Review

Microbiome and Behçet's disease: a systematic review

M. Joubert[1], M. André[1], N. Barnich[2], E. Billard[2]

[1]Department of Internal Medicine, Gabriel Montpied Hospital, Clermont Auvergne University, Inserm U1071, INRAe USC2018, M2iSH, Clermont-Ferrand;
[2]Clermont Auvergne University, Inserm U1071, INRAe USC2018, M2iSH, Clermont-Ferrand, France.

Morgane Joubert, MD
Marc André, MD, PhD
Nicolas Barnich, PhD
Elisabeth Billard, PhD

Please address correspondence to:
Morgane Joubert,
Service de Médecine Interne,
CHU Gabriel Montpied,
58 rue Montalembert,
63000 Clermont-Ferrand, France.
E-mail:
m_joubert@chu-clermontferrand.fr
ORCID iD: 0000-0002-0281-0459

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ABSTRACT

The aim of this review was to describe the changes in the microbiota of patients with Behçet's disease (BD) and the mechanisms involved in the relationship between the microbiome and immunity in BD. A systematic search for relevant articles was made on PubMed and the Cochrane Library database using the following terms: "microbiota AND Behçet's disease" or "microbiome AND Behçet's disease". Sixteen articles were included in a qualitative synthesis. This systematic review on the microbiome and Behçet's disease underlines the presence of gut dysbiosis in BD patients. This dysbiosis is marked by (i) a decrease in butyrate-producing bacteria, which could affect T cell differentiation and epigenetic regulation of immune-related genes, (ii) a modification of tryptophan-metabolising bacteria, which could be linked to dysregulated IL-22 secretion, and (iii) a decrease in bacteria known to have anti-inflammatory properties. Regarding oral microbiota, this review underlines the possible role of Streptococcus sanguinis through molecular mimicry and NETosis. Clinical studies of BD have shown that (i) need for dentistry is associated with a more severe course in BD, and (ii) antibiotic-supplemented mouthwash reduces pain and ulcers. Faecal transplantation of BD patients' microbiota into mouse models led to decreased SCFA production, neutrophil activation, and Th1/Th17 responses. Recipient mice showed exacerbated experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE). In Herpes Virus Simplex-1 (HSV-1) infected mice mimicking BD, administration of butyrate-producing bacteria improved symptoms and immune variables. The microbiome may thus be involved in BD through immunity regulation and epigenetic modifications.

Introduction

Behçet's disease (BD) is a systemic vasculitis involving arteries and veins with heterogeneity among patients in demographics, organ involvement, frequency and severity of relapses, disease course, and response to treatment. Clinical manifestations are oral and genital ulcers, cutaneous manifestations, ocular, articular, vascular, neurologic and gastrointestinal involvement (1).

Epidemiology shows large geographic variations in BD frequency, with prevalence rates per 100,000 inhabitants of 20–420 for Turkey, 1.5–15.9 for southern Europe, and 0.3–4.9 for northern Europe (2). Interestingly, ethnic disparities persist among higher-prevalence migrants or their descendants living in lower-prevalence areas (3) emphasising both a genetic and environmental factors role. Mortality is increased in BD owing to pulmonary artery and large vessel, neurological, and gastrointestinal involvements. BD-associated uveitis can cause blindness. Better knowledge of the aetiopathogenesis of BD is therefore important to improve therapies.

BD shares common features with autoimmune and autoinflammatory diseases and with spondyloarthropathies (4). Regarding innate immunity, vascular infiltration of activated neutrophils has been widely reported in BD (5, 6). High concentrations of pro-inflammatory cytokines, including IL-8, IFN-γ and TNF-α, could activate neutrophils (5, 7, 8). Reactive oxygen species (ROS), produced at the site of inflammation, cause endothelial dysfunction and tissue damage (9, 10) and induce NETosis, a programmed form of neutrophil cell death. Neutrophil extracellular traps (NETs) enable the capture of infectious agents but have deleterious effects by exposing potential autoantigens extracellularly. For example, 74% of NET-associated proteins are thought to be autoantigens in autoimmune diseases such as lupus,

