Behcet’s disease (BD) is a systemic inflammatory disorder with a diverse spectrum of clinical manifestations including mucocutaneous, ocular, vascular, gastrointestinal, musculoskeletal and central nervous system involvement [1]. A complex genetic background leading to a pro-inflammatory, innate-immune-system-derived activation perpetuated by adaptive immune responses against environmental and auto-antigens is accepted to be the hallmark of BD [2]. This review aims to make an in-depth critical analysis of current data for recent controversies on the role of innate immune system vs autoimmune in BD [3–5].

Recent epidemiological data

Although epidemiological data is scarce in BD, some recent observations from Japan suggest that the prevalence of BD in reducing among uveitis patients (23.2% in 1981–1983 vs 5.8% in 1999–2001) and the disease is becoming milder (ocular attacks and vision loss getting less frequent) [6, 7]. Since the genetic background of the Japanese population at risk is accepted to be fairly stable, environmental factors are implicated in this change. Male patients from Turkey who presented in the 1990s are also reported to have a lower risk of losing vision compared with patients presented in the 1980s. However, the authors preferred to explain this trend by a more aggressive treatment approach [8].

In another recent study from Turkey, BD patients were observed to have a lower monthly family income, lower wealth score and lower education with higher unemployment compared with those with ankylosing spondylitis and inflammatory bowel disease patients, suggesting again the role of environmental factors in disease pathogenesis [9].

Infectious aetiology

As BD starts mostly from the oral mucosal surface (oral aphthae as the first manifestation in 70% of the patients), oral microbial flora has long been implicated in BD pathogenesis. Oral manifestations are increased after dental manipulations, and hypersensitivity to streptococcal skin tests are shown [10]. Oral health parameters such as dental and periodental indices are impaired in BD and are associated with a more severe disease course [11]. Oral streptococcal colonization is increased in BD patients with a dominance of atypical streptococcal species in BD patients’ oral flora. Pustular skin lesions are also shown to be non-sterile in BD [12]. Although a wide variety of organisms such as Staphylococcus aureus, Propionibacterium acnes and coagulate negative staphylococca are cultured from BD lesions, gram-negative microorganisms such as E. coli and Prevotella species were also, suprisingly, present when compared with acne vulgaris.

Various immunological studies show an immune hyperreactivity to streptococci in BD. KTH-1 (a crude extract of Streptococcus sanguis SSF-83) causes increased IL-6 and interferon-γ (IFN-γ) secretion by peripheral blood (PB) T-cells of BD patients [13]. KTH-1 also up-regulates γδ-T-cells in short-term T-cell cultures and KTH-1-specific γδ-T-cell lines secrete pro-inflammatory mediators IL-6, CXCL-8 and tumor necrosis factor-α (TNF-α) [14]. Lipoteichoic acid, a streptococcal cell membrane antigen, is also demonstrated to cause increased CXCL-8 production from PB mononuclear cells of BD patients [15]. However, in addition to streptococcal antigens, E. coli and S. aureus also activate BD lymphocytes to release increased amounts of IFN-γ and IL-6 [16]. Comparison of PB lymphocyte changes after stimulation with streptococcal and E. coli extracts also gave similar proliferative responses [17]. As microbial antigens common to different species seem to drive a similar immune activation in BD, not the specific microorganism itself but its presence and persistence might determine its role in BD pathogenesis [2].

Recent reports of beneficial anti-bacterial therapy also support the role of streptococci in BD [18, 19]. However, studies, especially of longitudinal nature, that might show the association of oral colonization with oral ulcer development and whether anti-bacterial treatments effect oral bacterial colonization are lacking. Another crucial weakness of ‘infection’ theory is the effective role of TNF-α-antagonists in BD treatment, which are contraindicated in active infection.

Autoimmunity and BD

A seminal paper from Rose et al. [20] define as the direct proof of autoimmunity the transfer of autoimmune disease to normal recipients or animals, such as fetal heart block induction by anti-Ro antibodies. Indirect evidence is the transfer of disease by T-cells to severe combined immunodeficiency (SCID) mice as demonstrated for Grave’s disease, thyroiditis, systemic lupus erythematosus (SLE) or induction of disease in animals by autoantigens such as myastenia gravis or uveoretinitis models.

