Behcet’s Disease and Related Diseases
-Immune Reactions to Oral Streptococci in Their Pathogenesis

Fumio Kaneko, Ari Togashi, Erika Nomura, Koichiro Nakamura

ABSTRACT
Behcet’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 Brn3b 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 reactively 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

INTRODUCTION
Behcet’s disease (BD) is a chronic systemic inflammatory disorder characterized by the recurrent involvement of muco-utaneous [recurrent aphthous stomatitis (RAS), genital ulceration, erythema nodosum (EN)-like eruption, acne-like eruption, etc.], ocular, vascular, digestive and/or nervous system organs. RAS showing oral aphthous ulceration generally starts as an initial sign since childhood and/or youth before the systemic symptoms of BD patients. Although the actual etiology of BD is still unclear, the pathogenesis has been generally clearer by the etiological studies based on the genetic intrinsic factors and extrinsic triggering factors. As one of the triggering factors, the oral unhygienic condition may be suspected, because periodontitis, decayed teeth, chronic tonsillitis, etc. are frequently noted in BD patients. The proportion of Streptococcus sanguinis (S. sanguinis), which was previously recognized as species of the genus Streptococcus named “S. sanguis”, was significantly high...
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 \[14-16\]. 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 \[11,12,17\]. The cutaneous reactions to streptococcal antigens induced the clinical symptoms in some BD patients \[18\].

Non-BD patients with RAS (non-BD RAS) were also considered to react with streptococcal antigen \[19\], although several environmental factors are also to be a trigger of aphthous ulceration \[20\]. In vitro system, 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 \[21\]. The titers of serum-antibody against streptococci were also elevated in BD patients \[22\]. 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 \[23-25\].

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 Ningxiahui and Heilongjiang of China \[26,27\]. In Japan, the prevalence was suggested to be 13 per 100,000 as well as in Korea in the 1970s \[28\], but its rate has decreased lately, because the environmental conditions, such as oral health behaviors, etc., are changed \[29\].

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 \[1-4\] (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 \[20,29,30\]. 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 \[17,31\]. However, it is difficult to differentiate oral ulceration-lesions in patients with BD from non BD-RAS by the clinical and/or histological aspects.


Figure 1 a. Prick tests by bacterial antigens (1 × 10^9 org./mL) (Hollister-Stairs, USA). After 24-48 h, strong erythemaous reactions appeared by *Streptococcus* (S.) *sanguis*, S.sarivarius, S.faecalis, S.pyogenes and cell wall of S.sanguis and salivarius antigens except *Staphilococcus* (S.) aureus antigen and saline (control). b. The cutaneous reaction by 0.01 mL injection of *S.viridans* and *Staphilococcus 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, ×400). c. The vascular lesion was stained by anti-streptococcal antibody (Immunofluorescence, ×400).

Streptococci from saliva of a BD patient in SM agar

**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 μpores.

Incomplete BD female patient (35 F, YS)

**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[23], which is supposed to be due to Epstein-Barr viral (EBV) infection[18,34]. 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[23,44].

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[12-44], 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[35]. 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 factors[12,17,31]. It is of interest that GroEL of S. sanguinis and human heterogeneous 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[38,39].

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 RAS. Pathergy reaction, which is a non-BD EN is also unknown, but the majority of EN patients have evidence of recent streptococcal infection or have no identifiable causes[38,39].

Table 1 Self-salivary prick test in patients with aphthius ulcerations and controls.

<table>
<thead>
<tr>
<th>Age, Sex</th>
<th>Disease controls</th>
<th>Salivary prick: S: 20×20mm, SS: 2×2mm, CS: -, Pathergy... - HLA-B51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese lassification</td>
<td>Prick test: S: Patients (initials)</td>
<td>Small pustule (after 48 h)</td>
</tr>
<tr>
<td>Neuro BD</td>
<td>M Y</td>
<td>55</td>
</tr>
<tr>
<td>Incomplete 33 F</td>
<td>AT</td>
<td>22</td>
</tr>
<tr>
<td>26 F</td>
<td>MN</td>
<td>10</td>
</tr>
<tr>
<td>27 M</td>
<td>TG</td>
<td>11</td>
</tr>
<tr>
<td>47 M</td>
<td>Y</td>
<td>10</td>
</tr>
<tr>
<td>36 F</td>
<td>MY</td>
<td>5</td>
</tr>
<tr>
<td>46 M</td>
<td>KH</td>
<td>10</td>
</tr>
<tr>
<td>17 F</td>
<td>YT</td>
<td>5</td>
</tr>
<tr>
<td>35 F</td>
<td>Y</td>
<td>23</td>
</tr>
<tr>
<td>50 M</td>
<td>HS</td>
<td>13</td>
</tr>
<tr>
<td>Complete 23 M</td>
<td>OC</td>
<td>10</td>
</tr>
<tr>
<td>Recurrent aphthous stomatitis (RAS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 F</td>
<td>Y</td>
<td>8</td>
</tr>
<tr>
<td>28 F</td>
<td>Y</td>
<td>8</td>
</tr>
<tr>
<td>32 F</td>
<td>Y</td>
<td>-</td>
</tr>
<tr>
<td>29 M</td>
<td>ON</td>
<td>-</td>
</tr>
<tr>
<td>28 F</td>
<td>MS</td>
<td>3</td>
</tr>
<tr>
<td>10 F</td>
<td>AY</td>
<td>6</td>
</tr>
</tbody>
</table>