rheumatoid arthritis and vasculitis (11). An excess NETosis has been described in BD patients (9, 12) and could promote autoimmunity. Of note, mast cells have long been described in BD lesions (13) but their role remains elusive; this is illustrated by the fact that histamine-enriched food can be a trigger of oral ulcers in BD (14). Natural killer (NK) cells are increased in peripheral blood and BD lesions during the active phases of the disease (15) and contribute to the initiation of the Th1 response (16). Activated (γδ)T cells are increased in BD patients' peripheral blood and accumulate at inflammatory sites (17, 18). Anti-endothelial cell antibodies (AECAs) have been described in BD (19-21) and may trigger inflammation through complement or antibody-dependent cell toxicity, causing vasculitis. Genetic evidence links BD to human leukocyte antigens (HLAs), or the major histocompatibility complex (MHC). MHC class I-peptide complexes (pMHC I) are presented on all nucleated cells. For NK cells, MHC I is a cytotoxicity-inhibiting ligand (22). pMHC I is also a ligand for the TCR of $CD8^+$ T cells, determining their cytotoxic action toward target cells. The antigen processing for presentation on MHC class I involves the aminopeptidase ERAP1, which determines the peptide repertoire presented to $CD8^+$ T cells (22). Interestingly, there is an epistatic genetic interaction in BD between the main susceptibility nucleotide polymorphism HLA-B*51 and ERAP1 haplotype 10. In affected patients, ERAP1 activity is decreased and the peptide repertoire is altered (23). BD has thus been described as an MHC-I disease, along with ankylosing spondylitis (AS) and psoriasis (24). Following activation by an antigen-presenting cell, $CD4^+$ T lymphocytes adopt an effector (Th1, Th2, Th17, etc.) or regulatory (Treg) profile. Th1 cytokines are increased in peripheral blood, and mucosal and skin lesions of active BD patients (25). Circulating Th17 and regulatory T cells are increased and decreased, respectively (26). In children with acute and relapsing BD, Tregs incompletely counterbalance Th17 response (27). Current treatments of mucosal manifestations of BD include apremilast that downregulates Th1 and Th17 cell activity (28). Moreover, ustekinumab (anti-IL12/IL23) has shown efficiency in BD patients (29), and even if its effect is controversial, secukinumab (anti-IL 17) may have a beneficial effect at least in some BD patients (30, 31), underlining the importance of Th1/Th17 *versus* Treg imbalance in this pathology.

Carrying the HLA-B*51 allele confers a relative risk of developing BD of 5.8. Besides *ERAP1*, genome-wide association studies have identified various other polymorphisms in immune-related genes (*IL23R-IL12RB2, IL10, STAT4, CCR1-CCR3, KLRC4, TNFAIP3, FUT2*, *MICA*) (32,33). Many rare variants of autoimmune or autoinflammatory-related genes are found in BD patients (34). Some of these variants, especially those in *NOD2, PSTPIP1* and *MVK* (34), may play a role in BD pathogenesis.

Microbiota and especially intestinal microbiota is major player in autoimmune and autoinflammatory diseases, raising the question of their possible role in BD. Dysbiosis in salivary or gut microbiota might trigger inflammation by influencing immune responses. Accordingly, this systematic review set out to describe the alteration in BD patients' microbiota and discuss its effects on BD physiopathology through immune dysregulation.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to conduct this review, with pre-screening of all methods prior to the literature search (35). A systematic search for relevant articles was made on PubMed and the Cochrane Library database, without language limitation, using the terms: "microbiota AND Behçet's disease" or "microbiome AND Behçet's disease". Inclusion criteria were as follows: human and animal studies examining the gut or oral microbiota or microbiome in BD, interventional studies targeting microbiota. There was no restriction on sample size, participants, age, sex, or health status. Exclusion criteria were as follows: studies of other diseases, studies of other environmental factors, reviews. There was no registration on any international platform of prospectively registered systematic reviews. The following data were extracted from each study: title, authors, year of publication, country of origin of included patients, sex, mean age, study design, aims of study. For studies characterising microbiota, α diversity (defined by Chao-1 and Shannon indices), and modified bacterial abundances were also extracted, together with animal model in preclinical studies. In all, sixteen articles meeting search criteria were used for data collection (Fig. 1).

