

Insights into Behçet's disease

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Postep Derm Alergol 2012; XXIX, 6: 461-466

DOI: 10.5114/pdia.2012.32395

Abstract

Behçet's disease is a chronic relapsing multi-organ inflammatory disorder characterized as a triad of oral and genital ulcers, uveitis. Characteristic manifestations of Behçet's disease are joints, skin, central nervous system and gastrointestinal tract involvement. Behçet's disease has a complicated genetic etiology. However, epidemiological studies recommend that genetic factors have a significant role in its pathogenesis, the same as other autoinflammatory disorders. Antigenic stimuli, antigen-presenting cells, T cells, monocyte, neutrophil and endothelial cells are most important parts of the pathology of the disease. Inflammatory response was triggered by an infectious agent in a genetically susceptible host. Understanding the pathogenesis based on the molecular mechanism of the disease highlights the new therapeutic modalities. Enhanced inflammatory activity and over-expression of proinflammatory cytokines are the striking features of Behçet's disease, and they are accordant with the result in other auto inflammatory disorders. Moreover, there is evidence of antigen-driven immune response in Behçet's disease, but it probably advances in further innate immune reactivity. New therapeutic modalities target specific and nonspecific suppression of the immune system. The diagnosis is a clinical one, and although there is no single laboratory test enough for the diagnosis of Behçet's disease. In this paper, a new aspect of the studies on genetic susceptibility, immunopathogenesis of Behçet's disease and novel treatment modalities will be discussed.

Key words: Behçet's disease, autoinflammatory, immunogenetics, gene polymorphisms, cytokines.

Introduction

The new observed genetic origins of the autoinflammatory diseases have been related to the mutations in genes encoding a comparatively different family of proteins that have significant roles in the regulation of apoptosis, inflammation, and cytokine processing. Currently, the genetic defects alone are not specific to describe the estimated inflammatory manifestations sites in individual syndromes. Nevertheless, an association of different genes and their variable roles in the tissue-specific regulation of inflammation, associated genetic properties, and environmental aspects may contribute to the development of diverse groups of clinical manifestations. Though auto inflammatory diseases are rare disorders, identification of their pathogenic mechanisms may support us to comprehend the pathogenesis of more common inflammatory conditions [1].

Behçet's disease as an autoimmune disease

Behçet's disease (BD) is a persistent chronic vascular inflammatory disorder with a polymorphic clinical picture

described by relapsing and remission aphthous stomatitis, oral ulcers, ocular inflammation, genital ulcers, skin lesions, relapsing uveitis (may result in blindness), articular, neurologic, urogenital, vascular, intestinal, and pulmonary manifestations which frequently involve the joints, skin, central nervous system (CNS) and gastrointestinal tract [2, 3]. The diagnosis is mostly based on clinical data, and currently the most usually used diagnostic criterion is the International Study Group's classification, a definitive diagnosis requires recurrent oral ulcerations along with following: recurrent genital ulcerations, skin lesions, eye lesions and a positive pathergy test [4]. Despite the fact that prevalence of BD has been reported worldwide, peculiar ethnic distribution specially in Turkey, Iran, and Japan, which are along with Silk Road (an ancient trading route between the Mediterranean and East Asia), revealed distinct pattern in BD epidemiology. Mitochondrial DNA studies, which indicate trading of genes between Eastern Asians and Europeans travelling the Silk Road support that the discrete geographic spread of BD might have a genetic origin [5, 6]. It usually triggers in the third and fourth decades of life, affecting both sexes and rarely chil-

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dren. Overall, the disease is more severe in Mediterranean and Eastern cohorts than in Western populations, and it is generally more severe in males than females [7, 8]. The etiology and pathogenesis of BD remains indefinite, but a dysregulated immune response has been recommended as the underlying pathology, and can be activated by environmental pollution sources, mainly microbes, in genetically susceptible individuals. Whatever the stimulus is, the target tissue seems to be the small blood vessels, with different consequences of either vasculitis and/or thrombosis in many organ systems [9, 10]. Immune-mediated mechanisms play a key role in the pathogenesis of the disease, and inflammatory mediators get involved. Current studies have made clear details about the hypersensitivity of T lymphocytes to different types of antigens, which plays an important role in the pathogenesis [9].

We review the highlights of current studies on genetic susceptibility, the immunopathogenesis and novel treatment modalities of BD.

