Lesional activation of $T_c17$ cells in Behcet disease and psoriasis supports HLA class I-mediated autoimmune responses*

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Accepted for publication
10 July 2021

Funding sources
This work was supported by the Deutsche Forschungsgemeinschaft (grants PR 241/5-1 and S-2). S.Vural is supported by a TUBITAK 2219 postdoctoral research grant and a L’OREAL-UNESCO for Women in Science Fellowship of Turkey. M.H. is supported by a scholarship of the ‘Full Doctoral Study-Model’ provided by the LMU–China Scholarship Council Program.

Conflicts of interest
The authors declare they have no conflicts of interest.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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*Plain language summary available online

DOI 10.1111/bjd.20643

Summary

Background Behcet disease (BD) presents with lymphocytic and neutrophilic vasculitis of unknown aetiology. HLA-B*51, the endoplasmic reticulum aminopeptidase 1 (ERAP1), and interleukin 23 receptor (IL23R)/IL12R are genetic risk factors. IL-23 regulates IL-17A, which controls the recruitment and activation of neutrophils.

Objectives To determine pathological changes in BD skin lesions related to the complex genetic predisposition.

Methods We characterized the expression of IL-17A and IL-23A in various cell types by immunohistological double staining of sections from papulopustular skin lesions of acute attacks of BD and psoriasis vulgaris lesions, another HLA-class I-associated T-cell-mediated autoimmune disease in which excessive T-cell-derived IL-17A production promotes neutrophil activation.

Results We found that in BD lesions, as in psoriasis, actively expanding CD8$^+$ T cells were the predominant source of IL-17A. IL-17A$^+$ CD8$^+$ T (Tc17) cells outnumbered infiltrating IL-17A$^+$ CD4$^+$ T cells. Unlike the epidermal localization of CD8$^+$ T cells in psoriasis, Tc17 cells in BD lesions mainly infiltrated the perivascular tissue and the blood vessel walls of dermis and subcutaneous tissue. They co-localised with a marked IL-23A expression by CD11c$^+$ dendritic cells and CD68$^+$ macrophages. IL-17A expression was associated with extensive recruitment of neutrophils around blood vessels that formed neutrophil extracellular traps (NETs).

Conclusions In BD, the genetic predisposition may mediate antigen-specific activation and differentiation of a Tc17 response, possibly targeting endothelial (auto) antigens. Neutrophils recruited by IL-17A in this process may enhance tissue damage by extensive NET formation (NETosis). Thus, the IL-23/IL-17 axis presumably controls neutrophilic inflammation in BD vasculitis in the context of a predominant antigen-specific CD8$^+$ T-cell response.

What is already known about this topic?

- Behçet disease (BD) is a systemic vasculitis of unknown aetiology.
- HLA-B*51 and the endoplasmic reticulum aminopeptidase 1 (ERAP1), which cooperate in antigen processing and presentation to CD8$^+$ T cells, and interleukin 23 receptor (IL23R)/IL12R are the main risk genes.
- IL-17A is thought as a key cytokine in BD, which is produced under the control of IL-23 and activates neutrophils.
Behçet disease (BD) is a systemic vasculitis of unknown aetiology. It causes severe morbidity and mortality, particularly in young adults. Vascular inflammation presents with neutrophilic and lymphocytic vasculitis. Classified as a variable vessel vasculitis, it can involve all arteries and veins in mucocutaneous, ocular, articular and gastrointestinal tissues, and the central nervous system. In the absence of universally accepted diagnostic laboratory tests, papulopustular skin lesions (PPLs), erythema nodosum and aphthous oral and genital ulcers serve as diagnostic hallmarks of BD. Skin inflammation in BD extends from the vascularized dermis deep into subcutaneous adipose tissue.

BD is prevalent in countries along the historic Silk Road, including Turkey, Iran, Israel and Japan. Turkey has the highest prevalence, with 1–4 cases per 1000 inhabitants. In these countries, BD is a primary cause for visual loss in young people.