Results

Gut microbiota imbalance in BD patients

The human gut microbiota comprises approximately 100 trillion resident microorganisms, including archaea, bacteria, viruses, and fungi. The composition of the microbiota is moderately stable along the gut, but the absolute numbers of microorganisms vary from the mouth to the rectum (36). The gut microbiota consistently differs among individuals. It is acquired in early life via the commensal microbiota from the mother's skin, vagina, and faeces, and it matures during the first two years. Some bacterial species are found in nearly all individuals, and the human gut microbiota is dominated by three phyla: Firmicutes (30–50%), Bacteroidetes (20–40%) and Actinobacteria (1–10%). The gut microbiota forms a defensive barrier to infection. It has numerous protective, structural and metabolic roles (36) and is involved in immune development and function (37, 38). The gut microbiota influences metabolic processes in immune cells by producing active metabolites such as short-chain fatty acids (SCFAs), bile acids, and tryptophan metabolites (39).

Studies of gut microbiota and BD are listed in Table I. Microbiota analysis requires DNA extraction, polymerase chain reaction (PCR) amplification using primers targeting the 16S ribosomal RNA gene, and finally sequencing of some of the nine hypervariable regions (V_1-V_9) of the gene. Studies listed in Table I show an decrease of abundance

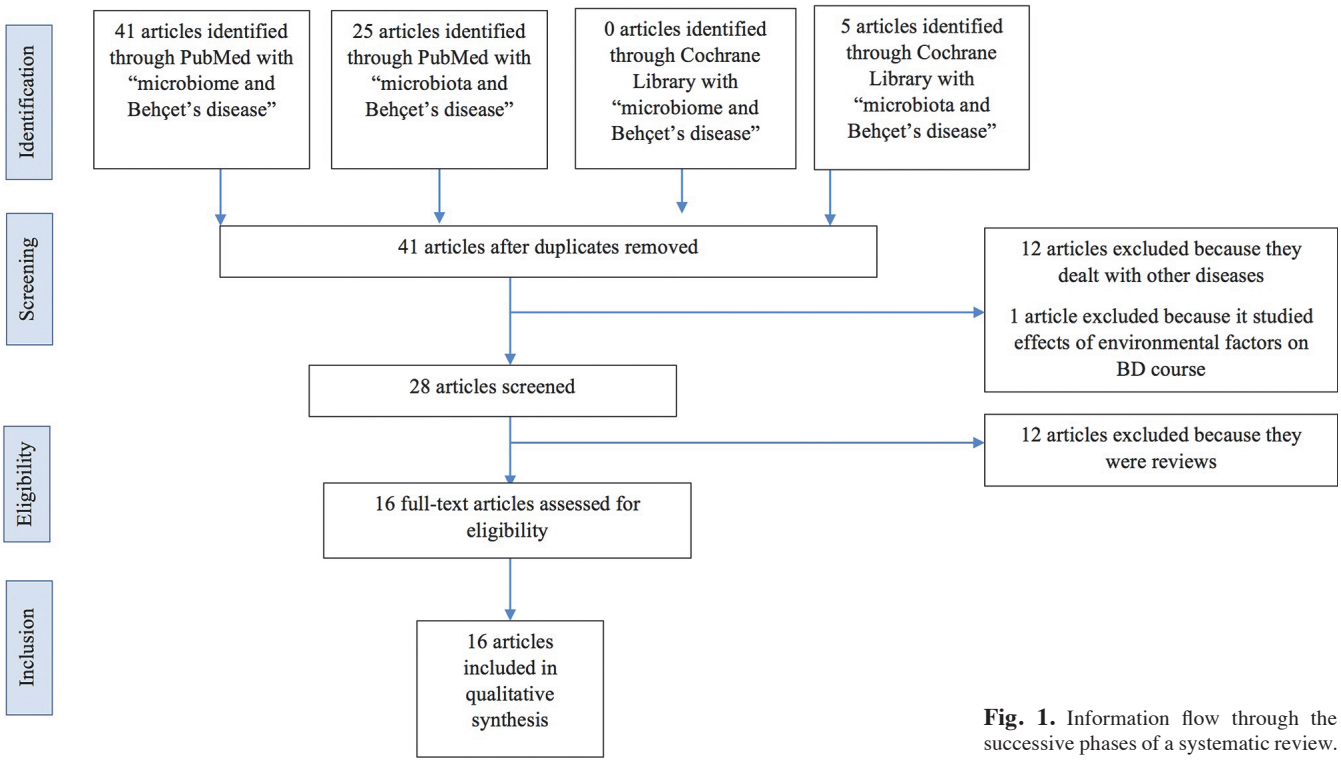

Fig. 1. Information flow through the successive phases of a systematic review.

of SCFA-producing bacteria such as *Roseburia* and *Subdoligranulum* (genus *Clostridium*), *Lachnospira*, *Bacteroides*, and *Akkermansia* in the gut microbiota of BD patients (40). SCFAs (acetate, propionate, and butyrate) are produced through fermentation of dietary fiber by the microbiota. Butyrate levels were significantly reduced in faecal samples of BD patients (40), as already described in inflammatory bowel disease (37) and diabetes mellitus (41). Butyrate promotes differentiation into regulatory T cells (Tregs) and inhibits pro-inflammatory gene expression through histone deacetylase (HDAC) regulation and subsequent epigenetic modifications (42). SCFAs also influence the metabolic status of T cells and modulate their function through epigenetic modifications (37, 43). Dysbiotic gut microbiota in BD patients may thus disrupt Treg versus Th1/Th17 homeostasis and promote pathological responses (40, 44, 45).