BD: Behcet’s disease; EN: erythema nodosum; F: female; M: male; dot: small spot; +: positive; -: negative; S: prick with self-saliva; SS: prick with sterilized self-saliva; CS: prick with control saline; nd: not done; The clinical type of BD is followed by the Japanese BD classification. * Same cases in Table 2.
Three of 11 BD patients were positive for Bes-1 DNA in mucosal and skin lesions of BD patients, also becomes a stress-induced factor in connection with MICA*009 expression by inflammatory cytokines including interferon (IFN)-γ, IL-12, IL-18, which are up-regulated in the remission of BD patients in vitro experiment. In our cases, about 33% of the patients (35.3%) were HLA-B51 possessor.

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.

Generally, the oral health is impaired in BD patients with the disease severity. Generally, 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. 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 lesions including aphthous and genital ulcerations and erythema was associated with HLA-B51 (Table 2) and the severity of the lesions including aphthous and genital ulcerations and erythema nodosum (EN)-like eruption by amplified polymerase chain reaction (PCR) using the primers: Bes-1-1 (5'-TAAATCCGCAAGCCAGCT-3') and Bes-1-2 (5'-CCCTTTCAGAAAAGCTCATAACGTC-3') encoding S. sanguinis. Bes-1-1 and Bes-1-2 DNA was also detected in the cytoplasm of monocytes adhering to the vascular walls and infiltrated around the vessels by PCR in situ hybridization.

**Table 2** Self-salivary prick test in BD patients with or without HLA-B51.

<table>
<thead>
<tr>
<th>Type of BD (Japanese classification)</th>
<th>Patients (initials)</th>
<th>Prick test (mm)</th>
<th>HLA-B51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete type</td>
<td></td>
<td>S  SS CS</td>
<td></td>
</tr>
<tr>
<td>Intestinal type</td>
<td>25 M OC</td>
<td>10 -</td>
<td>(B51)</td>
</tr>
<tr>
<td>Intestinal type</td>
<td>37 F NH</td>
<td>20 2 -</td>
<td>(B51,52)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>40 M HG</td>
<td>10 7 -</td>
<td>+ (B51,01,01)</td>
</tr>
<tr>
<td>Intestinal type</td>
<td>31 F MA</td>
<td>30 7 -</td>
<td>+ (B51,40)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>42 F MK</td>
<td>7 4 -</td>
<td>+ (B51,64,6B,48)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>36 F MY*</td>
<td>10 -</td>
<td>- (B54,57)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>33 F YK</td>
<td>10 -</td>
<td>-</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>33 F AT*</td>
<td>22 -</td>
<td>- (B40,48)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>26 F MN*</td>
<td>10 -</td>
<td>-</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>35 M VI</td>
<td>10 nd</td>
<td>- (B15,35)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>37 F HT</td>
<td>4 -</td>
<td>- (B40,44)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>36 M MK</td>
<td>4 3 -</td>
<td>- (B35,44)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>35 M KF</td>
<td>2 2 -</td>
<td>- (B46,54)</td>
</tr>
<tr>
<td>Incomplete type</td>
<td>35 F YO</td>
<td>14 -</td>
<td>- (B17,02,01)</td>
</tr>
</tbody>
</table>

Studies, still no clear causation is present. The Salivary prick test is considerable to make a differentiation of BD patients 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. The appearance of BD lesions is not directly correlated with HLA-B51 in the immunological background of the patients, but it was found that HLA-B51-restricted cytotoxic T lymphocytes (CTLs) played some roles in correlation with the stressed target tissues expressing major histocompatibility complex class I chain-related gene A (MICA) in BD pathogenesis. When the transmembrane-MICA is preferentially expressed on epithelial and endothelial cells by stress, they seem to be the candidates for the HLA-B51-restricted CTLs response. The endothelium are also considered to be the ligand for activating natural killer (NK) cells with NK2D molecule and CD8 T cells as CTLs. Regarding NK cell activation, inhibitory CD34/NKG2A and activating CD94/NKG2C molecules are alternatively expressed on NK, CD4 CD8 T cells, as indicating an imbalance in cytotoxic activity in BD patients [50]. However, the function of NK cells is supposed to be down-regulated in the active stage and to be up-regulated in the remission of BD patients [51]. On the other hand, the expression CD4+ T cells activated by inflammatory cytokines including interferon (IFN)-γ, IL-12, IL-23, etc. might be altered to Th17 cells, which release IL-17 in the BD lesions, as seen in autoimmune disorders [51].