Identification of possibly pathogenic self-reactive T or B cells in tissues (such as insulin-dependent diabetes) is the weakest of evidences for autoimmunity. Some circumstantial evidence for...
Autoimmunity and BD

A recently introduced concept to BD is ‘autoinflammation’. Autoinflammatory disorders are described as a group of inherited disorders characterized by episodes of seemingly unprovoked recurrent inflammatory attacks of innate nature, mainly by neutrophils [39]. In contrast to classical autoimmune disorders, no significant high-titre autoantibodies or antigen-specific T-cells are present. The prototype disorder in Middle Eastern populations is familial Mediterranean fever (FMF), a disease caused by mutations of MEFV gene, encoding the newly described pyrin/marenostrin protein. MEFV and pyrin are expressed at high levels in neutrophils, monocytes and dendritic cells but not in lymphocytes. The N-terminal of pyrin binds to another pyrin-domain-containing protein called apoptosis speck like protein containing a caspase-recruitment domain (CARD)(ASC), and through this interaction might regulate IL-1β processing, NF-κβ activation and apoptosis. However, both inhibitory and enhancing effects have been observed depending on the experimental system [40].

Behcet’s disease, with some of its clinical features such as recurrent non-scarring mucocutaneous lesions and non-deforming arthritis, and enhanced inflammatory response with the over-expression of pro-inflammatory cytokines, is described to be in this spectrum [4]. MEFV mutations are also observed more frequently in BD and associate with a more severe disease [41]. However, various clinical aspects differ between the two diseases, which is discussed in further detail previously [5]. Among these, pediatric onset and paroxysmal attacks of serosal inflammation and fever typical of autoinflammatory disorders are not characteristic of BD, whereas panuveitis, extensive vasculitis, hypercoagulability and a disease course getting milder in late ages are common [5]. Another crucial difference between FMF and BD is the prolonged inflammatory skin response. Pathergy test, a non-specific response to skin trauma, is typically described in BD and some neutrophilic dermatoses such as pyoderma gangrenosum and Sweet’s syndrome [1, 42]. Pathergy reactions are shown to be associated with skin flora, as extensive skin cleansing decreases the positivity of the test [43]. In a chronological study of pathergy, mixed neutrophil and T-cell infiltrations are observed as early as 4h, with a peak density in 24h in BD patients’ skin biopsies [42]. No pathergy skin response is reported in FMF [44], although erizipel-like skin lesions or rarely cutaneous vasculitis with neutrophil infiltrations are observed. Similar to pathergy test, skin responses to urate crystals are described in BD, which is again a highly specific response not observed in FMF [45, 46]. When innate responses to urate crystals are investigated, urate-derived superoxide production in neutrophils was found to be dose-dependent and very similar in magnitude in both BD and FMF, and was even higher in FMF monocytes [46]. Urate crystals are recently shown to activate NALP3 inflammasome, a protein complex of cryopyrin, ASC and a protein called CARDINAL (CARD-inhibitor of NF-κβ-activating ligand), causing the activation of caspase-1 complex and leading to the release of IL-1β [40, 47].

Pathways from innate to adaptive responses

Although there are clinical and inflammatory response similarities between autoinflammatory disorders and BD, presence of a prolonged inflammation such as non-specific (pathergy) or urate-induced skin responses suggests that innate and adaptive pathways are more integrated in BD. A unifying hypothesis for BD requires the explanation of these links between the two main arms of immune system. One explanation might be an unprovoked, uncontrolled innate-related inflammation causing an adaptive system activation only as a secondary response, as in autoinflammatory disorders [4]. An overactivated cytokine
cascade through IL-1, IL-6, IL-18, TNF-α and chemokines such as CXCL-8 might activate non-specific and non-pathogenic T- and B-cell responses in BD. As an example, increased CD3+/HLA-DR+ , CD4+/CD69+ , CD8+/CD25+ and CD8+/CD69+ T-cells are observed in the peripheral blood during FMF attacks; however, these adaptive responses are possibly not pathogenic [48].

