Etiopathogenesis and Immunology of Behçet's Disease

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The etiopathogenesis of Behçet's disease (**BD**), which is a chronic, recurrent and multi-systemic disease, is still not completely known. Environmental factors, genetic predisposition, cytokines, molecular factors, autoantibodies, T-lymphocytes, coagulation abnormalities, and activation of neutrophils are blamed in the etiopathogenesis.

ENVIRONMENTAL FACTORS

Currently, it has been suggested that the disease appears mostly in individuals with genetic predisposition as a result of various environmental factors. Several studies indicate that the risk of BD development decreases in those migrating to regions where the BD prevalence is lower, and high BD prevalence supports the fact that environmental factors play a role in the disease etiology [1]. Mostly, bacteria (*Streptococci, Helicobacter pylori, Mycoplasma fermentans, Mycobacteria* and *Borreliaburgdorferi*) and viruses (Herpes simplex virus (**HSV**) 1 and 2, hepatitis viruses, cytomegalovirus, Varicella zoster virus, Epstein-Barr virus, and parvovirus B19) among the environmental factors are blamed [2,3]. The existence of an increased skin reactivity to certain Streptococcus sanguis antigens in the skin and sanguineous monocytes in BD patients is also intriguing [4]. There are few studies investigating the relationship of BD with streptococcal infections and herpes simplex virus type 1; however, no direct relationship has been demonstrated, yet [5]. In a study investigating the role of infections in BD and neuro-Behçet's syndrome (**NBS**),

some evidences for the polyclonal immune activation have been shown in the serum samples of individuals with BD and NBS, rather than a specific virus effect [6]. Earlier, since there are oral aphthae in almost all individuals with BD, oropharyngeal pathogens were mostly accused in the pathogenesis [7]. An increase in oral ulcers and the activation of the disease after dental procedures also supported this opinion [3]. It was suggested that this situation occured as a result of the fact that microorganism-derived antigens proceeded to the blood circulation from the oral cavity [3]. The rate of *Streptococcus sanguinis* in the oral flora was also found to be higher in these patients, compared to healthy individuals [2]. In addition to streptococcal antigens, *Escherichia coli, Staphylococcus aureus*, and a non-peptide antigen, which is commonly found in many bacteria, activated γδ-T cells in these patients, which made us consider that the T lymphocytes of these patients were hyperactive not against specific bacteria, but against bacterial antigens in general [2,7]. Based on the observation of intracellular inclusion particles in the samples obtained from aphthous ulcers, Hulusi Behçet was the first physician to suggest that viral infections could play a role in the BD etiology and it was studied by many researchers [8]. HSV-1 genome was also detected in the orogenital ulcers of patients with BD [2,8].

The most widely adopted opinion on the role of microorganisms in the BD etiology is that the antigens (i.e., heat shock protein) of microorganisms proposed in the etiology are similar with human proteins, and the immune response appears as a result of emerging cross-reactivity [1]. Heat shock proteins (**HSPs**) are a group of proteins synthesized by all eukaryotic and prokaryotic cells due to physiological shock (i.e., heat, anoxia, and trauma) and microbial stimulations and expressed in the cell membrane [4,2]. These proteins protect cells from severe damage and apoptosis [2]. Firstly, bacterial 65-kDa HSP (**HSP65**) isolated from mycobacterium has a considerably similar amino acid sequence with human mitochondrial 60-kDa HSP (**HSP60**), and an immune response occurs due to the cross-reactivity between them [1]. It has been suggested that, as a result of this cross-reactivity, auto reactive T-cell clones specific to human HSP60 are formed, and immunopathological changes in BD appear [9]. HSP60 causes the production of pro inflammatory cytokines (i.e., interleukin [**IL**-]-6, 12, 15, and tumor necrosis factor-alpha [**TNF-α**]) by connecting to toll-like receptors 2 and 4, the expression of cell adhesion molecules (i.e., intercellular adhesion molecule [**ICAM**] and vascular cell adhesion molecules [**VCAM**]) and the formation of Th1 immune response [2]. The accumulation of microbial agents in the gingiva and aphthous ulcers of BD patients and the immune response occurring against the related microbial HSP cross-react with endogenous HSP and create auto reactive T cell clones, thereby, leading to immunopathological changes. In general, some others have proposed that BD initially manifests with recurrent mucosal ulcers and other clinical signs occur over time.

