Genetics and immunodysfunction underlying Behçet’s disease and immunomodulant treatment approaches

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ABSTRACT

Behçet’s disease (BD) is a chronic autoimmune condition primarily prevalent in populations along the Mediterranean Sea. The exact etiology of BD has not been fully explained yet, but the disease occurrence is associated with a genetic factor, human leukocyte antigen (HLA)-B51 antigen. Among the various immunodysfunctions that are found in BD, patients are increased neutrophil motility and superoxide production, as well as elevated production of tumor necrosis factor (TNF)-\(\alpha\) and decreased production of interleukin (IL)-10. Elevated levels of inflammatory cytokines like IL-1 and IL-17 in BD have been found associated with aberrant expression of microRNA. Gene polymorphisms in BD patients have been observed in molecules involved in responses to pathogens that can ultimately modulate the host anti-microbial response. Moreover, several single nucleotide polymorphisms (SNPs) have been reported in genes encoding chemokines and adhesion molecules; many of these changes manifest as increases in vascular inflammation and vascular damage. Lastly, genetic and epigenetic changes have been suggested as involved in the pathogenesis of BD. Modifications in DNA methylation have been found in BD patient monocytes and lymphocytes, leading to adverse function of these cells. This review presents a comprehensive compilation of the literature with regard to the immunodysfunction underlying BD, as well as of the genetics, newly described clinical specifications and novel treatment strategies using immunomodulators based on the current understanding of BD.

Introduction

Behçet’s disease (BD) is a chronic inflammatory disorder characterized by recurrent oral ulcers, genital ulcers, ocular involvement and skin lesions, with periods of remission and exacerbation. Other manifestations have been frequently reported in BD, including arthritis, vasculitis, epididymitis, gastrointestinal lesions and central nervous system lesions (Sakane et al. 1999; Hatemi et al. 2008). Epidemiological studies have documented that BD is comparatively frequent in countries between 30°N and 45°N latitude in countries around the Mediterranean, the Middle East, China and Japan, and along the ancient Silk Route (Verity et al. 1999).

BD is defined as a disorder with shared autoimmunity and auto-inflammatory characteristics. The major specifications of BD that cause classification of the disease as an autoimmune disorder include positive responses to classical immunosuppressive agents like cyclosporine and azathioprine (Evereklioglu 2005), and involvement of autoantigens in initiation of the disorder, namely heat-shock protein 60 (HSP60) (Direskeneli and Saruhan-Direskeneli 2003). Other features of the disease implying an auto-inflammatory role include high-titer autoantibodies or antigen-specific T-cells, a role for major histocompatibility complex (MHC) Class I molecules, clinical periods of recurrent inflammation characterized predominantly by neutrophils (Stojanov and Kastner 2005), an association with familial Mediterranean fever (FMF) and effectiveness with treatments containing anti-inflammatory agents (Cho et al. 2012).

Despite uncertainties about the etiopathogenesis of BD, recent findings from immunogenetic studies have contributed to resolving more pieces of the puzzle. In this review, current understanding of BD with regard to immunology, genetics, newly described clinical specifications and, ultimately, novel treatment strategies is discussed.

Epidemiology

Behçet’s disease (BD) is prevalent along the ancient “Silk Route” that extends from Japan to the Middle East and Mediterranean countries. In BD, sex distribution is almost equal, but there are some exceptions; while in some Middle Eastern and Mediterranean nations, male predominance has been seen, the inverse has been noted in Japan and Korea (Cho et al. 2012).
Among the affected countries, Turkey has the highest prevalence (420 cases per 100,000 inhabitants) (Azizleri et al. 2003). The prevalence of BD has been reported (per 100,000 inhabitants) to be 80 in Iran (Davatchi et al. 2010a), 13.5 in Japan (Nakae et al. 1993), 14 in China (Piga and Mathieu 2010), 19.5 in Saudi Arabia, 15.2 – 120/100,000 in Israel, 2.1/100,000 in Kuwait, 17 in Iraq and 7.6/100,000 in Egypt (Mahr et al. 2008). Within Europe, the reported prevalence (per 100,000 inhabitants) trends higher among people in the Mediterranean area; Italy 2.5, Spain 7.5 and France 2.4 excluding immigrants (Zouboulis et al. 1997; Salvarani et al. 2007; Mahr et al. 2008). In the Atlantic regions, values are estimated at 1.53 in Portugal, 0.64 in United Kingdom (UK), 0.55 in Germany and 0.27 in Scotland (Mahr et al. 2008; Davatchi et al. 2010a). The United States has a prevalence of 5/100,000 (Calamia et al. 2009).

Territorial variability in BD expression is a well-known epidemiological feature. BD shows associations with MHC Class I molecules, and HLA-B51 is the most strongly associated genetic factor to this disease (Ohno et al. 1982). The frequency of the HLA-B51 subtype among inhabitants along the Silk Road ranges from 20 to 25% in the general population and 50 to 80% among BD patients. In contrast, the frequency of HLA-B51 is 2–8% in Northern European and US general populations, but 15% among their BD patients (Dalvi et al. 2012). It was proposed that genetic factors may be more effective than environmental factors in impacting on the incidence of BD (Mahr et al. 2008). BD risk is not related to age and the disease has a primarily hereditary basis. For example, the prevalence of BD among Turks that immigrated to Germany is reduced (15.1/100,000) compared with the population in Turkey (80–420/100,000), but is still high in comparison with individuals who have German ancestry and live in Germany (0.30/100,000) (Papoutsis et al. 2006).

Though BD occurs sporadically, familial aggregation and a higher prevalence in siblings and parents is seen in some familial clusters of BD (Koné-Paut et al. 1999). Among different populations, familial aggregation of BD also varies (Table 1). Familial aggregation is 18.2% in Turks, 15.4% in Koreans, 2.6% in Chinese, 2.2% in Japanese and 0–4.5% in various European populations (Gollnickls et al. 1997; Fietta 2005). Among patients with early-onset BD (i.e. 18–40 years of age), familial aggregation was observed (Koné-Paut et al. 1999). The disease generally is more severe in patients with early onset (Yazici et al. 1984; Saricaoglu et al. 2006). Late-onset cases, i.e., after 55 years of age, have been reported rarely (Akpolat et al. 2002).

### Immunodysregulation in the course of developing/progressive BD

#### Immunology

Although the pathogenesis of BD is not completely understood, it is considered an inflammatory disorder at a crossroad between autoimmune and auto-inflammatory syndromes. Increased systemic levels of chemokines and inflammatory cytokines (including interleukin [IL]-1, IL-18 and tumor necrosis factor [TNF]-α) are characteristics of the profile associated with BD patients (Hamzaoui et al. 1990, 2002; Musabak et al. 2006; Htoon et al. 2011). TNF-α, encoded in the Class III region of the HLA complex adjacent to HLA-B, is a pro-inflammatory cytokine important in regulation of the immune response. It is involved in the activation of macrophages and apoptosis, and appears responsible for recurrent inflammatory reactions experienced by BD patients (Oztas et al. 2005; Ates et al. 2006). Members of the IL-1 family play a pivotal role in the inflammatory responses (Barksby et al. 2007). Most recent studies have implicated the IL-33 ligand for the ST2 receptor in the pathogenesis of BD (Schmitz et al. 2005; Chackerian et al. 2007; Palmer et al. 2008). IL-33 is a member of the IL-1 superfamily expressed by epithelial, endothelial, inflammatory and central nervous system cells. IL-33 expression is up-regulated by pro-inflammatory conditions and it can function both as a cytokine and as a nuclear factor regulating gene transcription. 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 immunosuppressants like azathioprine, cyclosporine, corticosteroids or anti-TNF-α monoclonal antibodies (mAb), present novel ways to help mitigate BD (Everecklougo 2005; Hamzaoui et al. 2015; Sket al. 2015).

