

## Review

# Autoimmunity vs autoinflammation in Behcet's disease: do we oversimplify a complex disorder?

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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 autoimmunity 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 is 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 periodontal 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 coagulase negative staphylococci are cultured from BD lesions, gram-negative microorganisms such as *E. coli* and *Prevotella* species were also, surprisingly, 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* SSH-83) causes increased IL-6 and interferon- $\gamma$  (IFN- $\gamma$ ) secretion by peripheral blood (PB) T-cells of BD patients [13]. KTH-1 also up-regulates  $\gamma\delta$ -T-cells in short-term T-cell cultures and KTH-1-specific  $\gamma\delta$ -T-cell lines secrete pro-inflammatory mediators IL-6, CXCL-8 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [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- $\gamma$  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- $\alpha$ -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 myasthenia 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

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autoimmunity are also described. These are lymphocytic infiltration of target organs, especially if there is a restriction in V-gene usage, associations with major histocompatibility complex (MHC) molecules and favourable responses to immunosuppression.

Behcet's disease does not have the classical clinical features of autoimmunity such as anti-nuclear antibody (ANA) positivity, female dominance and association with other autoimmune diseases such as Sjogren's syndrome [3]. A multi-systemic animal model of BD is also non-existent. However, BD has various aspects that deserve to be evaluated as 'autoimmune'. Some effective treatments in BD such as azathioprine and cyclophosphamide are classical immunosuppressives, and cyclosporine A is a T-cell inhibitor. However, TNF- $\alpha$ -antagonists are also an exception in this concept, as they act mainly as anti-inflammatory agents and are accepted to be contraindicated in autoimmune diseases such as SLE and multiple sclerosis.

From an immunological perspective, a possibly antigen-driven change in peripheral blood CD4+ and CD8+ T-cell repertoire with oligoclonal T-cell receptor V $\beta$  subset increases are observed in BD [21]. For an autoimmune aetiology, a general B-cell activation and autoantibodies against cell surface antigens such as anti-endothelial cell (AECA) and anti-lymphocyte antibodies are demonstrated [22, 23]. Later on, antibodies against specific antigens such as  $\alpha$ -tropomyosin,  $\alpha$ -enolase and, recently, kinectin were shown [24–26]. Among the candidate autoantigens, human heat-shock protein 60 (HSP60) is the most extensively investigated [27]. Four immunodominant epitopes of HSP60 are shown to cause T- and B-cell responses in studies from UK, Japan and Turkey [28–30]. A T-helper 1 (Th1) type, pro-inflammatory cytokine response to HSP60-derived peptide 336-51 with IFN- $\gamma$ , IL-12 and TNF- $\alpha$  production is demonstrated [31]. Txk, a tyrosine kinase expressed in Th1 cells up-regulating IFN- $\gamma$  gene transcription, also responds to peptide 336-51. However, when long-term T-cell lines were produced from PB mononuclear cells with repetitive stimulation, 336-51-responsive T-cell lines were present also in healthy controls, suggesting that healthy immune repertoire also responds to human HSP60 as an immunodominant antigen [32].

In terms of animal models for autoimmunity, HSP60 peptide 336-51 and  $\alpha$ -tropomyosin are both shown to cause uveitis in rats, but without any other clinical features of BD [24, 33]. An oral conjugate of peptide 336-51 and cholera toxin-B is shown to ameliorate uveitis in the animal model; a preliminary study is also reported in BD patients with uveitis [34].

Another crucial element of autoimmunity is MHC relationship. Most classical autoimmune disorders are shown to have an MHC class II association, leading to a disease-associated peptide hypothesis (MHC-class-II-associated presentation of pathogenic epitope to CD4+ T-helper cells) such as shared-epitope in rheumatoid arthritis. However, BD is associated with a class I antigen, HLA-B\*51 [1, 2]. Although this association is similar to spondyloarthropathies, possible immune mechanisms associated with HLA-B\*27 are not studied sufficiently in BD. Recently, a HLA-B\*51-restricted peptide from an MHC class I chain-related gene (MICA) antigen is shown to activate CD8+ T-cells with an up-regulated IFN- $\gamma$  response in BD patients [35]. Another study has previously shown that an HLA-B\*51-related peptide (also present in HLA-B\*27) causes the proliferation of PB mononuclear cells only in HLA-B\*51-positive BD patients with posterior uveitis [36]. As a genetic susceptibility associated with B\*2702 has also been implicated in BD, interactions of natural killer (NK) and T cells through the NK receptors may also be important in the pathogenesis of BD with an interaction between NK receptor KIR3DL1 and HLA-Bw4 motif [37, 38]. Other recently described HLA-B27-related immune mechanisms such as heavy-chain association or bacterial persistence are not studied in BD yet.

## Autoinflammation and BD

A recently introduced concept to BD is 'autoinflammation'. Autoinflammatory diseases 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-1 $\beta$  processing, NF- $\kappa$ B 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 overexpression 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 4 h, with a peak density in 24 h 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- $\kappa$ B-activating ligand), causing the activation of caspase-1 complex and leading to the release of IL-1 $\beta$  [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- $\alpha$  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 pathogenetic [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- $\alpha$  that attract monocytes and dendritic cells (DC), and influence whether macrophages differentiate to a predominantly pro- or anti-inflammatory state. IFN- $\gamma$  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 RNA 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 persistent 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- $\gamma$  or IL-5. These cells might represent a particular

TABLE 1. Mechanisms of responses from innate to adaptive immunity in Behcet's disease

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- A persistent bacterial stimuli (oral or skin infections) activating adaptive immune responses through PRRs [2, 17]
  - Uncontrolled innate-related inflammation (caspase pathway IL-1, IL-18) [4]
  - Neutrophil activation with T-cell derived chemokines (CXCL-8) [61]
  - Defective neutrophil apoptotic clearance and bacterial defence with MBL deficiency [58]
  - Bacterial  $\gamma\delta$ -T-cell activation and antigen-presentation [28]
  - HLA-B\*51-associated responses
    - Presentation of a Behcet-related peptide to CD8+ cytotoxic T-cells [35]
    - HLA-B common peptide activation of CD4+ T-cells [36]
    - Bw4-associated NK receptor activation [38]
- 

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  $\gamma\delta$ -T-cells. Although they possess a T-cell receptor, this T-cell subset is activated mainly by bacteria-originated molecules.  $\gamma\delta$ -T-cells are shown to activate dendritic cells recently and can make antigen presentation. They are also activated under stress conditions recognizing damaged cells [62]. Various studies demonstrated elevated  $\gamma\delta$ -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 streptococci 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]. Interactions between DCs and neutrophils, T, B, NK and  $\gamma\delta$ -T-cells determine the nature of immune responses that characterize the unique clinical syndrome complex of BD (Table 1).

### 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  $\gamma\delta$ -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- $\kappa$ B 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 antimicrobial and immunosuppressant therapies seem to be effective in BD and might pave the way for more specific immune interventions.

Rheumatology	Key messages
	<ul style="list-style-type: none"> <li>• It seems too simplistic to describe Behçet's disease as either an autoimmune or an autoinflammatory disorder.</li> <li>• 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.</li> </ul>

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