At the same time, opportunistic pathogens such as sulfate-reducing bacteria, *Stenotrophomonas spp.*, *Actinomyces spp.*, and *Paraprevotella spp.* are overrepresented in BD patients and could contribute to intestinal epithelial barrier damage and innate immune receptor stimulation (46). Of note, some pattern recognition receptors such as TLR2 and TLR4 are overexpressed in intestinal lesions of BD patients (47).

The studies listed in Table I also highlight a decrease in *Bacteroides* and *Clostridia* abundance in BD patients. Interestingly, these bacteria produce biologically active metabolites from tryptophan, an essential amino acid that humans must acquire through diet (39). Dietary tryptophan is mainly metabolised by the host into kynurenine and serotonin. Gut microbiota converts it into indole derivatives exerting anti-inflammatory effects in mammals (48). Among these, indoleacetic acid, indole-3-acetaldehyde, indole-3-aldehyde, and indole acrylic acid act as agonists for aryl hydrocarbon receptor (AhR) transcription factor, which influences T cell immunity and exerts anti-inflammatory effects through the regulation of IL-10 family member IL-22 (39). Intriguingly, circulating IL-22 is increased in active BD patients and correlates with small vessel inflammation (49). At the intestinal level, IL-22 exerts beneficial effects by reinforcing the epithelial barrier, protecting against bacterial infections and favouring mucosal healing. IL-22 signalling often results in reduced Th1 responses and contributes to inflammation resolution by promoting Treg differentiation or IL-10 secretion (50). Early treatment with IL-22 thus reduces the severity of experimental autoimmune uveitis (EAU) in mice (51). Increased IL-22 in the BD inflammatory context is thus unexpected. However, the same observations have been reported in Crohn's disease, and IL-22 is now considered to play a pathogenic role in some inflammatory contexts, in particular the skin in psoriasis. In BD, IL-22 could be produced to counteract inflammation-related damage to the mucosal barrier and endothelial cells. Of note, the concentration of biologically active IL-22 also depends on the secretion of a soluble receptor homolog, IL-22 binding protein (IL-22BP) (52). To our knowledge, IL-22BP has not been explored in BD: such a study would help to better understand the role of IL-22 in physiopathology and the link with dysbiosis.