Within a complex genetic predisposition, the human leukocyte antigen (HLA) class I allele, HLA-B*51 is the strongest risk gene and is present in 50–70% of patients with BD. The HLA class I association is a prominent feature because only three of > 19 000 currently defined HLA-class I alleles show strong associations with common diseases: HLA-B*51 is associated with BD; HLA-C*06:02 with psoriasis; and HLA-B*27 with ankylosing spondylitis. Genome-wide association studies (GWAS) have further revealed that all three diseases show epistasis (i.e. nonadditive gene–gene interactions) between the respective HLA-class I risk allele and variants of the endoplasmic reticulum aminopeptidase 1 (ERAP1). This interaction may indicate common disease mechanisms related to autoreactive activation of CD8+ T cells because ERAP1 generates antigenic peptides for HLA-class I presentation to CD8+ T cells. Indeed, we have demonstrated that ERAP1 controls the generation of the melanocyte autoantigen for the HLA-C*06:02-restricted psoriatic CD8+ T-cell response. BD and psoriasis share several additional features; neutrophil-predominant inflammation with substantial lymphocytic admixture is characteristic of both. Genetic susceptibility to, and the pathomechanisms of, both diseases are related to the interleukin (IL)-23/IL-17 axis. IL-23 maintains activation of Th17 cells (Th/c17) cells, and promotes their production of IL-17A, which causes the recruitment and activation of neutrophils. In psoriasis, CD8+ T cells from psoriasis patients produce IL-17A in response to stimulation by the melanocyte autoantigen, whereas the pathogenic role of IL-23 and IL-17, which are highly expressed in psoriatic skin lesions, is well established by therapeutic cytokine blockade. In patients with active BD, circulating T cells produce more IL-17A upon in vitro polyclonal or microbial stimulation, and increased serum IL-17A levels correlate with BD activity. Preliminary observations suggest that IL-17A or IL-12/IL-23 inhibition may be effective in suppressing BD attacks. IL-17A-producing CD4+ T cells (i.e. Th17 cells) are thought to be the main cause of neutrophil activation in BD. Involvement of CD8+ T cells has rarely been considered, although studies have revealed the ability of CD8+ T cells to produce IL-17, and the association with HLA-B*51 and its interaction with ERAP1 implicates an essential role of CD8+ T cells in BD pathogenesis.

To determine the role of the IL-23/IL-17 pathway in BD, we analysed the expression of IL-17A and IL-23A in PPLs of acute BD attacks. We observed a marked expression of IL-23A by dendritic cells (DCs) and, to a lesser extent, by CD68 macrophages in BD lesions. Lesional CD8+ T cells showed...
signs of antigen-specific activation and proliferation, and a marked expression of IL-17A, which were predominantly found perivascularly and even in the vessel walls. This coincided with the perivascular infiltration of neutrophils and formation of neutrophil extracellular traps (NETs), suggesting that lymphocyte vasculitis might mediate neutrophilic vasculitis. These results suggest that HLA-B*51, together with certain ERAP1 and IL23R/IL12R variants, mediates an autoreactive CD8+ T-cell response in the IL-23/IL-17A pathway as a key pathogenetic event in BD.

Patients and methods

Patients and patient samples

Samples from patients with BD and psoriasis obtained for diagnostic purposes at the Ankara University were included in the study. PPLs from acute attacks of BD only, with findings of vasculitis or dense perivascular inflammatory cell infiltration, were included. All patients fulfilled the international study group criteria for BD (Table S1; see Supporting Information). A diagnosis of psoriasis was established clinically and histopathologically.

Healthy skin samples were obtained from the discarded healthy skin of donors undergoing plastic surgery. All participants were white and gave their written, informed consent to take part. The study was approved by the Institutional Review Board of Ankara University and performed in accordance with the principles of the Declaration of Helsinki.