### Infectious/environmental factors and BD

It has long been postulated that an autoimmune process, possibly triggered by an infectious or environmental agent in genetically susceptible individuals, is important in the pathogenesis of BD (Behcet 1937; Gül 2001; Kulaber et al. 2007; Pay et al. 2007). A presence of herpes simplex virus (HSV) type 1 genome was found in peripheral blood lymphocytes of BD patients (Eglin et al. 1982; Studt et al. 1991). A 289-bp genomic sequence of type 1 HSV was also amplified (by PCR) from saliva fluid, as well as genital/gastrointestinal ulcers of some BD patients (Lee et al. 1996; Sohn 1997). The existence of an increased skin reactivity to certain Streptococcus sanguis antigens in the skin and sanguineous monocytes in BD patients is also intriguing (Mendoza-Pinto et al. 2010).

It has been reported that individuals from endemic areas who immigrated to areas with a low prevalence of BD had an intermediate risk to then develop it, implying environment could also play a key role in disease onset (Mendes et al. 2009). The most generally accepted theory for the role of infectious agents is that these antigens have high homology with human proteins like heat-shock protein 65 (HSP65). Mycobacterium-derived HSP56, which has high homology with human HSP60 protein, causes a cross-reaction leading to activation of γδ T-cells in BD patients.

### 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. However, the influence of ICAM-1 on BD is probably small. Increased E-selectin in BD may be a direct consequence of the leukocyte and endothelium activations and has a significant positive correlation with the erythrocyte sedimentation rate (ESR) and C-reactive protein.

| Table 1. Familial aggregation varies among different populations. |
|---------------------------------------------------------------|
| Population | Familial aggregation |
|------------|----------------------|
| Turkish    | 18.2%                |
| Koreans   | 15.4                 |
| Jews       | 13.2                 |
| Chinese    | 2.6                  |
| Japanese  | 2.2                  |
| European populations | (0–4.5%)            |
| **Stronger familial aggregation was observed in population with early onset of BD.** |
(CRP) levels in patients with BD (Sakane et al. 1999; Yazici and Esen 2008). Vascular endothelial growth factor (VEGF) is involved in inflammation with a powerful effect on endothelial cells. Mendoza-Pinto et al. (2010) reported a potential effect of genetic polymorphisms of VEGF on the expression of this molecule that may contribute to BD developing.

**Autoantibodies in BD**

Several studies have reported the presence of anti-endothelial cell antibodies (AECA) in patients with BD. Lee et al. (2003) found that the α-enolase was a target protein of antisera to endothelial cells in patients with BD. These researchers concluded that patients with BD may have circulating antibodies induced by antigens on endothelial cells. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) may be especially common in intestinal BD. Studies have reported both increased and normal levels of ASCA in BD patients. Because ASCA is found in 50–60% of patients with Crohn’s disease, it is necessary to assess other antibodies such as an antineutrophil cytoplasmic antibody (p-ANCA) in differentiating BD from inflammatory bowel disease (Filik and Biyikoglu 2008).

**Cytokines in BD**

Overproduction of select pro-inflammatory cytokines from several cell types appears to be responsible for the inflammatory responses in BD patients, with circulating levels of interferon (IFN)-γ, TNF-α, IL-6, IL-8 and IL-12 being unusually high. IL-8 acts as cofactor/costimulator of T<sub>H1</sub> cell IFN-γ production. IL-18 can directly stimulate IFN-γ synthesis by CD3<sup>+</sup> and natural killer (NK) cells. Both IFN-γ and CD40L activate macrophages and monocytes to produce reactive nitrogen species and cyto-/chemokines that, in turn, stimulate antigen-presenting cells to express MHC Class II molecules and costimulatory molecules—resulting in T-cell activation. It has been suggested IL-18 and IFN-γ contribute to local inflammatory responses in BD (Hamzaoui et al. 2002; Ben Ahmed et al. 2004; Hamzaoui et al. 2009).

**Role of γδ and other T-cells in the pathogenesis of BD**

Bank et al. (2003) showed that the frequency of γδ T-cells was higher in BD patients than in healthy controls. A higher number of these cells were associated with increased disease activity in patients with BD. Koarada et al. (2004) investigated relationships between production of T<sub>H1</sub>/T<sub>H2</sub> cytokines and cellular division, cellular kinetic and proliferation in patients with BD. These authors found that T<sub>H1</sub>-related responses of dividing CD4<sup>+</sup> T-cells played a predominant role in active BD. Koarada et al. (2004) concluded cell kinetics also had an important role in T<sub>H1</sub> cell differentiation and pathophysiology in cases of BD. Ling et al. (2007) assessed the role of CD3<sup>+</sup>CD8<sup>-</sup>CD4<sup>-</sup> double-negative (DN) T-cells in BD pathogenesis in 10 pediatric patients (age 12.2 ± 2.2 years, 7 patients were in remission and 3 were in exacerbation states), reporting that the proportion of CD4<sup>-</sup>CD8<sup>-</sup> DN T-cells was significantly increased in BD patients in comparison with healthy controls as well as with the exacerbated BD cases. Moreover, Ling and colleagues found that levels of DN T-cells were significantly increased in BD patients in remission, compared with levels in healthy controls.

**Activation of neutrophils in the pathogenesis of BD**

BD is also considered as a neutrophilic vasculitis in vasmuror vessels (Ling et al. 2007). Eksioglu-Demiralp et al. (2001) investigated roles of phagocytic neutrophil functions and surface molecules (i.e. CD10, C16, CD14) associated with neutrophil activation in BD patients. These researchers found a presence of proactive neutrophils in these subjects. The studies also showed increased generation of reactive oxygen species (ROS) by the BD neutrophil and it was surmised the ROS-mediated oxidative stress related to neutrophil activation may play a significant role in the disease pathogenesis. Yazici (2004) described the importance of neutrophil activation as a main source of oxidative stress through the oxidation of proteins and concluded that advanced oxidation products of proteins (AOPP) might be used as markers of BD status. Further, hyper-activated neutrophils secrete some cytokines that are both autocrine and also stimulate T<sub>H1</sub>L cells.

**Coagulation abnormalities in the pathogenesis of BD**

Thrombotic events can occur in as many as 25% of BD patients, though causes are unclear. Espinosa et al. (2002) found no abnormalities—except for elevated thrombin, fibrinolysis and thrombomodulin production—that were not related to thrombotic events in patients. A well-known risk factor for atherosclerosis/thrombosis is hyper-homocysteinemia. High levels of homocysteine (tHcy) can be an independent risk factor for thrombosis and may be responsible for endothelial damage in BD. tHcy may be an additional risk factor for the development of retinal vascular occlusive disease (Korkmaz et al. 2002). These researchers described two BD patients with a mutation in the prothrombin gene G > A20210, an established cause of thrombosis. Salvarani et al. (2000) found that reduced activated C protein (natural anticoagulant) levels were associated with a high incidence of venous thromboembolism in BD patients.

**Clinical features**

BD has 16 sets of diagnosis/classification criteria. Two of these are from an International Study Group (ISG) and the International Criteria for Behcet’s Disease (ICBD) (Davatchi 2011). The ISG criteria have five items (Davatchi et al. 2010b). Two items are mucous membrane manifestations, i.e., oral aphthosis (OA) and genital aphthosis (GA). The third item is skin manifestations comprising pseudofolliculitis (PF) and nodosum-like lesions (EN). The fourth item is ocular manifestation including anterior uveitis (AU), posterior uveitis (PU) and retinal vasculitis (RV). A presence of a pathergy phenomenon (PP) is the fifth item that is evaluated by a pathergy test (Davatchi et al. 2010b).