The decrease in tryptophan-metabolising bacteria abundance could also contribute indirectly to the massive IL-1β production in BD patients. IL-1β secretion results from NLRP3 inflammasome activation, and the tryptophan analogue Tranilast (*N*-[3´,4´-

Table I. Characterisation of gut microbiota dysbiosis in Behçet's disease patients compared to healthy or disease controls.

Authors	Population	V regions sequenced	_ Diversity	Increased bacterial abundance in BD patients	Reduced bacterial abundance in BD patients
Consolandi *et al*., 2015 (35)	**Italian** BD = 22 (12 male, 10 female) mean age 41.1 HC =16 (6 male, 10 female) mean age 43.4	V3-V4	NS		***Roseburia* (genus *Clostridium*)** ***Subdoligranulum* (genus *Clostridium*)**
Shimizu *et al*., 2016 (80)	**Japanese** BD = 12 (5 male, 7 female) mean age 48.8 HC = 12 (6 male, 6 female) mean age 48.6	NA	NA	*Actinobacteria* *Lactobacillus*	***Clostridia***
Ye *et al*., 2018 (40)	**Chinese** BD = 32 HC = 74 (matched on age, BMI, sex)	V3-V4	NA	*Bilophila spp*. *Parabacteroides spp* *Paraprevotella spp* *Stenotrophomonas spp*. *Actinomyces spp*. *Corynebacterium spp*.	***Clostridium spp*** *Methanoculleus spp*. *Methanomethylophilus spp*.
Shimizu *et al*., 2019 (81)	**Japanese** BD = 13 (5 male, 8 female) mean age 49.2 HC = 27 (12 male, 15 female) mean age 52.8	V1-V2	NS	*Eggerthella lenta* *Acidaminococcus bifidum* *Lactobacillus iners* *Streptococcus species* *Lactobacillus salivarius*	*Megamonas hypermegale* *Butyrivibrio* *Streptococcus infantis* *Filifactor*
Oezguen *et al*., 2019 (82)	**Turkish** Neurobehçet = 13 (8 male, 5 female) mean age 42.1 HC = 14 (10 male, 4 female) mean age 37.8	V3-V5	↓	*Parabacteroides* *Clostridiales* *Geminger* *Butyricimonas* *Actinobacteria* *Erysipelotrichaceae incertae sedis*	*Vampirovibrio* Unclassified *Lachnospiraceae* *Prevotella*
Tecer *et al*., 2020 (83)	**Turkish - Ankara** BD = 7 with uveitis (5 male, 2 female) mean age 35.6 FMF = 12 (6 male, 6 female) mean age 32.2 Crohn's disease = 9 (3 male, 6 female) mean age 35 HC = 16 (6 male, 10 female) mean age 39.4	NA	NA	*Veillonellaceae* *Succinivibrionaceae* *Prevotellaceae* *Lachnospiraceae*	*Bacteroidaceae*
Bilge *et al*., 2020 (84)	**Turkish** BD = 27 (17 male, 10 female) mean age 40.8 HC = 10 (6 male, 4 female) mean age 38.9	V3-V4	NS		*Bacteroides* *Cricetibacter* *Alistipes* ***Lachnospira*** ***Akkermansia*** *Sutterella* *Anaerofilum*
Van der Houwen *et al*., 2020 (47)	**Netherlands** BD = 19 (9 male, 10 female) mean age 50 HC = 17 (9 male, 8 female) mean age 47 **Italian** BD = 13 (6 male, 7 female) mean age 54 HC = 15 (7 male, 8 female) mean age 44	V3-V4	NA	*Actinomyces* *Libanicoccus* *Collinsella* *Eggerthella* *Enethrohabdus* *Canetibacterium* *Enterobacter*	*Barnesiellaceae* *Lachnospira*
Kim *et al*., 2021 (64)	**South Korea** BD = 9 (1 male, 8 female) mean age: 33 RAU = 7 (2 male, 5 female) mean age: 47 BD-matched HC = 9 (4 male, 5 female) mean age: 53 RAU-matched HC = 7 (3 male, 4 female) mean age: 44	V3-V4	NS	*B. uniformis* (active *vs*. inactive BD, and BD *vs*. HC) *Faecalibacterium prausnitzii* group *Bifidobacterium adolescentis* group salivary *Streptococcus pneumoniae* group *Streptococcus peroris* group *Neisseria sicca* group (BD with uveitis *vs*. BD without uveitis)	*Clostridium_g24* (BD with uveitis *vs*. BD without uveitis)