Genetic susceptibility of Behçet's disease

Behçet's disease is not a monogenic disorder and genetic disease with a Mendelian inheritance model. TNF- α overproduction has been associated with susceptibility and the severity in the lesion of BD. In the Turkish patients compared to controls, the TNF- α -1031C allele was associated with susceptibility to BD, but no significant difference was detected in the distribution of TNF promoter-308 and -376 polymorphisms, but a decreased frequency of the TNF-308A-378G haplotype was observed in patients. In Korean BD patients, no differences were found in the distribution of the TNF-308 G/A, TNF- α gene +252 G/A or TNFRSF1B/TNFR11 gene 196 R/M polymorphisms. In European BD patients, a high frequency of R92Q mutation in TNFRSF1A/TNFR1 gene was observed. In the Tunisian population, both the 1031T/C and 308A/GTNF- α gene promoter polymorphisms were found. The frequency of TNF- α -1031C allele was certainly significantly higher in BD patients than in healthy controls, whereas the frequencies of TNF- α 308G and TNF- β 252G alleles were similar in the two groups. A study in Iranian Azeri Turkish patients showed the relation between TNF- α 1031T/C and TNF- α 308G/A polymorphisms and susceptibility to BD with the frequency of the TNF- α 1031C allele being significantly higher in Behçet's patients than in healthy controls; whereas the frequency of the TNF- α 308A allele was similar in the two compared groups [11-15].

A study on interleukin (IL)-2 (-330, +166), IL-4 (-1098, -590, -33), IL-10 (-1082, -819, -592), IL-12 (-1188), IFN- γ (5644), transforming growth factor (TGF)- β (codon 10, 25), and IL-4RA (+1902) in Iranian patients with BD showed significant increase in frequency of IL-2 (-330) GG genotype, IL-4 (-33) CC genotype and TGF- β (codon 10) CC genotype. Meanwhile a significant decrease in the frequency of IL-4 (-33) TC genotype was detected in the patient group in comparison with normal controls. The genotype CC of

TGF- β at codon 10 was also significantly overrepresented in the patient group [16, 17]. The association of the IL-4 and IL-4R α gene polymorphisms with the susceptibility in BD Turkish patients and healthy control subjects, display that the frequency of IL-4 (-1098 TG and -590 CT) genotypes was higher in the patients [18]. The study on promoter region of the IL-10 encoding gene polymorphisms in patients with BD from Middle East and UK populations showed that the IL-10 (-1082AA) genotype was weakly associated with BD [19].

Hamzaoui *et al.* suggested a critical role of IL-17 in the pathogenesis of BD due to higher IL-17 serum level in active BD patients than control group. Chi *et al.* mentioned that the production of IL-23 and IL-17 by PBMCs was unregulated in patients with BD. Three single nucleotide polymorphisms (SNPs) including A126G, G155A, and A161G of the IL-17F gene was analyzed in Korean patients. Significant differences in the frequencies of allele and genotype in A126G SNP of IL-17 gene were found between BD patients and controls. The frequency of haplotype AA did not differ between patients with BD and controls [20, 21].

Disease susceptibility has always been associated with polymorphisms in the HLA-B gene, particularly HLA-B 51. Among the 24 already defined B 51 molecular subtypes (B 5101-B 5124), B 5101 and B 5108 were significantly associated with BD in Spanish, Italian, Greek, German, Iranian and Saudi Arabian patients, though Japanese patients were found to express the B 5101, but not B 5108 sub allele. In Israeli patients, HLA-B 51 and B 52 were both mainly associated with BD, being B 5101 and B 5201 the prevalent subtypes, while B 5108 and B 5104 were less expressed. In Chinese Han patients, B 5101 dominated, and a new association between BD and HLA-B 46 was found. Remarkably, in Turkish, Jordanian, Iranian and Japanese BD patients, the whole nucleotide sequences of HLA-B 510101 genes, with the promoter and intron regions, are identical, proposing that B 510101 may have the same phylogenetic source [22-31].

Recently, allelic variants of the HLA-G gene association with BD were studied. In Korean patients, the frequency of the HLA-G haplotype containing 3741+14 base pair (bp) and 1597 del C was increased. In Japanese patients with refractory ocular attacks, HLA-B 51, DQw3 alleles and the human complement factor 4 (C4) AQ0 allotype were significantly over-expressed. In Italian patients, a significant association between BD and the B51-DR5-DQw3 haplotype was observed. Moreover, in Turkish patients the B5-DR5 alleles were in strong positive linkage disequilibrium. In Chinese patients, the DR5-DQw1 and DRw8-DQw1 haplotypes were over-expressed. In Spanish patients, the DR11 and DQB1 0301 frequency was increased, and the DQB1 0303 allele was associated with severity of uveitis, whereas the DQ5 expression was decreased mainly in HLA-B 51-positive individuals [32-38]. Spanish BD patients linkage disequilibrium between TAP2B and HLA DQB1*0501 were found significance in the development of BD [39, 40].