A mandatory item in ISG criteria is the presence of OA. Two other items (GA and PP) were used for classifying a patient as having BD. Vascular manifestations (VM) are one of the characteristics of BD and were used in some criteria before the advent of ISG such as Mason and Barnes, Hewitt, Hubault and Hamza, Dilsen, Japan revised, and Dilsen revised criteria, hence VM have been added to the five items of the ISG criteria. VM are described as superficial phlebitis, arterial thrombosis, deep vein thrombosis, large vein thrombosis, arterial thrombosis and aneurysm. Hence, ICBD uses six items: OA, GA, skin (PF and EN), eye lesions (AU, PU and RV), VM and PP. In the ICBD, genital aphthous lesions and eye lesions get each 2 points and have more diagnostic value than the others manifestations. OA, skin
(PF, EN), VM and PP get each 1 point. By this, a patient is diagnosed/classified as having BD if get ≥ 3 points (Davatchi 2011).

**Mucocutaneous lesions**

Mucocutaneous lesions are a hallmark of the disease. The most frequent features of BD in all countries are oral ulcers (OU; 92–100%), genital ulcers (GU; 57–93%) and cutaneous lesions (38–99%) together with ocular (29–100%) and articular (16–84%) involvements. The most commonly observed cutaneous lesions are erythema nodosum (EN)-like lesions (15–78%) and papulopustular lesions (PPLs) (28–96%) (Alpsoy et al. 2007b).

**Oral aphthosis (OA)**

Oral aphthosis (OA) is the most common manifestation of BD (Khairallah et al. 2012). It is characterized by recurrent and painful aphthosis of the oral mucosa especially in the mucous membranes of the lips, buccal mucosa, tongue and soft palate. They can occur after local trauma or dental intervention (Mendes et al. 2009). OA often subsides spontaneously within 1–4 wk and recurs at intervals from days to months (Alpsoy et al. 2007b). OA can be categorized as: (1) minor (most common): < 1 cm diameter, (1–5 in number), shallow, surrounded by moderately painful, an erythematos halo, healing without scarring in 4–14 days; (2) major (less frequent): > 1 cm diameter, 1–10 in number, morphologically alike, more painful, persistent and may heal with scarring in 2–6 wk; or (3) herpetiform (the least common): 2–3 mm diameter, recurrent crops of numerous small and painful ulcers, which may become coalescent (Mendes et al. 2009).

**Genital aphthosis (GA)**

Genital aphthosis (GA) occurs in 57–93% of BD patients (Mendes et al. 2009). GA and OA are similar in appearance, but GA is larger with a more irregular border, commonly healing with scarring, and may not recur as often. The most common involved site in males is the scrotum (90%). They are deeper than an OA and heal within 10–30 days—with a tendency to scar. The labia majora are the most frequently involved site in females. Perianal and groin lesions can occur in both sexes.

**Pseudofolliculitis lesions (PPLs)**

Pseudofolliculitis lesions (PPLs) and acne-like lesions are the most common cutaneous manifestation (28–96%) and their distribution is more widespread than adolescent acne, affecting the limbs, face, trunk and buttocks (Mendes et al. 2009). The most frequent skin manifestations are erythema nodosum-like and pseudofolliculitis lesions. PPL and acneiform nodules can appear all over the body and they are not always hair follicle-associated (Saadoun and Wechsler 2012).

**Erythema nodosum (EN)**

Erythema nodosum (EN) lesions are frequently (15–78%) found in BD patients, especially females, and occur in approximately one-third of all patients (Mendes et al. 2009). EN occurs as bilateral, pretibial, painful and hot erythematous nodules. EN-like lesions can also be localized to the face, neck, forearms and buttocks. The lesions resolve spontaneously within 2–3 wk in pigmented ethnic groups. However, recurrence is also common.

**Papulopustular lesion (PPL)**

Papulopustular lesions (PPLs) are seen in 30–96% of cases with this disease (Mat et al. 2014; Alpsoy 2016). PPLs are sterile, folliculitis- or acne-like lesions; recent studies have shown a colonization of coagulase-negative *Staphylococcus* and *Prevotella* species. PPLs are mostly localized on the back, chest, shoulder area and, less commonly, on the face. PPLs appear as papules and become pustule over the course of 24–48 hr after appearing. Studies have shown that PPLs are more frequent in patients with positive skin prick test (SPT) and arthritis. There is no evidence of vascular injury in the PPL.

**Pathergy**

Pathergy phenomenon, which is also called skin pathergy test (SPT), is a non-specific skin hyper-reactivity induced by needle prick, minor trauma such as a bump, bruise or needle stick injury. The test positivity is defined as the development of a papule or a pustule at the needle prick or minor trauma site after 24–48 hr (Alpsoy 2016). The SPT might become negative if it is performed in patients under corticosteroids or immunosuppressive therapy (Khairallah et al. 2012). Pathergy positivity is very specific to BD patients, but its sensitivity varies in different countries. Patients in Turkey and Japan have 60–70% positivity. It is rarely observed in patients with BD from Northern Europe and North America (Seyahi et al. 2008).

**Ocular manifestation**

Eye involvement occurs in 30–70% of BD cases (Mendes et al. 2009). It is commonly bilateral and usually occurs 2–3 year after onset of BD symptoms. Half of all BD patients have ocular manifestation, and it is more frequent and severe among male and young patients (Seyahi et al. 2008). Anterior uveitis (AU) with hypopyon is rare, transient with a bad outcome, and is generally associated with severe retinal vasculitis. AU is observed only in about 33% of patients and can manifest with complications of synechia and glaucoma. Posterior uveitis (PU) is almost seen constantly in cases of ocular involvement. Inflammation in PU with retinal involvement can cause retinal exudates, venous thrombosis, hemorrhage, papilloedema and macular disease. The choroid is first involved with necrosis lesions. It has severe prognosis due to frequent relapses and partial recovery after treatment (Saadoun and Wechsler 2012).

**Vascular manifestations**

**Venous thrombosis**

Venous thrombosis occurs in 30% of BD cases (Mendes et al. 2009; Saadoun and Wechsler 2012). Vasculitis is a principal pathologic finding and vessels of all sizes are involved. According to studies, venous disorder is more common than arterial involvement and may be seen in 14%–39% of patients. Venous thrombosis in BD may affect many different sites including the inferior vena cava, superior vena cava, suprahepatic vessels,
pulmonary artery and cardiac cavities. The pathogenesis of venous thrombosis in BD is not known.

**Superficial phlebitis**

BD can affect vessels of any size and type (Jennette et al. 2013). The venous system is the major affected site, and superficial thrombophlebitis (TFB) is, indeed, the most frequent type of venous involvement. It commonly occurs in males and frequently confused with EN-like lesions. Because the superficial TFB could frequently be associated with other forms of vascular disease in BD, superficial TFB is clinically important. Sarica-Kucukoglu et al. (2006) indicated the prevalence and types of vascular involvement in BD; among 2319 patients, 14.3% had vascular involvements, and superficial TFB was the most common vascular symptom (53.3%).

**Arterial and cardiac involvement**

Arterial involvement is seen in 3–5% of BD cases (Saadoun et al. 2012). These “arterial aphthae” are localized on aorta, pulmonary arteries, renal and peripheral arteries. The pulmonary aneurysms have a severe prognosis and they can rupture suddenly (Saadoun and Wechsler 2012).

Cardiac involvement in BD has been seen to include instances of myocarditis, pericarditis, endocarditis, valve lesions, mitral valve prolapse, intracardiac thrombosis, myocardiopathy, endomyocardial fibrosis and coronary artery lesions (Mendes et al. 2009). Almost every form of cardiac involvement (i.e. angina pectoris, myocardial infarction, heart failure, pericarditis, valve prolapse) has been seen in patients with BD (Davatchi et al. 2010a). It may also involve three blood vessel layers, i.e., the tunica intima, tunica media and tunica adventitia (Wechsler et al. 1999; Geri et al. 2012). Myocarditis, endocarditis with mitral or aortic insufficiency, fibroelastic endocarditis complicated by relapsing pericarditis and intramural thrombosis sometimes associated with coronary involvement are also sometimes observed. Aneurysms/thrombosis of the coronary arteries also occurs that may cause sudden death (Saadoun and Wechsler 2012).