BD: Behçet's disease; HC: healthy controls; FMF: familial Mediterranean fever; RAU: recurrent aphthous ulceration; NS: non-significant; NA: not available.
V*n*: hypervariable regions of the 16S ribosomal RNA gene sequenced in each study.
In bold, bacteria involved in SCFA production.
↓ decreased with $p<0.05$.

dimethoxycinnamoyl]-anthranilic acid) has recently been shown to inhibit NLRP3 inflammasome assembly and subsequent caspase-1 activation and IL-1β production (53, 54). If some tryptophan derivatives produced by microbiota also exert this effect, then the decrease in tryptophan-metabolising bacteria in BD patients could release NLRP3 inhibition and promote IL-1β secretion.

Finally, the studies listed in Table I show a decrease in *Akkermansia* and *Barnesiellaceae* in the gut microbiota of BD patients (55). These bacteria may exert protective anti-inflammatory effects by down-modulating pro-inflammatory cytokines (56, 57).

Taken together, the studies listed in Table I do not convey a consistent picture of dysbiosis in BD patients, possibly because of confounding ethnic or environmental factors, but suggest a role for gut microbiota in BD. Faecal transfer experiments in animal models would be of great interest to firmly establish the causal role of gut microbiota in BD. Incidentally, microbiota may be involved in some epidemiological features of BD, such as regional disparities, that are usually attributed to genetics. Several studies have shown a lower incidence of BD in the immigrants from the prevalent areas compared to the same ethnic living in the homeland (58), suggesting the contribution of the environmental factors to the regional disparities, unfortunately microbiota has never been considered nor characterised in these studies. Moreover, various phenotypes of BD can be distinguished with different therapeutic strategies (59). It would thus be relevant to stratify BD patients in future microbiota studies according to their clinical features, which would require a large enough cohort size.

Oral microbiota imbalance in BD patients

The human oral cavity contains various microbial habitats, including the teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils (60). The oral microbiota has been extensively characterised by both cultivation and culture-independent molecular methods. Unfortunately, the vast majority of unnamed oral taxa are referenced by clone numbers or 16S rRNA GenBank accession numbers, often without taxonomic anchors (60). In March 2020, the Human Oral Microbiome Database (www.homd.org) listed 784 bacterial taxa and 1,567 genomes in the human oral microbiome taxonomic hierarchy, mostly belonging to the Firmicutes phylum and to the *Streptococcaceae* family. The tongue, teeth, mucous membranes, palate and gums harbour distinct microbiota organised in biofilms (61). Their composition is affected by environmental factors such as exposure to oxygen, dental plaque, breastfeeding, and dental hygiene habits. Altered oral microbiota has been observed in various diseases such as diabetes mellitus, bacteraemia, endocarditis, cancer, autoimmune diseases, and preterm births (62).

Table II summarises all the studies characterising oral microbiota in BD patients. Ribosomal 16S rRNA comprises nine variable loops (V_1-V_9). At present, oral microbiome sequencing targets either the $V_{1–2}$ or the $V_{3–4}$ regions as described in the preceding section (62). However, V_1 region sequencing should be used to differentiate *Streptococci*. Region V_2 best identifies Gram-negative *Porphyromonas* and *Fusobacterium* (63), $V_{1–3}$ *Streptococcus, Fusobacterium, Prevotella, Porphyromonas* and *Bacteroides*, and $V_{4–6}$ *Prevotella, Porphyromonas, Treponema, Enterococci* and *Campylobacter*-like oral inhabitants (62).