Endothelial nitric oxide synthase (eNOS) gene polymorphism was associated with BD susceptibility in Italian and Korean, but not in Turkish patients. In a recent study, Glu298 Asp polymorphism of the eNOS gene was also associated with BD in Turkish patients, yet no association was found with clinical parameters of the disease. In a Japanese population, however, there was no significant difference in the frequencies of eNOS gene polymorphisms between patients with BD and controls [41-43]. Analysis of the potential association of the PD-1 and its ligand genes with BD in a Chinese Han population in four single-nucleotide polymorphism (SNPs) rs2227981 and rs10204525 of PD-1, rs1970000 of PD-L1 and rs7854303 of PD-L2 showed that there were no significant differences in the genotype and allele frequencies of PD-1 rs2227981 and rs10204525 between the Behçet's patients and controls. A similar result was found for PD-L1 rs1970000 versus healthy controls. Only the C allele and the CC genotype of PD-L2 rs7854303 were identified in patients and controls [44-46].

Laboratory findings

Laboratory findings are non-specific in BD. Homocysteine levels in active BD patients were significantly higher than in healthy controls [47]. Lower nitric oxide and neopterin levels were detected in BD patients compared with healthy controls [48]. Other studies on pathogenic aspects of the disease pointed to significant oxidative stress in patients with BD, the severity of which may arise from impaired antioxidant mechanisms [49]. There may also be a possible primary relation between BD and parvovirus B19, particularly in no ulcerative skin lesions of the disease [50]. Patients with active BD had higher serum prolactin levels than the inactive and control groups [51]. Moderate anemia of chronic disease is common, and a neutrophil leukocytosis is seen in 15% of patients. Serum immunoglobulin may be non-specifically elevated. Autoantibodies such as rheumatoid factor, anti-nuclear antibody and anti-neutrophil cytoplasmic antibody are generally negative [52]. Prominently, non-specific markers of inflammation such as C-reactive protein level and erythrocyte sedimentation rate can be normal in spite of active urogenital, ocular or CNS disease [53].

Etiopathogenesis

The origin of BD is unclear, but an autoimmune reaction initiated by infectious or environmental stimuli in a genetically predisposed individual seems most expected [54]. Primary studies isolated possible HSV-1 from oral ulcers HSV-1. DNA has been recognized in peripheral blood lymphocytes and monocytes of patients with BD. HSV-1 raised up circulating antibodies to HSV-1 were proven in patients with BD. HSV-1 DNA was detected in saliva, genital and gastrointestinal ulcers, not in oral ulcers of BD patients [55]. Following stimulation with streptococcal-related antigens, inflammatory cytokines like IL-1, IL-6, IL-8, interfer-

on γ , and TNF- α , by peripheral blood mononuclear cells and T-lymphocytes of patients with BD is enhanced [56]. Moreover, neutrophil activation in BD is correlated with the proportion of *S. sanguis* within the oral flora. Increased serum levels of IgA antibodies against the mycobacterial 65-kDa heat-shock protein (HSP; which cross-react with strains of *S. sanguis*) have been elevated in patients with BD. The expression of HSP60 in epidermal regions is upregulated at lesion sites in BD, and antibodies to streptococcal HSP60 might cause tissue damage. Antibodies reactive with uncommon serotypes of *S. sanguis* KTH-1, KTH-2 and KTH-3 were detected in the sera of BD patients and healthy controls [10, 57].

Immunology

Patients with active BD had significantly higher CD4 (+) CD25 (+) T cells compared with healthy controls. The Th1/Th2 lymphocytes balance plays an important role in the promotion and regulation of autoimmunity. Interleukin-12, a prototype of T helper type 1 (Th1) immune reaction, may have an important role in the pathogenesis of the disease. Streptococcal antigens stimulate expression of IL-12 p40 mRNA and protein, in combination with IL-12 p70 induction, in peripheral blood mononuclear cells [58]. Levels of IL-18, another Th1 cytokine, were higher in BD patients [59]. High serum IL-15 levels were identified in BD patients, also in the cerebrospinal fluid from neuro-BD patients. The Th2 cytokine IL-4 was detected in oral ulcers of BD patients. Hyperactivity of the neutrophils is detected in BD. Activated neutrophils secrete some cytokines and stimulate Th1 cells [60]. Activity of the BD increases with the high level of γ/δ positive T cells in circulation and mucosal lesions. CD+8 γ/δ positive T cells rather than CD4+ T cells were activated *in vivo* in BD patients [61]. Tumor necrosis factor- α gene, which is closely related to the HLAB 51 gene, played the main role in producing proinflammatory cytokine in BD. An overproduction of proinflammatory cytokines from cellular resources may be responsible for the inflammatory reaction in BD, with interferon- γ , TNF- α , IL-6, IL-8, and IL-12. These proinflammatory cytokines are elevated and probably contribute to neutrophil and endothelial cell activation [62]. Specially IL-12, and IL-18 produced by APCs, is regulating the neutrophil function and skewing of immune response [63].