**Articular manifestations**

Articular manifestations are noted in ≈50% of BD patients and often take the form of arthritis, arthralgia and/or synovitis (Seyahi et al. 2008). Arthralgia, which occurs in 45% of BD cases—with or without swelling, redness and hydroarthrosis—can also be seen in BD patients (Davatchi et al. 2010a). Smaller joints may be affected: the most frequently involved sites are knees, followed by the ankles, wrists and elbows. Spondyloarthropathy associated with HLA-B27 positivity has been reported in 2% of BD patients. Osteonecrosis of the hip and knees has also been observed. Articular manifestations often occur in conjunction with erythema nodosum and thrombophlebitis (Koné-Paut et al. 1998). Patients with BD and arthritis also have more acne lesions.

**Neurologic manifestations**

Neurologic manifestations are seen in 20–40% of BD cases, particularly in men (Akman-Demir et al. 1999). These often occur 1–10 yr after the first symptom of BD and manifest as parenchymal (80% of patients have parenchymal brain involvement) and non-parenchymal (i.e. cerebral venous thrombosis or an arterial aneurysm) lesions (Wechsler et al. 2002; Mendes et al. 2009). Neurological involvements may present as increased intracranial pressure due to dural sinus thrombosis. Cerebrospinal fluid (CSF) in affected BD patients is frequently normal and may have high protein levels or increased number or neutrophils and/or lymphocytes (Mat et al. 2014). In addition, psychiatric symptoms including personality changes may develop. The prognosis is severe, but improvement is known to occur with aggressive rapid therapy using immunosuppressive drugs (Saadoun and Wechsler 2012).

**Other organ system manifestations**

Gastrointestinal involvement is present in 7–34% of BD cases (Koné-Paut et al. 1998; Davatchi et al. 2010a). Interestingly, this complication is frequent in patients from Japan but quite rare in those in Turkey (Seyahi et al. 2008). It is difficult to distinguish between BD and inflammatory diseases of the GI because of shared intestinal and extraintestinal symptoms (Yurdakul et al. 1996), including perforation. The ileum is the most commonly affected part of the GI tract, but the cecum and other parts of the colon may also be adversely impacted. Clinical features include vomiting, anorexia, diarrhea, dyspepsia and abdominal pain.

Pulmonary manifestations have been reported in 0.3–18% of BD cases (Khairallah et al. 2012). The main manifestation that has been reported is hemoptysis (Hamuryudan et al. 2004). Pleural effusions are rare but when they happen, often lead to embolisms or increased incidences of infections. Renal involvement is seen rarely in BD subjects. Epididymitis, occasionally recurrent, occurs in 4–11% of the patients. Orchitis (usually bilateral) and epididymitis have been reported to occur in 2.4–28% of male BD patients (Khairallah et al. 2012).

**Role of genetic factors in BD development**

Evidence suggests that genetic factors play an important role in the development of BD. In particular, it has long been known that genes of major histocompatibility complex (MHC) are involved in BD pathogenesis (Ohno et al. 1982; Verity et al. 1999). More recently, a genome-wide association study (GWAS) identified an association between BD and other putative genes (Fei et al. 2009; Wallace 2009; Meguro et al. 2010; Mizuki et al. 2010; Remmers et al. 2010). There is a strong association between the HLA-B*51 allele (located in MHC locus), on chromosome 6p and BD (Ohno et al. 1982). Several studies subsequently confirmed this association in different populations of BD patients (de Menthon et al. 2009; Meguro et al. 2010; Pineton de Chambrun et al. 2012). Two subtypes of HLA-B*51 allele, HLA-B*5101 and HLA-B*5108, have been specially implicated (Kera et al. 1999; Yabuki et al. 1999a, 1999b; Takemoto et al. 2008). The MHC region also contains MHC Class I chain-related (MIC-A and MIC-B) genes (MHC Sequencing Consortium 1999). Multiple studies have shown an association between BD and a MICA allele (MICA*009) that presents with a triple repeat microsatellite polymorphism in the transmembrane portion of MICA (A6) (Wallace et al. 1999; Mizuki et al. 2001; Park et al. 2002; Mok et al. 2003). Recent studies reported additional independent associations with positions in the MHC Class I region. Specifically, HLA-B*15, HLA-B*27, HLA-B*57 and HLA-A*26 were deemed independent risk factors while HLA-B*49 and HLA-A*03
protective alleles in the context of BD (Takeuchi et al. 2015). Tumor necrosis factor (TNF) is encoded in the Class III region of the HLA complex, adjacent to HLA-B, and many studies reported it as both positional and functional candidate gene in the onset and progression of BD (Ahmad et al. 2003). A recent meta-analysis demonstrated a relationship between TNF gene polymorphisms (including 1031 C, -238 A, -857 T) and the risk of BD (Touma et al. 2010).

The role of the BD pathogenesis and different non-MHC genes encoding cytokines (such as for IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, IL-23 and transforming growth factor [TGF]-β), chemokines (e.g. CCR1-CCR3, CCR5), cell membrane receptors (TNFRSF1A, TLR2, 4, 7, 9), immunoregulatory proteins (IRF1, IRF5, CTLA-4, NF-kB), extracellular proteins (like ICAM-1, MMP-9), oxidative stress involved proteins (glutathione transferase and myeloperoxidase), and others including those for DEFA1,NEMO, MEVF, NOD2, TLR4 and FUT2 have been evaluated in many studies—with conflicting results (Pineton de Chambrun et al. 2012; Hisamatsu et al. 2014; Mat et al. 2014; Takeuchi et al. 2015).

### Table 2. SNPs associated with genome-wide significance for BD susceptibility.

| Gene          | Variant | Allele | Location | OR      | Risk allele function                                                                 | References |
|---------------|---------|--------|----------|---------|---------------------------------------------------------------------------------------|------------|
| KIAA1529      | rs2061634 | G      | Missense | 2.04    | Potential to regulate immune response                                                 | Fei et al. 2009 |
| LOC100129342  | rs11206377 | G      | Intergenic | 1.84    | Potential to regulate immune response                                                 | Fei et al. 2009 |
| IL23R, IL12RB2 | rs1495065   | G      | Intragenic | 1.35    |                                                                                       | Remmers et al. 2010 |
| IL10          | rs1518111  | A      | Intron   | 1.28    | Low expression in monocytes                                                            | Remmers et al. 2010 |
|               | rs1800871  | C      | Promoter | 1.45    | Low expression in monocytes                                                            | Muzik et al. 2010 |
| GIMAP6        | rs10266069 | A      | Intergenic | –       | T-cell aberration                                                                     | Lee et al. 2013 |
| GIMAP7        | rs1916012  | A      | Intergenic | –       | T-cell aberration                                                                     | Lee et al. 2013 |
| UBASH3B       | rs4936742  | T      | Intergenic | 1.71    | Abnormal ubiquitin.                                                                  | Fei et al. 2009 |
| UBAC2         | rs9513584  | G      | Intron   | 1.61    | Abnormal ubiquitin.                                                                  | Fei et al. 2009 |
| TNFAIP3       | rs9494885  | C      | Intergenic | 1.81    | Increases STAT4 and IL-17 expression                                                  | Li et al. 2013 |
| STAT4         | rs7574070  | A      | Intergenic | 1.27    | Low expression in macrophage mitochondria                                              | Kirino et al. 2013a, 2013b |
| CCR1          | rs7616215  | T      | Intergenic | 1.39    | Low expression in monocytes; reduces monocyte chemotaxis                              | Hou et al. 2012a |
| CCR1/CCR3     | rs13092160 | T      | Intergenic | 3.13    | Decreases expression in PBMC                                                         | Hou et al. 2012a |
| KLRC4         | rs2617170  | C      | Missense | 1.28    | Tag for haplotype associated with/increased PBMC cytotoxicity                      | Kirino et al. 2013a, 2013b |
| MEFV          | rs6949V    | V      | Missense | 2.65    | Increase LPS response                                                                | Kirino et al. 2013a, 2013b |
| ERAP1         | rs17482078 | T      | Missense | 4.56    | Tag for haplotype with reduced peptide trimming activity                               | Kirino et al. 2013a, 2013b |
| FUT2          | rs681343   | T      | Synonymous | 1.30    |                                                                                       | Remmers et al. 2010, Xavier et al. 2015, Kappen et al. 2015, Kirino et al. 2013a, 2013b |
| IL12A         | rs17810546 | A or G | Intergenic | 1.66    |                                                                                       | Kirino et al. 2013a, 2013b |
| IL23R         | rs381Q     | G      | Missense | Protect | Reduces IL-23-dependent IL-17 (R381Q)                                               | Kirino et al. 2013a, 2013b |
| TLR4          | rs7613560  | G      | Intergenic | Protect | Low response to LPS                                                                 | Kirino et al. 2013a, 2013b |
| NOD2          | rs702W     | G      | Missense | Protect | Low response to MDP                                                                  | Kirino et al. 2013a, 2013b |
| CPVL          | rs1957651  | C      | Intragenic | 2.26    | Affect function of any of the peptides cleaved by enzyme and interferes with macrophage function | Fei et al. 2009 |

