, Caille A, Heid E, Grosshans E. Erythema nodosum and associated diseases. A study of 129 cases. Int J Dermatol 1999; 37: 667-72.

Shojania KG. Erythema nodosum. Up To Date www.uptodate.com 2016.

International Study Group for Behcet’s Disease. Criteria for diag-

nosis of Behcet’s disease. Lancet 1990; 335: 1078-80.

Haim S, Solod JB, Friedman-Birnbaum R, Liching. Histological and direct immunofluorescence study of cutaneous hyperreactivity in Behcet’s disease. Br J Dermatol 1976; 95: 631-5.

Jorizzo JL, Abernathy JL, White WL, Mongeldorf HC, Zouboul-
is CC, Sarica R, Gafney K, Mat C, Yazici H, Al Lalaan A, Assad-
Kahalil SH, Kaneko F, Jorizzo EAF. Mucocutaneous criteria for the diagnosis of Behcet’s disease: an analysis of clinicopathologic data from multiple international centers. J Am Acad Dermatol 1995; 32: 968-76.

Srhat-Inaloz H, Evereklioglu C, Unal B, Kirtak N, Eralp A, In-

Apollo RN. The significance of immunohistochemistry in the skin pathergy test and Behcet’s syndrome in Britain. APLAR J Rheumatol 2007; 10: 333-5.

Davies PG, Fordham JN, Kirwan JR, Barnes CG, Dinning WJ. The pathergy test and Behcet’s syndrome in Britain. Ann Rheum Dis 1984; 43: 70-3.

Friedman-Birnbaum R, Bergman R, Aizen E. Sensitivity and specificity of pathergy tests results in Israeli patients with Behcet’s
cocal antigen in skin lesions from patients with Behcet’s disease. *J Appl Res* 2003; 3: 232-8.
65 Tojo M, Zheng X, Yanagihori H, Oyama N, Takahashi K, Nakamura K, Kaneko F. Detection of herpes virus genomes in skin lesions from patients with Behcet’s disease and other related inflammatory disease. *Acta Derm Venereol* 2003; 83: 1-4.
66 Sohn S, Lee ES, Bang S, Lee S. Behcet’s disease-like symptoms induced by the herpes simplex virus in C57BL/6J mice. *Eur J Dermatol* 1998; 8: 21-3.
67 Xiang M, Zhou L, Meng Y, Eddy RI, Shows TB, Nathans J. Brn 3b: POU domain gene expressed in a subset of retinal ganglion cells. *Neuron* 1993; 11: 689-701.
68 Jindal S, Dudani AK, Singh B, Harley CB, Gupta RS. Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. *Mol Cell Biol* 1989; 9: 2279-83.
69 Lamb JR, Young DB. T cell recognition of stress proteins: a link between infections and autoimmune disease. *Mol Biol Med* 1990; 7: 311-21.
70 Lehner T, Lavery E, Smith R, van der Zee R, Mizushima Y, Shinick T. Association between the 65-kilodalton heat shock protein, Streptococcus sanguis, and the corresponding antibodies in Behcet’s syndrome. *Infect Immun* 1991; 59: 1434-41.
71 Pervin K, Childstone A, Shinick T, Mizushima Y, van der Zee R, Hasan A, Vaughan R, Lehner T. T cell epitope expression of mycobacterial and homologous human 65-kilodalton heat shock protein peptides in short term cell lines from patients with Behcet’s disease. *J Immunol* 1993; 151: 2273-82.
72 Ergun T, Ince U, Ekisigou-Demiralp E, Direskeneli H, Gurbuz O, Gurses L, Aker F, Akoglu T. HSP90 expression in mucocutaneous lesions of Behcet’s disease. *J Am Acad Dermatol* 2001; 45: 904-9.
73 Direskeneli H, Ekisigou-Demiralp E, Yavuz S, Ergun T, Shinick T, Lehner T, Akoglu T. T cell responses to 60/65 kDa heat shock protein derived peptides in Turkish patients with Behcet’s disease. *J Rheumatol* 2000; 27: 708-13.
74 Imamura Y, Kurokawa MS, Yoshikawa H, Nara K, Takada E, Masuda C, Tsukikawa S, Ozaki S, Matsuda T, Suzuki N. Involvement of Th1 cells and heat shock protein 60 in the pathogenesis of internal Behcet’s disease. *Clin Exp Immunol* 2005; 139: 371-8.
75 Suzuki N, Sakane T. Characterisation of heat shock protein specific T cells in patients with Behcet’s disease. *Rev Rheum* 1996; 63: 551-63.
76 Direskeneli H, Saruhan-Direskeneli G. The role of heat shock proteins in Behcet’s disease. *Clin Exp Rheumatol* 2003; 21 (Suppl. 30): 544-8.
77 Shaker O, Ay El-Deen MA, El Hadid H, Grace BD, ElSherif H, Abdel Haim A. The role of heat shock protein 60, vascular endothelial factor and antiphospholipid antibodies in Behcet’s disease. *Br J Dermatol* 2007; 156: 32-7.
78 Yi SW, Kim EH, Kang HY, Kim YC, Lee ES. Erythema nodosum: clinicopathologic correlations and their use in differential diagnosis. *Yonsei Med J* 2007; 48: 601-8.
79 Ikunor T, Pabuccuglu U, Akin C, Lebe B, Gunes AT. Histopathologic and direct immunofluorescence findings of the papulopustular lesions in Behcet’s disease. *Eur J Dermatol* 2006; 16: 146-50.
80 Hu W, Hasan A, Wilson A, Stanford MR, Li-Yang Y, Todryk S, Whiston R, Shinnick T, van der Zee, Lehner T. Experimental mucosal induction of uvetis with the 60-kDa heat shock protein-derived peptide 336-351. *Eur J Immunol* 1998; 28: 2444-55.
81 Phipps PA, Stanford MR, Sun JB, Xiao BG, Holmgren J, Shinick T, Hasan A, Mizushima Y, Lehner T. Prevention of mucosally induced uveitis with a HSP60-derived peptide linked to cholera toxin B subunit. *Eur J Immunol* 2003; 33: 224-32.
82 Stanford M, Whittall T, Bergemeier LA, Lindbald M, Lundin S, Shinnick T, Mizushima Y, Holmgren J, Lehner T. Oral tolerization with peptide 336-351 linked to cholera toxin B subunit in prevent-
Kaneko F et al. Immune reactions to oral streptococci in their pathogenesis