The oldest study reports abnormal abundance of *Streptotococcus sanguinis* on patients' ulcers. The role of *Streptococcus* in BD has been suspected since the 90s, supported by BD clinical manifestations during tests of hypersensitivity to streptococcal antigens (64). *Streptococcus sanguinis,* formerly *S. sanguis,* is historically the agent most often incriminated in BD. This Gram-positive, non-sporulating, facultative anaerobic, chain-forming bacterium is a pioneer germ of dental plaque biofilm (65). *S. sanguinis* could lead to an immune cross reaction through molecular mimicry between bacterial and human heat shock proteins (HSPs) (66). HSPs are conserved in microorganisms and mammals and are powerful antigens for T cell activation. The HSP-60 family includes HSP-65 (65 kDa), which is shared by mycobacteria and several strains of S*treptococci* and its human equivalent HSP-60 (60 kDa), mainly expressed within mitochondria. There is about 60% identity between these proteins (66). Anti-HSP-60/65 antibodies have been described in BD patients with neurologic involvement (67), suggesting that bacterial HSPs may be initiator antigens of BD, secondarily causing, by cross-reactivity, proliferation of T cells self-reactive to human HSP-60. However, although various auto-antibodies have been identified in small groups of BD patients, none of them seem highly prevalent, nor do they target the bacterial epitope (68). One study in BD patients (68) identified antibodies directed against the middle neurofilament (NF-M) constituting filamentous neuronal compounds in the brain, retina, and skin. There is significant identity between human NF-M and bacterial HSP-65. NF-M immunoreactive sera cross-reacted with bacterial HSP-65 (68). To our knowledge, although much evidence seems to support involvement of bacterial HSP-65, nothing firmly demonstrates that primary immunisation of patients occurs against *S. sanguinis* HSP-65. Other bacteria could also contribute to this cross-reaction phenomenon. In particular, the impact of BD-associated HLA-B*51 and ERAP-1 polymorphisms on this molecular mimicry process remains to be explored.

Streptococcus sanguinis also induces NETosis (69), thus favouring exposure of self-antigens in the extracellular space and promoting inflammation, tissue damage, and autoimmune manifestations. No literature has reported on the impact of either microbiota or *Streptococcus sanguinis* on the excess NETosis reported in BD. Finally, *S. sanguinis* was increased in the saliva of orally active compared to orally inactive BD patients. *Akkermansia* and *Muribaculaceae,* previously known as the S24-7 family, are underrepresented in BD patients' oral microbiota (70), as they are in inflammatory bowel diseases (71). Pili-like proteins from *Akkermansia muciniphila* increase epithelial barrier function through the induction of IL-10 (72). For unknown reasons, salivary *Rothia*

Table II. Characterisation of oral microbiota dysbiosis in Behçet's disease patients compared to healthy or disease controls.

Authors	Population	Samples	V regions equenced	A Diversity	Increased bacterial abundance in BD patients	Reduced bacterial abundance in BD patients
Seoudi *et al*., 2015 (65)	**UK** BD = 54 (35 male, 19 female) mean age: 41.7 HC = 25 (15 male, 10 female) mean age: 38 RAS = 8 (5 male, 3 female) mean age: 43.5	Saliva Oral mucosa swab Brush biopsies from ulcer and non-ulcer sites	V4	NA	RAS + BD *vs*. HC, non-ulcer sites: *Rothia denticariosa* (phylum *Actinobacteria*) BD *vs*. RAS, ulcer sites: *Streptococcus salivarius* BD *vs*. HC, ulcer sites: *Streptococcus sanguinis*	RAS + BD *vs*. HC, oral mucosa: *Neisseria* and *Veilloneilla*
Coit *et al*., 2016 (79)	**Turkish** BD = 31 (15 male, 16 female) mean age: 36.5 HC = 15 (7 male, 8 female) mean age: 37.8	Saliva	V4	↓	*Haemophilius parainfluenzae* (species)	*Leptotrichia* *Alloprevotella* (genus)
Balt *et al*., 2020 (61)	**Mongolian** BD = 47 (16 male and 31 female) mean age: 44.2 HC = 48 (17 male and 31 female) mean age: 38.4	Saliva	V3-V4	NS		*Akkermansia* S24-7 family (*Muribaculaceae*)
Kim *et al*., 2021 (64)	**South Korea** BD = 9 (1 male, 8 female) mean age: 33 RAU = 7 (2 male, 5 female) mean age: 47 BD-matched HC = 9 (4 male, 5 female) mean age: 53 RAU-matched HC = 7 (3 male, 4 female) mean age: 44	Saliva	V3-V4	NS	*Lachnoanaerobaculum* (BD *vs*. HC) *Rothia mucilaginosa* (BD *vs*. RAU)	*Veillonella* (BD with uveitis *vs*. without uveitis)