Semi-quantification of immunofluorescence staining of the skin samples

Immunofluorescence staining, isotype control stainings and semi-quantification were performed as previously described. Briefly, heat-induced antigen retrieval was done using Tris-ethylenediaminetetraacetic acid buffer (pH 9°) at 120°C for 15 min; proteolytic antigen retrieval was performed with proteinase for 10 min. Sections were incubated with primary antibody or isotype controls at 4°C for 12–60 h, which was determined according to single staining condition for each antibody. IL-23A antibody was stained using the tyramide signal amplification method (PerkinElmer, Waltham, MA, USA). The antibodies used are summarized in Table S2 (see Supporting Information). For each specimen, 6–8 pictures from the epidermis, perivascular infiltration in the dermis and subcutaneous fat tissue with ×200 magnification were hand-counted separately by two scientists blinded to the study design, and the average counts per field in each sample were used in the statistical analysis. The labelled streptavidin biotin method was used for CD8 immunohistochemical staining.

Statistical analysis

When a significant Kruskal–Wallis H-test P-value was obtained (P < 0.05), a post-hoc comparison with a Mann–Whitney U-test was performed with SPSS (Version 26; IBM Statistics, Armonk, NY, USA). Bonferroni correction was used to evaluate the results. Two-tailed P-values < 0.016 were considered to be statistically significant.

Results

Increased interleukin-17A production in Behçet disease skin lesions

The production of IL-17 has previously been examined in peripheral blood mononuclear cells and cerebral lesions from patients with BD. We investigated the pattern of IL-17A+ cells in PPLs with vasculitis, a typical manifestation of BD, and in psoriasis vulgaris (PV) lesions. Both showed abundant IL-17A+ cells compared with healthy control (HC) skin [HC vs. BD P = 0.003; HC vs. PV P = 0.001 (Figure 1a, b)] but had different distribution patterns. In BD, IL-17A+ cells predominantly infiltrated around blood vessels in the dermis (PV vs. BD P = 0.035) and the subcutaneous tissue (PV vs. BD P = 0.002), and were largely absent in the epidermis [Figure 1c and Figure S1 (see Supporting Information)]. In PV lesions, IL-17A+ cells predominated in the epidermis (PV vs. BD P = 0.003) and the upper dermis but were scarce in the subcutis (Figure 1a, c and Figure S1). Thus, the distribution of IL-17A production corresponded to the respective sites of inflammation and tissue alterations in both disorders.

Enhanced interleukin-17A production by CD4+ cells in Behçet disease

Previous studies attributed the production of IL-17 in BD to CD4+ T cells. In our immunohistological stainings, inflammatory BD and psoriatic lesions showed significantly greater numbers of infiltrating CD4+ T cells than HC skin [HC vs. BD P = 0.002; HC vs. PV P = 0.008 (Figure 2a, b)]. Double staining for CD4 and IL-17A revealed that in BD PPLs, IL-17A+ CD4+ T cells mainly infiltrated the dermis and subcutis, and their frequency was significantly increased compared with HC skin (HC vs. BD P = 0.002; Figure 2a, c). In PV, CD4+ T cells were mainly present in the upper dermis. Most of them were IL-17A+, although IL-17A+ CD4+ T cells tended to be more abundant than in HC skin (HC vs. PV P > 0.016). These findings support previous reports that CD4+ T cells produce IL-17A in BD, while the contribution of CD4+ T cells to lesional IL-17 production is modest in psoriasis.

Activated CD8+ T cells are significant producers of interleukin-17A in Behçet disease

The association of BD with HLA-B*51 and ERAP1 variants indicates a causative role of CD8+ T cells in the pathogenesis of BD. As described previously, CD8+ T cells were found to infiltrate the vascular walls, perivascularly, around eccrine sweat glands, and diffusely distributed in the mid-to-deep dermis and subcutaneous adipose tissue (Figure 3a, b). Compared with
healthy skin, BD and PV lesions both showed increased infiltration of CD8[^8] T cells [HC vs. BD P = 0.003; HC vs PV P = 0.009 (Figure 3b, c)]. CD8[^8] T-cell infiltration was denser in BD than in PV lesions (PV vs. BD P = 0.0012). CD8[^8] T cells in PV were mainly located in lesional epidermis and dermis (Figure S1; see Supporting Information). In BD, the highest CD8[^8] T-cell density was observed in the subcutis and, to a lesser extent, in lesional dermis (Figure S1). The numbers of IL-17A[^] CD8[^8] T cells were higher in