...ing relapses of uveitis in Behcet’s disease. Clin Exp Immunol 2004; 137: 201-8.

83 Chen ZW, Letvin NL. Adaptive immune response to V 2V 2 cells: A new paradigm. Trends Immunol 2003; 24: 213-9.

84 Bank I, Duvelevani M, Livneh A. Expansion of T-cells in Behcet’s disease: Role of disease activity and microbial flora in oral ulcers. J Lab Clin Med 2003; 141: 33-41.

85 Lew W, Chang JY, Jung TY, Bang D. Increased expression of interleukin-23 p19 mRNA in erythema nodosum-like lesion of Behcet’s disease. Br J Dermatol 2008; 158: 505-11.

86 Oguma K, Shin R, Yokota K. Studies on immunological responses by bacterial antigens in Behcet’s disease. Report of the Research Group for Behcet’s Disease organized by the Japanese Ministry of Health, Labour and Welfare 2006-2007, 2008; 31-3 (in Japanese).

87 Kaneko F, Togashi A, Nomura E, Nakamura K, Isogai E, Yokota K, Oguma K: Role of heat shock protein derived from Streptococcus sanguinis in Behcet’s disease. J Medical Microbiol Diagnosis 2012; S.2, http://dx.doi.org/10.417/2161-0703,S2-001

88 Zarember KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol 2002; 168: 554-61.

89 Yavuz S, Elbirci Y, Tulunay A, Eksioglu-Demirap E, Direskeneli H. Differential expression of toll-like receptor 6 on granulocytes and monocytes implicates the role of microorganisms in Behcet’s disease etiopathogenesis. Rheumatol Int 2007; DOI 10.1007/s00296-007-0470-y

90 Durranni O, Wallace GR, Hamburger J, et al. Toll-like receptors (TLRs) expression in oral ulcer biopsies from Behcet’s disease (BD) patients: a role for the innate immune system in BD. Clin Exp Rheumatol 2004; 22(Suppl. 34): S1-93.

91 Inanc N, Mumcu G, Birtas E, Bilir Y, Yavuz S, Fresko I, Direskeneli H. Serum mannose-binding lectin levels are decreased in Behcet’s disease and associated with disease severity. J Rheumatol 2005; 32: 287-91.

92 Lynch NJ, Roscher S, Hartung T, Morath S, Matsushita M, Maennel DN, Kurya M, Fujita T, Schwaebel WJ. L-ficolin specifically binds to lipoteic acid, a cell wall constituent of Gram-positive bacteria and activates the lectin pathway of complement. J Immunol 2004; 172: 1198-202.

93 Wang H, Nakamura K, Inoue T, Yanagihori H, Kawakami Y, Hashimoto S, Oyama N, Kaneko F, Fujita T, Nishida T, Mizuki N. Mannose-binding lectin polymorphisms in patients with Behcet’s disease. J Dermatol Sci 2004; 36: 115-7.

94 Chen X, Katoh Y, Nakamura K, Oyama N, Kaneko F, Endo Y, Fujita T, Nishida T, Mizuki N. Single nucleotide polymorphisms of Ficolin 2 gene in Behcet’s disease. J Dermatol Sci 2006; 43: 201-5.

95 Calguneri M, Ertelni I, Kiraz S, Ertelni I, Benek-Li M, Karaarslan Y, Celik I. Effect of prophylactic benzathine penicillin on mucocutaneous symptoms of Behcet’s Dermatology 1996; 192: 125-8.

96 Mumcu G, Inac N, Yavuz S, Direskeneli H. The role of infectious agents in the pathogenesis, clinical manifestations and treatment strategies in Behcet’s disease. Clin Exp Rheumatol 2007; 25: S27-33.

97 Kaneko F, Oyama N, Yanagihori H, Isogai E, Yokota K, Oguma K: The role of streptococcal hypersensitivity in the pathogenesis of Behcet’s disease. Eur J Dermatol 2008; 18: 498-98.

98 Kaneko F, Togashi A, Saito S, Sakuma H, Oyama N, Nakamura K, Yokota K, Oguma K: Behcet’s disease (Adamantiades-Behcet’s disease). Clin Devol Immuno ID681956, 7 page, doi: 10: 1155/2011/681956, 2011.

99 Mizushima Y, Matsumura W, Mori M. Chemotaxis of leukocytes and colchicine treatment in Behcet’s disease. Br J Rheumatol 1979; 6: 108-9.

100 Caso F, Cesta L, Rigante D, Lucherini OM, Caso P, Bascherini V, Frediani B, Cimaz R, Marrani E, Nieves-Martín L, Attenu M, Raffale CGL, Terantino G, Galeazzi M, Punzi L, Cantarini L. Biological treatments in Behcet’s disease: Beyond anti-TNF therapy. Hindawi Publication Corporation, Mediators of Inflammation, 2014, Article ID 107421, 14 pages, http://dx.doi.org/10.1155/2014/107421

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