As to Th17 cells, a prominent increase was identified in the peripheral blood of patients with active BD. Th17 cells regulate inflammation via production of distinct cytokines such as IL-17 family. Previous studies confirmed that Th17 cells are pathological in several human autoimmune and inflammatory diseases [64]. Th17 cells predominantly produce IL-17A-F, IL-21, IL-22 and TNF- α . High level of TBX 21 (Th1), RORC (Th17) and Foxp3 (Treg) were confirmed in neuro-BD [65]. The presence of the IL-21 and IL-17-A producing T cells was demonstrated in the cerebrospinal fluid, brain parenchyma inflammatory infiltrates, and intrac-

erebral blood vessels of patients with active BD and central nervous system involvement [21]. Interleukin-21 represents a promising objective for novel therapy in BD [66].

Vascular endothelial growth factor (VEGF) was significantly increased in the cerebrospinal fluid in neuro-BD. Based on this, VEGF might be associated with the increased percentages of CD4 cell subpopulation [67]. The relation between serum vitamin D concentrations and BD activity was studied. Active BD was associated with lower serum vitamin D levels [68]. In BD, studies on chemokine have been carried out, IL-8 being the first identified. Definitely, IL-8 levels in the serum of BD patients have shown the relation with disease activity and C-reactive protein [62]. Another potent chemoattractant for neutrophils is the chemokine GRO, which is functionally associated to IL-8, in the serum of BD patients with uveitis [69].

Treatment of Behçet's disease based on immunopathogenesis

In general, BD patients have been treated for soothing the inflammatory symptoms and suppression of the immune system. Conventional therapeutic approaches suppress the activity of the leucocytes and lymphocytes in T-cell-mediated diseases. Generally, infliximab is a first choice medicine in BD treatment. The dosing regimen for infliximab was 5 mg/kg *i.v.* at weeks 0, 2, 6, and every 8 weeks subsequently and most of these patients were treated with infliximab; remission of oral ulcers, genital ulcers, erythema nodosum, and other skin lesions were seen in 91%, 96%, 81%, and 77% of them, respectively [70, 71]. Etanercept was administered subcutaneously (*s.c.*) in a dose of 25 mg twice a week or 50 mg once a week. Etanercept was found effective in sustaining remission for mucocutaneous findings significantly in patients [72]. Adalimumab was administered *s.c.* as 40 mg every 15 days. Complete remission was achieved in all patients treated with adalimumab [73]. Rituximab was found effective in retinal vasculitis and ocular manifestations in BD. In patients with ocular lesions of BD, rituximab was used with cytotoxic drugs such as methotrexate, prednisone, and cyclophosphamide [74]. Anti-CD52 antibody therapy (CAMPATH 1-H) for T-cell depletion will be a potential alternative treatment for refractory BD [75]. Tocilizumab is a humanized anti-IL-6 receptor antibody, which binds both to soluble and to membrane-bound IL-6 receptor. Tocilizumab is a new therapeutic regimen for neuro-BD because IL-6 has a crucial role in the neuroimmunology of neuro-BD [76]. Two new orphan medicines, rilonacept (Regeneron) and canakinumab (Ilaris), are a human anti-IL-1 β monoclonal antibody and result in suppression of inflammation in patients with disorders of auto inflammation [77]. Toleration therapy by Heat shock proteins (HSPs) are synthesized when cells are exposed to non-specific stimuli such as trauma, heat, and infection HSP has played a key role in pathogenesis of BD. Oral administration of the HSP-60, the 336–351 sequence peptide linked

to recombinant cholera B-toxin B subunit (CTB) was found effective in inhibiting the development of uveitis [78-80].

Conclusions

Behçet's disease is a chronic inflammatory disorder of the oral and genital mucosa, skin, eyes, joints, blood vessels, lungs, brain and intestines. However, epidemiological studies recommend that genetic factors have an important influence on its pathogenesis and hence the disease is similar to auto-inflammatory disorders. Behçet's disease is more predominant in definite geographic regions and in certain ethnic groups. The role of the HLA-B 51 gene has been confirmed, although its contribution to the genetic susceptibility to BD was estimated to be only 19%. The disease susceptibility and severity is affected by polymorphisms in genes encoding for host effector molecules, such as TNF, TAP proteins. The mechanism of neutrophil activation and T-cell hypersensitivity in BD was unclear. The findings of recent studies have confirmed that the production of a variety of cytokines by T cells was activated with multiple antigens and activation of neutrophils. As in other auto-inflammatory disorders, exaggerated inflammatory response and over-expression of pro-inflammatory cytokines are proven. Further characterization of inflammatory features of BD developed better treatment options. Eventually, BD is expected as a polygenic disease and further investigations are required to elucidate the contributing role of immunogenetic predisposing causes and environmental triggers in its pathogenesis. As to treatment, anti-TNF- α therapy has been effective for mucocutaneous symptoms and sight-threatening panuveitis in BD.

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