GENETIC PREDISPOSITION

Behçet's disease is a polygenic disease and does not have a specific Mendelian genetic pattern. Most cases are sporadic. Familial cases with regional differences have been also reported. Familial

cases are more frequently seen in Turkey, Arab Countries and Far East, and the age of onset is younger in these cases. The family history is more frequent in juvenile BD [4]. The presence of familial cases, extraordinary geographic distribution of the disease, and strong relationship with major histocompatibility component (**MHC**) allele make us consider that genetic factors may also have a role in the etiopathogenesis [4]. It is thought that the strongest genetic predisposition factor for BD is HLA-B51 allele [2]. More frequent association of HLA-B51 with HLA-B5101 and HLA-B5108 alleles has been also reported. Therefore, although it is considered that the strongest genetic predisposition factor for BD is HLA-B51 allele, this allele is positive only in 60% of individuals with BD [2, 10]. In addition, HLA-B51 positivity is more frequent in male patients and is related to an increase in the possibility of genital ulcers, and eye and skin involvement. However, the gastrointestinal system involvement is less frequent in patients with HLA-B51 positivity [11]. Despite the present data, there is a limited number of data to provide the use of HLA-B51 positivity in BD as a diagnostic and prognostic marker [12]. Also, HLA-B51 frequency varies among societies. The association between BD and HLA appears to be lower in the United States and United Kingdom than in the Middle East and Japan. Genetic polymorphisms of various cytokines, their regulators, and promoters are being studied. In a genome-wide association study in BD, the most significant association was found with the MHC region in chromosome 6, mostly due to HLA-B51 [13]. A TNF-α promoter gene located closely to HLA-B51, MHC Class 1 chain-related gene, and HLA-A26 gene located close to the telomeric end are the other MHC genes which are considered to be related to BD [12,9]. Apart from MHC, IL-10 and IL-23R mutations have been also reported to be the other genes playing a role in the BD pathogenesis [2,12,14].

CYTOKINES

Behçet's disease is an autoimmune and auto inflammatory syndrome. Alterations in the immune system due to the triggering factors in individuals with a genetic predisposition to BD are responsible for the symptoms of the disease and organ injuries. Many pro inflammatory cytokine levels such as IL-1, IL-6, IL-8, IL-12, IL-15, IL-18, and TNF-α, interferon gamma (**IFN-γ**) released from different cells are found to be higher in the serum of patients with BD [15]. In a study, increased IL33 levels were found to be related to the disease activity and neurological involvement [14]. In another study, IL8 levels in patients with active BD with the vessel involvement were reported to be significantly higher compared to those without the vessel involvement [16]. In another study, among a number of serum cytokines studied, including IL-6, only IL-8 was found to be significantly increased in patients with BD both with and without neurologic involvement, but there was no correlation between IL-8 levels and clinical severity [6]. Furthermore, increased TNF levels in the serum samples of patients with BD indicate that TNF-α inhibitors are effective in the treatment of patients with BD with uveitis in the first-line setting, suggesting that TNF-α also plays a role in the pathogenesis of BD [14]. Domiciled cells in involved sites play major roles in the pathogenesis of BD by actively helping in the recruiting, activating and promoting survival of inflammatory cells. Based on these findings, treatments that modulate the cytokine network, such

as use of immuno suppressants like azathioprine, cyclosporine, corticosteroids or anti-TNF-a monoclonal antibodies (**mAb**), present novel ways to help mitigate BD [17].

MOLECULAR FACTORS

Molecular factors related to the pathogenesis of BD a soluble form of intercellular adhesion molecule 1 (**ICAM-1**) can be detected in peripheral blood of patients with a variety of inflammatory disorders, including BD. The ICAM-1 has likely to have a mild effect. Increased E-selection in BD may be a direct consequence of the leukocyte and endothelium activations and has a significant positive correlation with the erythrocyte sedimentation rate and C-reactive protein levels in patients with BD [18]. Vascular endothelial growth factor (**VEGF**) induces inflammation with a strong effect on the endothelium cells. Mendoza-Pinto et al. reported a potential effect of genetic polymorphisms of VEGF on the expression of this molecule that may contribute to BD developing [4].