HSP-65/60 derived from microorganism including S. sanguinis and damaged human tissues, which is actually detectable in the oral mucosal and skin lesions of BD patients, also becomes a stress-inducible factor in connection with MICA*009 expression [26,27]. Generally, it has been reported that antigen presenting cells (APCs) expressing IL-12 are thought to be activated in BD patients with HLA-B51 in the active stage, as seen in transgenic mice [52]. However, 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 [53].

**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[61-63] 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[61,12,17,68], which is so-called “oral bacterial allergic reaction”. Isogai et al[69] demonstrated that the symptoms mimicking BD appeared in germ-free mice when *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 *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 *S. sanguinis* (serotype KTH-1) prepared by Yoshikawa et al[70]. 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)[63]. 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[64], although it is reported that the animal models infected by HSV demonstrated to mimic BD like symptoms[69].

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 the human intraocular ganglion peptide, Brn-3a and Brn-3c[67]. The peptide of Bes-1 (229-243) was also found to be correlated with the peptide of HSP-60 (336-351)[69]. These results suggest that Bes-1 derived from oral *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 conditions[68] and they may be involved in the pathogenesis of some autoimmune diseases[69]. The serum levels of IgA to mycobacterial HSP-65, which cross-reacts with selected strains of *S. sanguinis*, are significantly increased and HSP-60 was also detected in various lesions in BD patients[61,71]. On the other hand, 4 peptides of HSP-65 (111-125, 154-172, 219-233 and 311-326) derived from *S. sanguinis*, which are 50-80% homology to the counterpart human HSP-60, were recognized as immuno-dominant agents for T and B cell responses[61,62,68]. The 4 peptides of HSP-65 were also shown to significantly stimulate and undergo CD4+ and CD8+ T cell apoptosis in PBMCs from BD patients and HSP-60 also seemed to stimulate them[71]. On the contrary, the other two peptides of HSP-65 (21-35 and 401-415) corresponding to human HSP-60 (425-441) are reported not to stimulate PBMCs from BD patients and healthy individuals[69]. The peptide of HSP-60 (336-351) was also identified to be highly homologous to T cell epitope[68,70]. Whole HSP-60 is, however, supposed to induce vascular endothelial growth factor (VEGF) which activates, impairs and propagates the vascular endothelial cells[70]. It may also lead to thromboreliebitis and vasculitis by damaging endothelial cells in BD patients, although non-BD EN does not seem to be associated with thromboreliebitis[68,70].

It is of interest that the peptide of HSP-60 (336-351) linked to recombinant cholera toxin B subunit (rCTB) reduced the uveitis induced by whole HSP-60, although the peptide without the adjuvant is reported to induce uveitis in Lewis strain rats[61,62]. 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 genitalic ulcers without any side-effects. In those patients with uveitis and extra-articular manifestations, a lack of the peptide-specific CD4+ 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[60]. The HSPs presented by APCs can directly stimulate γδ T cells which play important roles in the oral mucosal immunity as the first defense against microorganisms. It is thought that Vγ9Vδ2 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[81]. 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 a 4 bp insertion within IL-12p40 (IL-12B) was significantly higher in the HLA-B51 negative BD patients. The expression of IL-12B mRNA and protein levels were also significantly increased in PBMCs from BD patients without HLA-B51 by stimulation with *S. sanguinis* antigen[80]. The expression of IL-23, which is composed of a shared p40 subunit of IL-12 and p19 subunit of IL-23 was also increased in EN-like lesions of BD patients[61,63].

The therapeutic approaches using the peptide of HSP (336-351) linked to rCTB were applied for BD patients with advanced uveitis, as “oral toleration” demonstrated by Stanford et al[82]. 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[30,67].

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.[10]. 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[90]. In RAS lesion of BD patients, expression of TLR-9 [unmethylated CpG DNA, bacteria and virus] has been also found[90]. 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[37,38,79] and the titer 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[91]. 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 binds to carbohydrate on the microbial cells and 3 different types of FCN are detected. FCN 1 and 2 are involved 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 2 binds to lipoteichoic acid on the cell wall constituent in all Gram-positive bacteria and activate immune cells to produce proinflammatory cytokines[92]. 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[90]. 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[90]. 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.[11,12,17,68,70] 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.[11,12,17,68,70] 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[92]. Other studies also showed that combination therapy, colchicine and benzathine penicillin, was effective to suppress BD symptoms compared to colchicine monotherapy[93,94]. 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, nervousystem 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].

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CONFLICT OF INTERESTS

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

REFERENCES

5 Altenburg A, Papoutsis N, Orawa H, Martus P, Krause L, Zoubou-
Kaneko F et al. Immune reactions to oral streptococci in their pathogenesis.


Kaneko F et al. Immune reactions to oral streptococci in their pathogenesis

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


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