**Genome-wide association studies, genetic susceptibility and HLA typing**

Genome-wide association studies (GWASs) provide impartial catalogs of genes involved in human disease, using high-throughput genotyping platforms and analysis of single nucleotide polymorphisms (SNPs) that form much of the 0.1% genetic difference across the human genomes (Jorde and Wooding 2004). Recently, GWASs about BD have been performed in multiple ethnic groups, including Japanese, Chinese, Korean, Turkish and Iranian populations (Fei et al. 2009; Mizuki et al. 2010; Remmers et al. 2010; Hou et al. 2012a, 2012b; Kirino et al. 2013a; Lee et al. 2013; Kappen et al. 2015; Xavier et al. 2015). Several significantly associated loci ($p < 5 \times 10^{-8}$) that were identified are provided in Table 2.

BD has an unusual geographic distribution, extending from the Mediterranean basin to Japan, between 30°N and 45°N latitudes. Healthy people in this area have a higher frequency of HLA-B51 (Ohno et al. 1982; Verity et al. 1999). BD is not a normal genetic disease with a Mendelian inheritance model and most patients do not have a family history. However, a familial clustering of BD patients has long been seen, and an increased...
disease risk has been noted among first-degree relatives (Gül et al. 2000). Sibling recurrence risk rate was found to be 4.2% in Turkey, and a higher rate of 10% for juvenile patients has been reported. A sibling risk ratio for BD was estimated as 11.4–52.5 in Turkey. This finding indicates a strong genetic background of BD. A genetic anticipation in the early-onset form of the disease in the children of affected parents was observed in some families (Stewart 1986; Fresko et al. 1998).

An association of HLA-B51 with BD is strong evidence supporting involvement of genetic factors (Gül 2001). Cross-reactivity between HLA-B51 and organ-specific antigens and a polymorphic HLA-B sequence common in HLA-B51, HLA-B27 and several other HLA-B alleles (B27PD), which show amino acid homology with retinal soluble antigen (S-Ag)-derived peptide, are some of the other mechanisms postulated in the pathogenesis of BD (Wildner and Thurau 1994). TNF polymorphisms and HLA-B*5701 were associated with disease susceptibility in Caucasians from the UK (Ahmad et al. 2003). Investigation of the genomic segment between the TNF and HLA-B loci revealed MICA association with BD; however, its participation has been considered to be due to linkage disequilibrium with the HLA-B51 gene (Marshall Sara 2004).

Other studies indicated that MIC genes (i.e. MICA and MICB) encode a polypeptide similar to the MHC Class I molecules expressed predominantly on fibroblasts, epithelial cells, endothelial cells, monocytes and gastrointestinal cells. This may be recognized by γδ T-cells and natural killer (NK) cells that have cytotoxic functions and whose levels are increased in the peripheral blood of BD patients. However, a proportional increase in levels of T-cells expressing activation markers (CD29 and CD69) and that produce TNF-α and IFN-γ was seen in response to derived HSP65 peptides in BD patients compared with controls (Verjans et al. 2002). There are several genes located outside the MHC region that have been proposed to be involved in BD pathogenesis. These are genes coding for ICAM-1, coagulation factor V and endothelial nitric oxide synthase (eNOS). Other studies indicated that mutations in the Mediterranean fever gene MEFV—originally linked to FMF—were additional genetic susceptibility factors in BD (Imurzaloglu et al. 2005; Ayesh et al. 2009). The frequency of the most penetrant p.Met694Val mutation of MEFV gene was found to be increased in Turkish BD patients (Kirino et al. 2013b). The association between BD and alleles in the promoter region of TNF, including TNF*B1 and TNF*B2, has been confirmed in Japanese patients with BD. These alleles are associated with high TNF production by the monocytes and are more prevalent in BD patients. Researchers also identified several genes associated with susceptibility to BD by GWAS including the IL23R, STAT and IL-10 genes (Mizuki et al. 2005; Hou et al. 2012a, 2012b). Copy number variation (CNV) of the DEFA1 gene that encodes α-defensin-1 was indicated to correlate with intestinal involvement in BD. Moreover, familial cases of BD with intestinal lesions have been found associated with NEMO mutations (Takada et al. 2010; Ahn et al. 2012). Endoplasmic reticulum aminopeptidase 1 (ERAP1) polymorphisms, which are recessively inherited risk factors only in HLA-B51+ individuals, may affect the enzymatic activity and possibly the peptide specificity of the enzyme, revealed the important role of the peptides loaded onto antigen-binding groove of HLA-B 51 (Gül 2015).

**Trisomy 8 and intestinal ulcers**

Some BD patients have been identified to suffer from bone marrow disorders such as myelodysplastic syndromes (MDS) and aplastic anemia. The most common chromosomal abnormality in BD patients with MDS is trisomy 8 (Tada et al. 2005). Trisomy 8 may also be associated with the development of intestinal ulcers in patients with MDS (Kimura et al. 2001). The mechanisms by which trisomy 8 is associated with intestinal ulcers has not been detected; however, autoimmune mechanisms may play a role in the development of hematopoietic disorders such as MDS and aplastic anemia (Hsu et al. 2002; Voulgarelis et al. 2004).

**Use of immunomodulant treatments to mitigate BD**

In general, the main aim of the treatment of BD should be the inhibition of irreversible organ damage—especially during the early active phase of the disease. In practicality, the choice of treatment for BD is generally based on the clinical appearance and the location(s) affected in the given patient. Close monitoring of the patient is mandatory to reduce the frequency/severity of attacks as well as to avoid complications. While a particular treatment is based on suppression of inflammatory attacks, the actual treatment selected is most often based on the organ(s) involved and severity of the involvement, frequency of recurrences, disease duration, age onset and sex. For example, in the case of mild symptoms, a first-line therapy for oral and genital isolated ulcerations is topical steroids or sulfonamide solution. A detailed list of approved treatments for BD patients is summarized in Table 3. As noted earlier, treatments that modulate the cytokine network, such as azathioprine, cyclosporine, corticosteroids or anti-TNF-α monoclonal antibodies (mAb), present some of the more novel ways to help mitigate BD (Evereklioglu 2005; Hamzaoui et al. 2015; Skef et al. 2015).