AUTOANTIBODIES

Anti-endothelial cell antibodies (**AECAs**) were also described in BD as in many types of vasculitis, and several studies reported the AECA frequency as 18 to 50% in patients with BD. In the aforementioned studies, it was suggested that these antibodies might be primarily related to the disease activity, particularly in patients with vascular involvement [2,4]. When patients with BD and with and without neurologic involvement along with healthy controls were screened using a protein macro array and then with confirmatory immuno histochemistry and immunoblotting studies, the mitochondrial carrier homolog 1 (Mtch1) autoantibody was detected and appeared to be highly sensitive and specific for NBS and BD [19]. This antibody, as well as another autoantibody against annexin V in BD [20], have targets that are associated with apoptosis. Furthermore, the levels of these antibodies were correlated with the severity of the disease, and this increased the possibility of NBS to be included in pathogenic mechanisms [21].

On the other hand, since there are Anti-Saccharomyces cerevisiae antibodies in approximately 50 to 60% of patients with Crohn's disease, it is necessary to evaluate other antibodies such as anti-neutrophil cytoplasmic antibodies to distinguish BD from inflammatory intestinal disease [22]. A Retinal-S antigen is localized to the photoreceptor of the retina, and has been shown to be a potent uveitic auto antigen [23]. Although, the presence of auto antibodies against endothelial antigens and retinal proteins in some tissues such as the oral mucosa makes us consider that there may be a failure in the humoral immune response, indicating that existing evidences for the fact that auto antibodies play a role in the BD pathogenesis, the evidence is limited [2,7].

T-LYMPHOCYTES

They are major cells including γδ and cytotoxic T cells, Th1, Th17 and regulatory T cells (**Treg**) which play a role in the BD pathogenesis [2].

Cytotoxic T Cells (γδ T cells, CD56+ cells, CD8+ CD56+ and CD56 + T cells)

While γδ T cells play a role only in the immune response against infections by recognizing bacteria-derived antigens, they also play a role in the autoimmunity by recognizing autologous antigens [1,4]. These cells, which play a major role in the primary defense mechanism in the mucosal immunity, also play a role in the BD pathogenesis accompanied by many mucosal lesions [1,2,4]. The number of γδ T cells in the circulation of patients with BD and mucosal lesions has increased, and this increase has been associated with the disease activity [4]. The fact that infliximab, which is an anti-TNF-α antibody and is effective in the BD treatment, inhibits the increase in the number of γδ T cells, activation, and cytotoxic activity, indicating the importance of these cells in the BD pathogenesis once again [2]. Natural killer T cells regulate Th1 or Th2-mediated immunity and play a role in the control of autoimmune diseases. These cells increase in the cerebrospinal fluid of untreated active NBS patients and in the aqueous humor liquids of patients with uveitis. In addition, CD8 bright CD56+ T cells exhibit a strong cytotoxic effect with both fas-ligand and perforin-dependent pathways [2].

Th1 Cells

There are several studies indicating that there is a relationship between the severity of Th1-type immune response and disease activity [1]. It has been suggested that Th1 cytokine levels such as IL-12, IL-18, TNF-α, and INF-γ increase in the lesions of patients with active BD and peripheral blood [2,24]. High lymphocyte populations have been indicated in BD with an imbalance between Th1- and Th2- phenotype lymphocyte components of the immune response. However, as T lymphocytes are particularly responsive to antigens of viral or bacterial pathogens, the skew is therefore suggested toward Th1-phenotype lymphocyte response followed by an infiltration into the affected regions [25]. The immunogenetic findings are providing clues that both innate and adaptive immunity are involved in the pathogenesis of BD and confirming the importance of the predominant polarization towards helper T cell Th1 versus Th2 cells, and the involvement of Th17 cells [26]. Although a Th1 predominant cytokine profile and elevated IL-17 levels are examples in favor of the adaptive system, the primed state of neutrophils, circulating polyclonal gamma delta T cells in the serum, and the decreased mannose-binding leptin levels are in favor of the action of the innate immune system [5,26].