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**Figure 1** Pronounced infiltration of interleukin (IL)-17A[^] cells into Behçet disease (BD) lesional skin, particularly in the subcutis. Healthy control (HC) skin (n = 5), psoriasis vulgaris (PV) lesional skin (n = 10) or BD lesional skin (n = 7) were stained with IL-17A antibody and visualized by fluorescence conjugated secondary antibody. The results were photodocumented by fluorescence microscopy and evaluated. (a) Representative immunofluorescence staining of IL-17A-expressing cells (red) in HC, PV and BD skin. Dotted lines indicate basal membranes. 4',6-Diamidino-2-phenylindole (DAPI) stained nucleoli. Magnification × 200. (b) Numbers of IL-17A[^] cells were counted per field of view and compared between groups by the Mann–Whitney U-test with Bonferroni correction (P < 0.016). Each dot represents one individual and also healthy control and the bar marks the median. (c) Numbers of IL-17A[^] cells were compared in the epidermis, dermis and subcutis between groups. Data are shown as median and interquartile range.
BD lesions and PV lesions than in HC [HC vs. BD P = 0.003, HC vs. PV P = 0.003 (Figure 3b, d)], and higher in BD lesions than in PV lesions (PV vs. BD P = 0.001). Staining with the proliferation marker Ki-67 showed that CD8+ T cells in the perivascular cellular infiltrate of BD lesions were activated and proliferated (Figure 3e). Thus, in BD PPLs, activated CD8+ T cells are predominant producers of IL-17A. These findings suggest that antigen-experienced CD8+ T cells play a prominent role in the inflammation in BD.

**CD56+ cells infiltrate perivascularly in Behçet disease skin lesions**

To determine IL-17 production by natural killer (NK) cells and NK-like T cells, for which a role in BD has been suggested, we next co-stained sections with antibodies for IL-17A and CD56. In BD PPLs, but not PV lesions, CD56+ cells were significantly overrepresented compared with HC skin [HC vs. BD P = 0.01; HC vs. PV P = 0.057 (Figure S2a, b; see Supporting Information)]. Thus, the increased density of CD56+ cells in BD skin lesions corresponds to the high frequency of NK cells in the blood of patients with BD. IL-17A expression by CD56+ cells in BD lesions was not statistically significantly increased (HC vs. BD P = 0.024; Figure S2a, c). These findings suggest that, in BD, CD56+ cells produce less IL-17A than CD8+ T cells.