Generally, azathioprine, a purine analog, acts to inhibit purine synthesis necessary for the proliferation of cells, including leukocytes and lymphocytes. This mode of action is based upon integration into replicating DNA and, consequently, the arrest of de novo purine production and ultimately that of DNA. Azathioprine is often used alone or in combination with other immunosuppressive therapies to prevent organ rejection after transplantation and to treat an array of autoimmune diseases apart from BD, including rheumatoid arthritis (RA), pemphigus, systemic lupus erythematosus (SLE), atopic dermatitis, myasthenia gravis, etc. (Evans 2004). It is also a steroid-sparing agent useful in the treatment of inflammatory bowel diseases (IBD such as Crohn’s disease and ulcerative colitis) and for multiple sclerosis (MS) (Karran and Attard 2008). Pharmacologically, azathioprine is a pro-drug that must be metabolized to 6-mercaptopurine (6-MP) through reduction by glutathione and other thiol-containing compounds and then enzymatically converted into 6-thiouric acid, 6-methyl-6-MP and 6-thioguanine (6-TG). 6-MP is also metabolized analogously to natural purines, yielding thioguanosine triphosphate (TGTP) and thio-oxynucleosine triphosphate (TdGTP) via thioinosine monophosphate (TIMP). When the latter is methylated to MeTIMP, this acts as the true purine synthesis inhibitor by blocking amidophosphoribosyltransferase (Maltzman and Koretzky 2003). TGTP itself is incorporated into RNA, compromising its functionality; TGTP also interacts with GTP-binding Rac1 and so blocks up-regulation of Bcl-xL—sending activated T-cells into apoptosis. Apart from these DNA/RNA-based effects, azathioprine also blocks downstream effects of CD28 costimulation, a process required for T-cell activation (Cara et al. 2004). Clearly, the effects of azathioprine most strongly impact on proliferating cells, such as T- and B-cells, and in so doing can mitigate symptoms of BD.
Table 3. List of the drug agents and their suggested mechanism of action in patients with BD.

| Organ involvement | Drug                  | Dose                  | Comment                                                                 |
|-------------------|-----------------------|-----------------------|-------------------------------------------------------------------------|
| Eye involvement   | Azathioprine          | 2.5 mg/kg/d           | Used for any patient with BD affecting posterior segment. Agent rapidly suppresses inflammation; potential side effects include cataracts, glaucoma, hypertension, diabetes, electrolyte abnormalities, cushingoid appearance, reduced host resistance and osteoporosis (Ozguler and Hatemi 2016). Frequently used in combination with other medications because alone often fails to prevent relapses (Evereklioglu 2005). |
|                   | Corticosteroids       | Oral prednisolone (1 mg/kg/d) | Initial agent for ocular involvement. Decreased hypopyon. Uveitis attacks based on randomized controlled trial (Ozguler and Hatemi 2016). Is pro-drug converted to 6-mercaptopurine and inhibits the T- and B-cell proliferation (Freudenberger et al. 2016). |
|                   | IV Methylprednisolone (1 g, 3 d) | Used for any patient with BD affecting posterior segment. Agent rapidly suppresses inflammation; potential side effects include cataracts, glaucoma, hypertension, diabetes, electrolyte abnormalities, cushingoid appearance, reduced host resistance and osteoporosis (Okada 2000; Hatemi et al. 2008). |
| Refractory eye involvement | Methotrexate          | Oral (7.5 mg/wk)      | Used in combination with azathioprine/corticosteroids. Usually treatment of choice shows its effect rapidly (Ozyaygan et al. 1992). Reduces frequency and severity of ocular attacks (BenEzra et al. 1988; Masuda et al. 1989). Hypertension, renal dysfunction and nephrotoxicity are concerns (Cantarini et al. 2015). |
|                   | Cyclosporine A        | 2–5 mg/kg/d           | Used in combination with azathioprine/corticosteroids. Usually treatment of choice shows its effect rapidly (Ozyaygan et al. 1992). Reduces frequency and severity of ocular attacks (BenEzra et al. 1988; Masuda et al. 1989). Hypertension, renal dysfunction and nephrotoxicity are concerns (Cantarini et al. 2015). |
|                   | Infliximab            | IV: 5 mg/kg every 6–8 wk | Used in combination with azathioprine and corticosteroids. Infliximab in combination with other immunosuppressants is promising for refractory eye disease. Decreases frequency of uveitis attacks (Sifakis et al. 2007). IFN-α alone or in combination with corticosteroids is a second choice in eye disease. Studies shown that IFN-α2a seemed more effective than IFN-α2b (Köter et al. 2004). Potential side effects: depression, cytopenia and possible myelosuppression. |
|                   | IFN-α                 | –                     | These agents interfere with DNA replication and impair lymphocyte proliferation and functions. Combined treatment with corticosteroids used in treatment of refractory eye disease and CNS involvement (Evereklioglu 2005). Side effects: bone marrow suppression, hepatotoxicity, secondary malignancies and infertility (Marshall Sara 2004). |
|                   | Alkylating agents (cyclophosphamide and chlorambucil) | –                     | These agents interfere with DNA replication and impair lymphocyte proliferation and functions. Combined treatment with corticosteroids used in treatment of refractory eye disease and CNS involvement (Evereklioglu 2005). Side effects: bone marrow suppression, hepatotoxicity, secondary malignancies and infertility (Marshall Sara 2004). |
| Major vessel disease (Hatemi et al. 2008) | Corticosteroids       | –                     | Management of acute deep vein thrombosis in BD. Management of pulmonary and peripheral arterial aneurysms. |
|                   | Azathioprine          | 2.5 mg/kg/d           | Management of acute deep vein thrombosis in BD. Management of pulmonary and peripheral arterial aneurysms. |
|                   | Cyclophosphamide      | Oral (2 mg/kg/d)      | Management of acute deep vein thrombosis in BD. Management of pulmonary and peripheral arterial aneurysms. |
|                   | IV (0.75–1.0 g/m²) every 4 wk | Management of acute deep vein thrombosis in BD. Management of pulmonary and peripheral arterial aneurysms. |
|                   | Cyclosporine A        | 2–5 mg/kg/d           | Management of acute deep vein thrombosis in BD. Management of pulmonary and peripheral arterial aneurysms. |
| Anticoagulants    | –                     | –                     | No controlled data or evidence of benefit of antplatelet, anticoagulant or antifibronolytic agents in management of deep vein thrombosis and are thus not recommended. Possibility of coexisting pulmonary arterial aneurysm can cause fatal bleeding. |
| Gastrointestinal involvement | –                     | –                     | No evidence-based treatment for management of GI involvement in BD. Except in emergencies, try agents such as sulfasalazine, corticosteroids, TNF-α antagonists, azathioprine or thalidomide fist (Hatemi et al. 2008). Can be used to manage arthritis in large joints, such as the knees and ankles. Colchicine inhibits neutrophil migration by interfering with microtubule formation. Most common side effects are gastrointestinal, i.e., nausea, vomiting, diarrhea, abdominal pain. May be tried in rare cases with resistant, disabling long-lasting attacks. (Hamuryudan et al. 1994; Alpsoy et al. 2002) |
| Joint involvement | Colchicine            | 1–2 mg/d              | Colchicine inhibits neutrophil migration by interfering with microtubule formation. Most common side effects are gastrointestinal, i.e., nausea, vomiting, diarrhea, abdominal pain. May be tried in rare cases with resistant, disabling long-lasting attacks. (Hamuryudan et al. 1994; Alpsoy et al. 2002) |
|                   | IFN-α                 | –                     | May be tried in rare cases with resistant, disabling long-lasting attacks. (Hamuryudan et al. 1994; Alpsoy et al. 2002) |
|                   | Azathioprine          | 2.5 mg/kg/d           | May be tried in rare cases with resistant, disabling long-lasting attacks. (Hamuryudan et al. 1994; Alpsoy et al. 2002) |
|                   | TNF-α blockers        | –                     | May be tried in rare cases with resistant, disabling long-lasting attacks. (Hamuryudan et al. 1994; Alpsoy et al. 2002) |
|                   | Benzathine penicillin | 1.2 × 10⁶ U/3 wk      | Combined treatment (colchicine + benzathine penicillin) more effective in reducing frequency of arthritic episodes (Calgueneri et al. 1996; Oliveira et al. 2011). |
| Organ involvement                                | Drug                  | Dose          | Comment                                                                                                                                                                                                 |
|------------------------------------------------|-----------------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Neurological involvement                        | Corticosteroids       | 1 mg/d        | Used for parenchymal involvement (Hirohata et al. 1998; Ozguler and Hatemi 2016). Used for dural sinus thrombosis.                                                                                  |
| Neurological involvement                        | IFN-α or Azathioprine | –             | For parenchymal involvement                                                                                                                                                                           |
| Neurological involvement                        | Cyclophosphamide      | –             | Methotrexate (folate analog) reported useful in treatment of CNS and parenchymal involvement (Hirohata et al. 1998; Evereklioglu 2005).                                                             |
| Neurological involvement                        | TNF-α antagonists     | –             | For parenchymal involvement                                                                                                                                                                           |
| Neurological involvement                        | Methotrexate          | –             | Rarely used because of high risk of serious adverse effects including myelotoxicity and increased risk of malignancies.                                                                           |
| Neurological involvement                        | Chlorambucil          | –             | First-line treatment for isolated oral and genital ulcers (Alpsoy et al. 1999).                                                                                                                     |
| Neurological involvement                        | Local corticosteroids | –             | Methotrexate (folate analog) reported useful in treatment of CNS and parenchymal involvement (Hirohata et al. 1998; Evereklioglu 2005).                                                             |
| Neurological involvement                        | Colchicine            | 1–2 mg/d      | Preferred when dominant lesion is erythema nodosa. Is effective on arthralgia and can reduce occurrence of GU, EN and arthritis. Combined treatment (colchicine + benzathine penicillin) very effective in reducing duration of OU and EN, and frequency of GU (Aktulga et al. 1980; Çağrıner et al. 1996; Yurdakul et al. 2001). |
| Mucocutaneous involvement (should be treated   | Azathioprine          | 2.5 mg/kg/d   | May be used in patients with resistant skin and mucosa. Effective in preventing mucocutaneous lesions and reducing occurrence of OU and GU (Yazici et al. 1990). |
| Mucocutaneous involvement (should be treated   | IFN-α                 | $6 \times 10^6$ U/d, 39 wk | May be used in patients with resistant skin and mucosa findings. Affects pain and healing time of OU, and frequency of GU and PPL (Alpsoy et al. 1999, 2002; Yağışdağ and Uzun 2012). |
| Mucocutaneous involvement (should be treated   | TNF-α antagonists     | –             | Effective for oral and genital ulcers (Alpsoy et al. 1999).                                                                                                                                          |
| Mucocutaneous involvement (should be treated   | Sucralfate suspension | –             | Decreased frequency of oral ulcers, erythema nodosa and PPLs (Alpsoy et al. 1999).                                                                                                               |
| Mucocutaneous involvement (should be treated   | Minocycline           | –             | Decreased occurrence of OU, nodular skin lesions and PPLs (Melikoglu et al. 2005).                                                                                                               |
| Mucocutaneous involvement (should be treated   | Etanercept            | 25 mg/d—29 wk | Decreased occurrence of OU, nodular skin lesions and PPLs (Melikoglu et al. 2005).                                                                                                               |
| Mucocutaneous involvement (should be treated   | Thalidomide           | 100–300 mg/d  | Patients with resistant skin and mucosa findings can be treated with it. Effective in preventing mucocutaneous lesions (Yazici et al. 1990). It increases frequency of nodular lesions. Potentially serious adverse events such as teratogenicity and peripheral neuropathy limit use. Because of side effects, it is not first-line therapy. |
| Mucocutaneous involvement (should be treated   | Apremilast            | 30 mg/2X/d    | Reduces pain of OU and the numbers of OU and GU (Hatemi et al. 2015).                                                                                                                            |
| Mucocutaneous involvement (should be treated   | Pentoxifylline        | –             | Pentoxifylline inhibits synthesis of several cytokines, including TNF-α. Has been used in treatment of oral and genital ulcers (Yasui et al. 1996).                                                   |
| Mucocutaneous involvement (should be treated   | Dapsone               | 100 mg/d, 3 mo| Has anti-inflammatory properties; shown to improve oral, genital and cutaneous lesions (Sharquie et al. 2002).                                                                                       |
Cyclosporine (cyclosporine A, CsA) displays immunosuppressive properties in great part via effects on the calcineurin/NFAT pathway. Ultimately, these effects block the transcription of cytokine genes in activated T-cells, including those for IL-2 and IL-4 (Kang et al. 2007). It is well known that CsA-through formation of a complex with cyclophilin-inhibits phosphatase activity of calcineurin that controls nuclear translocation and subsequent activation of NFAT transcription factors (Matsuda and Koyasu 2000).