Th17 Cells

Th17 is a recently described adjuvant subtype of T cells, which is responsible for many autoimmune/inflammatory diseases [2]. It is another cytokine-stimulating IL-21, Th17 differentiation created by active CD4+ T cells [2]. As a result of the fact that IL-21 levels in the peripheral blood of patients with BD increased in relation to the disease activity, and CD4+T cells were stimulated with IL-21, the differentiation of lymphocytes in the direction of Th17 and Th1 increased, while the number of Treg cells decreased [2,27]. The Th17 Treg cell rate was also

reported to be higher in the peripheral blood of patients with active BD and in the CSF of NBS [2]. In a study, the number of Th17 and Treg cells returned to normal after IL-21 blockage [24]. Therefore, it was concluded that IL-21 blockage might be a promising target in the BD treatment [2].

Regulatory T Cells

Treg cells are a subgroup of T cells playing a major role in the protection against autoimmune diseases and expressing CD4, CD25, and FOXP3. Several studies indicate that the antigen-specific activation of Treg inhibits the antigen-specific activation of other T cells and, thus, it suppresses antigen-specific T cell responses both in mice and humans [28].

COAGULATION ABNORMALITIES

Thrombotic events in BD are seen in approximately 25% of patients. Deep and superficial venous thrombosis is more frequent, compared to arterial thrombosis [2,10]. However, the exact mechanism of increased thrombosis disposition is unknown. Failed procoagulant, anticoagulant, and fibrinolytic factors may be also responsible for thrombosis disposition along with endothelium cell damage and vasculitis, which is the main pathological process of the disease [10].

Thrombomodulin is a cell surface glycoprotein present on the vascular endothelium surface. The high level of thrombomodulin in the peripheral blood of patients with BD may be responsible for endothelial cell damage [10]. Thrombophilic factors such as factor V Leiden and prothrombin G20210A gene have not been linked to BD, although Behçet's patients with coexisting thrombophilic factors may be at higher risk for thrombosis [30]. Levels of the fibrinolytic inhibitors thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 were higher in Behçet's patients than in controls, particularly in those patients with thrombosis [31].

Hyperhomocysteinemia is a well-known risk factor for atherosclerosis and thrombosis. Increased plasma homocysteine levels in patients with BD accompanied by thrombosis suggest that it may be an independent risk factor for thrombosis in BD [2,4,10]. There is also an increase in the fibrinolysis inhibitor factors in BD and a decrease in the tissue plasminogen activators [2].

Currently, it is thought that the thrombosis seen in BD is due to immune-mediated endothelial dysfunction, which increases in the presence of other risk factors that are associated with BD [4,29].

NEUTROPHIL ACTIVATION

It has been suggested that neutrophil hyper activation has an important role in the BD pathogenesis. An increase in the expression of CD11a, CD10, and CD14 present on the neutrophil cell surface and regarded as neutrophil activation indicators confirms that [2]. Cell surface markers indicative of activation, such as CD64, are increased in patients with active disease to levels similar to those of patients with sepsis [32].

The presence of an immune-mediated mechanism in which cells presenting antigens in BD and T lymphocytes release cytokines to induce neutrophil hyperactivation and activated neutrophils produce cytokines stimulating Th1 cells is in question [2,4]. There is a generalized derangement of the lymphocyte and neutrophil populations during the course of BD, which is characterized by elevated peripheral white blood cell count, activated monocytes, increased neutrophil motility with infiltration into the cutaneous and ocular lesions, and increased circulating proteins such as C3, C4, C5, IgA, haptoglobin, and orosomucoid [33]. Active monocytes produce a number of pro inflammatory cytokines, such as IL-1, IL-6, IL-8, TNFα, and granulocyte-macrophage colony stimulating factor, and these cytokines contribute to neutrophil activation by their augmented interactions with endothelial cells, causing tissue damage possibly by priming neutrophils [34].

It was indicated that whether there is BD or not, neutrophil functions increase in individuals with HLA-B51 positivity [2]. Another finding indicating neutrophil activation in BD is that myeloperoxidase and superoxide levels released from active neutrophils increase. Increased levels of reactive oxygen products are also an important indicator of neutrophil activation and a valuable finding, revealing the effect of neutrophils on the pathogenesis of the disease [2,4].

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