**Mast-cell density is similar in healthy skin, and psoriasis vulgaris and Behçet disease lesions**

Mast cells reportedly release IL-17 in psoriatic inflammation. We examined mast cells with a tryptase antibody.
Figure 3 Enhanced interleukin (IL)-17A production and activation of CD8$^+$ cells in Behçet disease (BD) and psoriasis vulgaris (PV) vs. healthy control (HC) skin. HC skin ($n=5$), PV lesions ($n=7$) or BD lesions ($n=7$) were stained with CD8 and IL-17A antibodies, and evaluated. (a) Representative CD8 immunohistochemical staining of the epidermis, dermis and subcutaneous area in BD papulopustular skin lesions. (b) Representative immunofluorescence staining of CD8$^+$ T cells (green) expressing IL-17A (red; overlay with green appears yellow) in the epidermis, dermis and subcutaneous fat levels of the skin. Dotted lines indicate basal membranes. 4',6-Diamidino-2-phenylindole (DAPI) stained nucleoli. Magnification $\times$ 200. (c) Numbers of CD8$^+$ T cells were counted per field of view and compared between groups by the Mann–Whitney U-test with Bonferroni correction ($P<0.016$). Each dot represents one individual; the bar marks the median. (d) Numbers of IL-17A$^+$ CD8$^+$ T cells were compared between groups by the Mann–Whitney U-test. (e) Representative image of CD8$^+$ T cells (red) expressing the proliferation marker, Ki-67 (green), in HC skin, PV lesions and BD lesions.
Figure 4 Interleukin (IL)-17A decorates neutrophil extracellular traps (NETs) in Behçet disease (BD) skin lesions. Neutrophils were visualized by staining with antibodies for elastase and IL-17A in healthy control (HC) skin (n = 5), psoriasis vulgaris (PV) skin lesions (n = 5) or BD lesions (n = 7). (a) Representative immunofluorescence staining of elastase-positive cells (green) for IL-17A (red; overlay with green appears yellow). Dotted lines indicate basal membranes. 4',6-Diamidino-2-phenylindole (DAPI) stained nucleoli. Magnification × 200. (b) Numbers of elastase-positive cells were counted per field of view and compared between groups by the Mann–Whitney U-test with Bonferroni correction (P < 0.016). Each dot represents one patient; the bar marks the median. (c) Numbers of IL-17A+ elastase-positive cells were compared between groups by the Mann–Whitney U-test.
The distribution of tryptase+ cells was similar in BD lesions, PV lesions and HC skin [HC vs. BD P = 0.315; HC vs. PV P = 0.905 (Figure S3a, b; see Supporting Information)]. IL-17A+ tryptase+ cells were significantly increased in PV compared with BD and HC skin [HC vs. PV P = 0.016; PV vs. BD P = 0.003 (Figure S3a, c)] but not in BD lesions compared with normal skin. Therefore, mast cells do not appear to contribute actively to the IL-17A–neutrophil pathway in BD.

Massive neutrophil infiltration and neutrophil extracellular trap formation in Behcet disease skin lesions

Next, we investigated the presence of neutrophils. Elastase staining revealed neutrophil infiltration into lesional psoriatic epidermis, which is a hallmark of PV. We observed a prominent neutrophilic infiltration in BD vs. HC and PV skin [HC vs. BD P = 0.003; PV vs. BD P = 0.010 (Figure 4a, b; see Supporting Information)]. The numbers of IL-17A+ neutrophils were increased in BD lesions vs. HC skin, and tended to be increased in PV [HC vs. BD P = 0.004; HC vs. PV P = 0.056 (Figure 4a, c)]. Between 8% and 15% of neutrophils in BD lesions stained positively for IL-17A, presumably corresponding to receptor-bound IL-17A. Notably, we observed a pronounced formation of NETs directly in BD PPLs (Figure 4d). NET structures were strongly decorated by IL-17A, whereas neutrophils without NET formation in BD were overall negative for IL-17A (Figure 4a, d). These findings emphasize that IL-17A from T cells recruits and activates neutrophils in BD,44 contributing to tissue damage by the formation of NETs.

Pronounced infiltration of interleukin 17A+ CD8+ T cells characterizes Behcet disease and psoriasis vulgaris lesions

Quantitative comparison determined the main producers of IL-17A. In healthy skin, a few mast cells expressed IL-17A, and their numbers did not increase in BD (Figure S4; see Supporting Information). In psoriasis, as shown previously,37,38,42,43 mainly CD8+ T cells and some mast cells produced IL-17A. In BD, disease-specific production of IL-17A can be predominantly attributed to CD8+ T cells and – to a somewhat lesser extent – to CD4+ T cells (Figure S4).

CD11c+ dendritic cells dominantly express interleukin 23A in the papulopustular skin lesions of Behcet disease

The production of IL-17A by lymphoid cells requires IL-23.45 We investigated the cellular expression of IL-23 using an IL-23A-specific antibody. We observed high numbers of IL-23A+ cells in the dermis and subcutaneous tissue of BD lesions (Figure 5). In agreement with previous studies,46–48 keratinocytes and cells in the upper dermis expressed IL-23A in PV lesions (Figure 5). IL-23A and IL-17A co-localized in skin lesions of both BD and PV. In PV, the co-localization of IL-17A and IL-23A in the epidermis and the upper dermis is consistent with the epidermal localization of psoriatic inflammation, while in BD the abundant presence of IL-17A and IL-23A throughout the mid-to-lower dermis and subcutaneous adipose tissue corresponds to the tissue damage in BD.