As general anti-inflammatory agents, corticosteroids (mineralo- and glucocorticoids) have an effect on protein synthesis at the cellular level by impacting gene transcription. As there are thousands of papers dealing with mechanisms of effects on the immune system induced by corticosteroids, readers seeking more information are directed to Coutinho and Chapman (2011), Chatham (2017), and Cain and Cidlowski (2017), or other recent reviews on the topic.

Topical steroids can modify functions of epidermal and dermal cells as well as of leukocytes that precipitate in both proliferative and inflammatory skin diseases. Receptors for corticosteroids exist in the cytoplasm; after passage through the cell membrane, corticosteroid receptor complexes are formed into the nucleus and bind DNA, primarily for genes encoding STAT family proteins, pro-inflammatory transcription factors, as well as NF-κB and AP-1. Various mechanisms seem to be involved in these signaling events and, in turn, eventually impinge on the activity of various kinases, such as AKT, PI3K and various forms of MAPK (Oakley and Cidlowski 2013). Corticosteroids also stimulate production of lipocortin glycoprotein. Lipocortin prevents the of phospholipase A₂ activity, thereby reducing the formation/release of arachidonic acid precursor for prostanooids and leukotrienes. These actions of corticosteroids on arachidonic acid metabolism and on cytokine formation ultimately give rise to the important anti-inflammatory, immunosuppressive and antimicrobial special effects (Kragballe 1988; Castiblanco and Foster 2014) that make them useful for the treatment of BD.

Infliximab is a monoclonal TNF-α-blocking antibodies (composed of human constant and murine variable regions) that neutralizes the biological activity of TNF-α by binding to the soluble and transmembrane forms of TNF-α, thereby reducing normal levels of binding of TNF-α binding to receptors. This, in turn, results in reductions of normal TNF-α-induced events, such enhanced production of other pro-inflammatory cytokines (i.e. IL-1 and IL-6), enhanced leukocyte migration (via increases in endothelial penetration secondary to increased expression of adhesion molecules by endothelial cells and leukocytes), activation of neutrophils/eosinophils and the induction of acute phase reactants as well as of tissue-degrading enzymes (by synovio- and/or chondrocytes) (Danese 2008). Moreover, the use of infliximab also prevents TNF-α promotion of T₁₁7 cell differentiation. Data have suggested that inhibition of this type of differentiation may protect BD patients from severe ocular inflammation (Sugita et al. 2012).