However, the situation can be more complex in BD. Neutrophils, although accepted as primary effector cells of inflammation, are usually neglected in their role in later stages of immune activation and response [49]. They have the capability to present antigen under inflammatory conditions with MHC class II and costimulatory molecule expressions. They generate chemotactic signals such as TNF-α that attract monocytes and dendritic cells (DC), and influence whether macrophages differentiate to a predominantly pro- or anti-inflammatory state. IFN-γ and B-lymphocyte stimulator (BLYS) are also released by neutrophils and cause proliferation and maturation of T and B cells, respectively [49]. In this context, neutrophil activation, cytokine release and antigen presentation may link innate immune system to adaptive responses and by definition gives a broader role to neutrophils than ‘autoinflammation’, which is accepted to be a limited inflammatory response without an effective adaptive component. Behcet’s disease in this respect require a more critical analysis of neutrophil activation, and BD neutrophils may have a different profile compared with autoinflammatory disorders [50].

An intriguing hypothesis might be the role of a ‘persistent infection’ in BD. Cryopyrin-associated inflammasomes in neutrophils can be activated by bacterial peptidoglycans (PGN), bacterial DNA and various gram-positive bacterial toxins [49, 51]. Pathways of inflammasome and recently described pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs) intersect as both are sensors of bacterial products. The augmented adaptive responses in BD compared with autoinflammatory disorders can be the result of persistant oral and skin infections discussed above. In addition to adaptive responses to bacterial and mammalian ‘cross-reactive’ epitopes of human HSP60, a direct activation of innate immunity through TLRs by HSP60 is also shown [27, 52]. HSPs released from necrotic (but not apoptotic) cells are observed to activate DCs [53]. Recently, HSP60 is also shown to induce DC maturation with increased MHC class II, CD40, CD54 and CD86 expressions and allogeneic T-cell proliferation with a Th1 bias [54]. We have also recently shown that both human HSP60 and streptococcal extracts activate TLR-6 on BD neutrophils [55]. As another link between oral diseases, TLRs and HSPs, human T-cell proliferative responses to human HSP60 is increased in patients with periodontal disease and this proliferation can be inhibited with anti-TLR2 antibodies [56].

As neutrophils arrive very early to initiate inflammation in tissues and live too briefly [49], clearance of apoptotic material by complement system proteins such mannose-binding lectin (MBL), surfactant protein-A and SP-D are critical in suppressing inflammation. An adaptive response related to neutrophils in BD may be promoted by aberrant phagocytosis of apoptotic neutrophils by dendritic cells, as shown in ANCA-associated vasculitis [57]. In this context, serum MBL levels are shown to be decreased in BD patients, and MBL deficiency may prolong the exposure of neutrophil-related antigens to adaptive immune system [58]. A lower bacterial clearance due to low MBL levels may also predispose to bacterial infections and a higher prevalence of S. mutans colonization is observed in patients with low MBL levels in BD [59].

Another model points to a possible role of T-cells for neutrophil activation in BD. A principal source of CXCL-8, the major neutrophil chemoattractant in BD peripheral blood, is lymphocytes [60]. Recently, skin-derived T-cell clones from BD patients were shown to produce CXCL-8 and GM-CSF, but failed to secrete IFN-γ or IL-5. These cells might represent a particular subset as they differ from both Th1 as well as Th2 and are associated with a unique, neutrophil-rich sterile inflammation [61]. Auto- (HSP60, retinal-S antigen) or microbial-derived antigen-stimulated T-cell lines mainly of Th1 phenotype were also demonstrated in other studies in BD [2].