Light microscopy examination of haematoxylin and eosin-stained sections of BD lesions revealed dense perivascular infiltrates of histiocytes (Figure 6a). Staining with the macrophage marker CD68 or the DC marker CD11c revealed dense infiltration of CD11c+ DCs and CD68+ macrophages in the dermis and subcutis of BD lesions [Figure 6a, b; Figure S5 (see Supporting Information)]. Both cell types also massively infiltrated the vessel walls in BD. In HC skin, CD68+ macrophages were occasionally positive for IL-23A in the upper dermis (Figure 6a). As previously reported,46–48 CD11c+ DCs and some CD68+ cells expressed IL-23A in psoriatic lesions (Figure 6a, b). Co-staining for IL-23A revealed that the majority of the CD11c+ DCs and a minor fraction of CD68+ macrophages expressed IL-23A in BD PPLs (Figure 6a, b).

Discussion

The characterization of lesion-infiltrating cells may improve our understanding of BD pathogenesis. This study explains several genetic and clinical features of BD in a histomorphological context. Improvement of BD by IL-17A blockade and normalizing IL-17 levels during remission or after therapy indicated a role of IL-17 in disease progression.49 Our results emphasize that CD8+ T cells are a major source of IL-17A in BD. We found that the IL-17A-producing cells densely infiltrating into the subcutis and dermis of BD lesions were predominantly CD8+ T cells. These Tc17 cells outnumbered the Tδ17 cells, which had formerly been regarded as the major source of IL-17A in BD.34–36,50 In agreement with a previous study,39 our data showed that CD8+ T cells were the dominant T-cell subset in BD PPLs.

Gene–gene interaction between HLA-B*51 and ERAP1 in genetic susceptibility to BD suggests that the presentation of ERAP1-dependent (auto)antigens from cytoplasmic proteins by HLA-B*51 might activate CD8+ T cells against certain target cells. Indeed, the expression of proliferation markers on lesional CD8+ T cells further supports the hypothesis that HLA-class I-restricted antigen-specific activation of CD8+ T cells drives the immunopathogenesis in BD lesions.

Genetic predisposition to BD involves association with the IL23R/IL12RB2 locus.18,19 The associated variants were located on the IL23R side, suggesting that IL23R rather than IL12RB2 is relevant in BD susceptibility.18,19 This suggests that immune activation in BD involves the IL-23/IL-17 pathway. Marked expression of IL-23A by CD11c+ DCs and, to a lesser extent, by CD68+ macrophages in PPLs factually demonstrates that mainly DC-derived IL-23 maintains the IL-17 production in BD. In accordance with previous studies in psoriasis,46–48 staining of PV lesions revealed IL-23A expression by CD11c+ DCs and CD68+ cells with a DC morphology in the papillary dermis. As subpopulations of DCs can express CD68, a classic...
macrophage marker. IL-23A+ CD68+ cells in PV may represent DCs. Thus, IL-23A may be produced by similar cell populations of monocyte lineage in both PV and BD.

High IL-17A production by CD8+ T cells in BD PPLs likely causes the lesional infiltration of neutrophilic granulocytes. These neutrophils contribute to the tissue damage in BD by the formation of NETs. NETs are fibrous networks of decondensed chromatin that are released from disintegrating neutrophils and are decorated with cytokines and antimicrobial peptides. While NETs are protective in microbial infections, uncontrolled NETosis in non-infectious, sterile inflammation can cause severe tissue damage. Blood neutrophils from patients with several diseases, including BD and psoriasis, form NET-like structures upon in vitro chemical stimulation, suggesting possible disease relevance. We observed fully developed NET structures and thus provide the first evidence that NET formation occurs directly in BD tissue lesions. Inhibition of NETosis is therapeutic for autoimmune diseases in experimental animal models. Thus, NETosis may offer a therapeutic approach for BD. In fact, this is presumably a mechanism of action underlying the therapeutic efficacy of colchicine, the first-line systemic treatment for BD recommended by the European League Against Rheumatism. Colchicine reduces NETosis by destabilizing the cytoskeleton.