Beyond the agents/class of agents noted above, other less familiar treatments have evolved to relieve symptoms of BD in affected patients. For example, it is well known that vitamin D deficiency is related to increases in the prevalence of autoimmune and inflammatory disorders (Cantorna and Mahon 2004). Beyond its involvement in calcium homeostasis, vitamin D is thought to be important in the pathogenesis of autoimmune/inflammatory pathologies including RA, SLE, IBD and MS. In vivo treatment of MS and RA patients with 1,25-(OH)₂-D₃ resulted in reduced levels of autoimmune T-helper (T₁₁) cells and the intensity of their cell-related responses (Paolino et al. 2016). This occurred, in part, through induction of processes that targeted CD4⁺ T-cells, T-regulatory (TREG) cells and dendritic cells (DC). Treatment with 1,25-(OH)₂-D₃ was shown to suppress the immune system and led to amelioration of complications and manifestations of RA, MS and BD (Cutolo et al. 2006; Munger et al. 2006).

Because of these and other associated immunomodulating effects (Leventis and Patel 2008), and the known potential role of alterations in vitamin D receptors and BD occurrence (i.e. Kolahi et al. 2015) as well as the importance of maintaining vitamin D levels in BD patients (Balta et al. 2014; Khabazzi et al. 2014), vitamin D supplementation has increasingly been evaluated to treat these patients, in particular, with regard to helping endothelial cells regain somewhat normal functionalities (Can et al. 2012; Gunor et al. 2016). In these studies, it was seen that the use of the vitamin did help to mitigate vascular dysfunctionality, although the authors cautioned this regimen could not be considered a primary treatment modality.

Trental (pentoxifylline), a methylxanthine derivative, imparts a variety of anti-inflammatory effects (Hassan et al. 2014) and was begun to be more widely investigated for its potential use in treating BD starting in the mid-1990s (see Arici et al. 1997; Hamuryudan et al. 1997; Wolchok 1997; Pizarro et al. 2000; Hassard et al. 2001). Pentoxifylline acts as an immunomodulant in that while it can cause increases in leukocyte deformability and chemotaxis, more importantly, it can induce decreases in neutrophil degranulation, endothelial leukocyte adhesion, activation of T- and B-cells, natural killer (NK) cell activity, release of superoxide and other ROS, monocyte production of select cytokines (e.g. TNF-α, IL-1, IL-6) and leukocyte responsiveness to TNF-α and IL-1 (Samlaska and Winfield 1994; van Furth et al. 1997). As a result of the latter, it has been suggested that pentoxifylline might be used as an alternative to some TNF-blocking agents (Koenig et al. 2006; Appenzeller and Hazel 2011). An in vitro study using lymphocytes/granulocytes isolated from BD patients showed that the use of pentoxifylline could cause significant inhibition of cell expansion, down-regulation of TNF receptor expression and inducible degranulation of perforin (Accardo-Palumbo et al. 2007). Most interestingly, that same study suggested pentoxifylline was capable of interfering with the functions (re: activation) of BD patient Vγ9/Vδ2 T-cells.

Pentoxifylline also causes decreases in blood viscosity (Porsche and Stefanovich 1978), in part via causing decreases in platelet aggregation, by impacting on source cell production of thromboxane and prostacyclin, plasminogen activator, plasmin and antithrombin III, as well as of fibrinogen, α₁-antitrypsin, α₂-antiplasmin and α₂-macroglobulin (Dettellbach and Aviado 1985). Such changes could help mitigate thrombosis associated with BD flare-ups. Pentoxifylline is also a non-selective inhibitor of cyclic-3’,5’-phosphodiesterase (PDE), which gives rise to a broad spectrum of effects on cell proliferation and inflammation, important underlying steps in BD pathologies. Likewise, as the drug causes diminished expression of adhesion molecules like intercellular adhesion molecule 1 (ICAM-1) on keratinocytes and E-selectin on endothelial cells (Kano et al. 1997), so this too represents an important blocking event against BD episodes.

Among lesser known/used products for treatment of BD have been dapsone and thalidomide. Dapsone [diaminodiphenyl sulphone (DDS)] is an anti-inflammatory and antimicrobial agent most often used in combination with rifampicin and clofazimine for treatment of leprosy or prevention of toxoplasmosis in
immunocompromised subjects. Dapsone can reduce tissue damage that might occur during inflammatory episodes (as occur in BD) by suppressing the activities of neutrophil myeloperoxidase and/or eosinophil peroxidase. It can also cause suppression of granulocyte hypochlorous acid production and reactive oxygen species formation, reductions in neutrophil activity, and also inhibit chemoattractant-induced signal transduction within granulocytes (Tan 2012; Wozel and Blasum 2014). Even though these latter studies are from the past decade, the known immunomodulating effects of dapsone allowed it to become a potential drug of interest in treatment of BD starting in the early 2000s (see, as examples, Thomas-Golbanov and Sridharan 2001; Sharquei et al. 2002; Evereklioglu 2004, 2005).

Thalidomide imparts immunosuppressive effects and anti-inflammatory actions, in great part due to its ability to inhibit monocYTE production of TNF-α (Deng et al. 2003). Thalidomide also suppresses angiogenesis by inhibiting secretion of TNF-α and IFN-γ which up-regulate endothelial cell integrin expression essential for new vessel formation. Thalidomide brings about these effects by selectively enhancing the degradation of TNF-α and IFN-γ mRNA and inhibiting production of their respective proteins (Hassan et al. 2015). Through these effects, thalidomide ultimately modulates T-cell functions, decreases the numbers of T-helper (and concurrently results in increases in the numbers of T-suppressor) cells and helps cause a shift in the Th1:Th2 paradigm (Wines et al. 2002; Hassan et al. 2015). The drug also blocks leukocyte chemotaxis and phagocytosis, an outcome associated with decreasing integrin β-chain production. In doing so, this mitigates the potential for damage to blood vessels/vascularity of the type commonly seen in BD patients.

Finally, though not expounded upon here in detail, another new area of intense interest for the immunomodulatory treatment of BD has been the potential use of stem cells. In general, allogeneic mesenchymal stem cells (MSCs) are multipotential non-hematopoietic cells that can impart immunosuppressive effects on both the innate and acquired immune systems in their host (Jones and McTaggart 2008; Li et al. 2010; Sioud et al. 2010; Trento and Dazzi 2010; English and Mahon 2011; Spaggiari and Moretta 2013; Stagg and Galipeau 2013). These cells have a potential to migrate to injured tissues and prevent the production/release of pro-inflammatory cytokines as well as other immunomodulatory agents. As there have been many reports on the use of MSC for the treatment of several immune-based pathologies, i.e., Crohn’s Disease, systemic sclerosis, osteoarthritis, etc. (Al-Maawali et al. 2016; Chang et al. 2016; Maria et al. 2016; Forbes 2017), the possibility exists that the use of MSC may have therapeutic value for control of BD as well. This belief is based in part on findings that MSCs have been shown to possess the potential to repress immune responses by inhibiting differentiation of dendritic cells and suppressing the functions of T-, B- and NK cells (Mazaheri et al. 2012).

Conclusions

Although the exact pathogenesis of BD is not fully understood, a variety of investigations have demonstrated genetic factors could be a major player in disease susceptibility. However, environmental factors have increasingly been suggested as contributory for disease development. Interesting geographical distribution of BD along the Silk Road has been considered as evidence supporting an environmental influence. At the immune system level, inflammatory imbalances, as well as potential key roles for γδ T-cells, have become important foci for studies of the pathogenesis of BD. Based on many of those related findings, therapeutic approaches to help treat BD have continued to advance.

Acknowledgements

The authors would like to thank the faculty members at Mashhad University of Medical Sciences for their assistance. This study was partly supported by Medical Genetics Research Center.

Disclosure statement

The authors declare no conflict of interest. The authors alone are responsible for the content of this manuscript.

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