Another important cell subset that links innate and adaptive responses is γδ-T-cells. Although they possess a T-cell receptor, this T-cell subset is activated mainly by bacteria-originated molecules. γδ-T-cells are shown to activate dendritic cells recently and can initiate antigen presentation. They are also activated under stress conditions recognizing damaged cells [62]. Various studies demonstrated elevated γδ-T-cell presence in BD patients [17, 63]. They are increased in skin biopsies together with HSP60 expression in BD [64]. These cells respond to both streptococcia and HSP60-derived peptides [17, 28] and might participate in tissue destruction and presentation of self and foreign antigens to adaptive immune cells.

As all activated cells require antigen-presentation first, DC maturation is now accepted to be the critical step for the induction of adaptive responses and BD is possibly no exception. Both pathogen and autoantigen-driven T-cell polarization are controlled by DCs through DC-priming receptors including PRRs and tissue factors such as cytokine and chemokines [65].

Table 1. Mechanisms of responses from innate to adaptive immunity in Behcet’s disease

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<th>Mechanism/Response</th>
<th>Description</th>
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<td>A persistant bacterial stimuli (oral or skin infections) activating adaptive immune responses through PRRs [2, 17]</td>
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<tr>
<td>Uncontrolled innate-related inflammation (caspace pathway IL-1, IL-18) [4]</td>
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<tr>
<td>Neutrophil activation with T-cell derived chemokines (CXCL-8) [61]</td>
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<td>Defective neutrophil apoptotic clearance and bacterial defence with MBL deficiency [58]</td>
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<tr>
<td>Bacterial γδ-T-cell activation and antigen-presentation [28]</td>
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<td>HLA-B*51-associated responses</td>
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<tr>
<td>Presentation of a Behcet-related peptide to CD8+ cytotoxic T-cells [35]</td>
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<tr>
<td>HLA-B common peptide activation of CD4+ T-cells [36]</td>
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<td>Bw4-associated NK receptor activation [38]</td>
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Future pathways to explore

As an effective adaptive response seems to be required for the prolonged immune activation in BD, mechanisms supported by the literature above are possibly not mutually exclusive. APCs such as dendritic cells and keratinocytes, neutrophils, CD4, CD8 and γδ-T-cells are present in BD lesions with confusing histopathological data according to the age (24–72 h) and type (folliculitis vs erythema nodosum) of the lesions. As dissecting each mechanism separately (single cytokine or chemokine measurements) seems insufficient to view the whole picture in BD, immune mediators and pathways such as apoptosis, NF-κB or TLR signalling should be investigated with new techniques such as multiplex bead immunoassays, mRNA oligoarrays or whole-genome microarrays [66, 67].

Conclusions

An immune response is possibly triggered by two main mechanisms. According to the ‘danger theory’ by Matzinger [68], the immune system responds to the alarm signals of injured host-cells, which activate antigen-presenting cells. ‘The pattern-recognition theory’ places the role of microbial ‘non-self’ as the dominant stimuli for innate immune system, which in turn triggers an adaptive response [69]. Human HSP60 can be an example of
The first and various microbial antigens such as streptococcal lipoteichoic acid of the second type of stimuli for innate and possibly adaptive immune responses in BD pathogenesis. In this context, it might be too simplistic to describe BD as either an autoimmune or an autoinflammatory disease. A new category should possibly be defined for diseases like BD, which are unlikely to be classical autoantigen-derived autoimmune diseases. An infectious agent is possibly required to trigger the inflammation, but unlike classical autoinflammatory disorders, an adaptive response is also sustained through bacterial persistence or autoantigen-activated dendritic, T or B cells. Clarification of these mechanisms might help to elucidate how both anti-microbial and immunosuppressant therapies seem to be effective in BD and might pave the way for more specific immune interventions.

<table>
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<th>Key messages</th>
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<td>• It seems too simplistic to describe Behcet’s disease as either an autoimmune or an autoinflammatory disorder.</td>
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<tr>
<td>• An infectious agent is possibly required to trigger the innate-derived inflammation, but an adaptive response might also be sustained through ‘bacterial persistence’ or autoantigen-activated antigen-presenting cells.</td>
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The authors have declared no conflicts of interest.

References

Autoimmunity vs autoinflammation in BD