The comparison with psoriasis allows a fine dissection of the pathogenetic processes in BD. In psoriasis, epidermal melanocytes are target cells of the psoriatic autoimmune response and thus determine the site of the psoriatic CD8+ T-cell response. In the pathogenesis of BD, the relationship between neutrophilic and lymphocytic vasculitis is unresolved. In BD PPLs, CD8+ T-cell infiltration and neutrophil recruitment were found exclusively in vascularized skin layers. The predominant localization of IL-17A-producing CD8+ T cells in the vessel walls and in perivascular infiltrates in deep dermal and subcutaneous tissue correlates with the actual tissue damage in BD. The localization of the Tc17 response in vessel walls and around blood vessels suggests autoantigenic target cells in vascularized tissues or directly in the blood vessel walls. Thus, one may speculate that the infiltration of neutrophilic granulocytes and the induction of neutrophilic vasculitis in BD may be secondary to lymphocytic vasculitis caused by an autoimmune response of Tc17 cells against endothelial antigens. Further studies to identify potential autoantigens and target cells of CD8+ T-cell response in BD should therefore include the endothelium.

In the context of the strong genetic association with HLA-B*51 and ERAP1 revealed by GWAS, this study proposes that CD8+ T-cell-mediated immune responses against (auto)antigens, probably from endothelial cells, may drive the neutrophilic inflammation in BD. Abundant IL-23A production by infiltrating CD11c+ DCs likely promotes IL-17A production by CD8+ T cells in acute BD attacks. The Tc17 response around blood vessels and in vessel walls could then cause infiltration of neutrophils, possibly with the histological appearance of

Figure 5 Representative immunofluorescence staining with interleukin (IL)-23A antibody or corresponding isotype in healthy control (HC) skin, psoriasis vulgaris (PV) and Behçet disease (BD) lesional skin in the epidermis, dermis and subcutaneous fat tissue. Dotted lines indicate basal membranes. 4',6-Diamidino-2-phenylindole (DAPI)-stained nucleoli. Magnification × 200.
Figure 6 Representative immunofluorescence staining with interleukin (IL)-23A (green) and CD68 or CD11c (red; overlay with green appears yellow) antibodies of healthy control (HC) skin, psoriasis vulgaris (PV) and Behçet disease (BD) skin lesions in the epidermis, dermis and subcutaneous fat tissue. Dotted lines indicate basal membranes. 4',6-Diamidino-2-phenylindole (DAPI) stained nucleoli. Magnification × 200.
neutrophilic vasculitis. The neutrophils further form prominent NETs that may contribute to BD tissue damage. These insights may also help to establish BD-specific diagnostic tools. By analysing pathogenomic BD lesions, our data may have important implications for understanding the pathomechanisms that cause tissue damage in BD, and provide a rationale for therapies targeting the IL-23/IL-17 axis or NETosis.

Acknowledgment

Open Access funding enabled and organized by Projekt DEAL.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Table S1 Patient characteristics.
Table S2 Primary and secondary antibodies used in the immunofluorescence staining.

Figure S1 The localization of interleukin-17A+ CD8+ T cells was compared between groups in the epidermis, dermis and subcutis.

Figure S2 CD56+ cells were increased in Behçet disease skin lesions.

Figure S3 Mast cells are not increased in Behçet disease skin lesions.

Figure S4 Cellular source of interleukin-17A in healthy skin, psoriatic skin lesions and Behçet disease.

Figure S5 Representative haematoxylin and eosin stain of lesional dermis in Behçet disease and subcutaneous fat tissue and immunofluorescence staining with interleukin-23A (red) and CD68 or CD11c.

Powerpoint S1 Journal Club Slide Set.

Video S1 Author video.