BD: Behçet's disease; HC: healthy controls; NA: not available; NS: non-significant; RAS: recurrent aphthous stomatitis; RA: recurrent aphthous ulceration.
V*n*: hypervariable regions of the 16S ribosomal RNA gene sequenced in each study. ↓: decrease with $p<0.05$.

mucilaginosa was more abundant in the active BD group than in the active recurrent aphthous ulceration (RAU) group (73). *Rothia denticariosa* colonisation was increased at non-ulcer sites in BD compared to the ulcerated mucosa of orally active BD (74). The oral mucosa of healthy controls (HC) was enriched in *Neisseria* and *Veillonella* compared to BD and RAU patients (74).

Overall, the literature points to an alteration of oral microbiota in BD, but with no consistency among studies. We note that all studies were based on sequencing of the V_3 and V_4 regions of 16S rRNA only, whereas the V_1 region is the best choice for differentiating oral *Streptococci* (62). It would be useful to amplify and sequence the whole 16S rRNA gene in future studies on BD patients' oral microbiota.

Changing microbiota to change disease course

Four studies summarised in Table III directly or indirectly analysed the role of the gut or oral microbiome in BD, by acting on hygiene practices or modulating microbiota-secreted metabolites. Since butyrate-producing bacteria are decreased in the gut microbiota of BD patients, two studies evaluated the effect on disease course of butyrate supplementation or fibre-rich diets increasing butyrate production (75, 76). This led to a significant reduction in corticoid use and BD disease activity (77), though without significant changes in blood inflammatory variables. Dental caries and need for tooth extraction were associated with a more severe BD course, suggesting a possible positive impact of more stringent hygiene practices (78).

Mouthwash containing betamethasone thus showed greater efficacy against pain and ulcers when antibiotics (*e.g.* doxycyclin and nystatin) were added, underlining the role of microorganisms in BD pathogenesis.

Faecal transplantation of BD patients' microbiota to animal models

Different models, all with their limitations, were used to evaluate the causal role of microbiota in BD (79). Table IV summarises the animal studies that investigated the role of microbiota in BD. Transplantation of faeces from BD patients in antibiotic-treated mice led to decreased SCFA production and altered intestinal permeability, and favoured neutrophil activation and Th1/Th17 responses compared to mice receiving faeces from healthy controls (80). Subsequent induction of experimental autoimmune uveitis (EAU) (46, 80) or experimental autoimmune encephalomyelitis (EAE, (80) by immunisation with interphotoreceptor retinoid binding protein or with myelin oligodendrocyte glycoprotein, respectively, showed that both diseases were exacerbated in BD-recipient mice.

Another study used HSV-1 infected mice, which developed BD symptoms after 5–16 weeks. Differences in gut microbiota were observed between normal and BD mice (81). Administration of *Eubacterium rectale*, one of the most prevalent bacterial species in the human colon and a major contributor to butyrate production (82), improved BD symptoms and immune variables in this model.

Table III. Clinical studies evaluating the impact of microbiota modulation on BD.

Authors	Population	Main goals	Main results	Comments
Emmi *et al*., 2020 (68) *	**Italian** BD = 17 (9 male, 8 female) mean age: 45.6 2 groups: - Habitual diet supplemented with oral butyrate (2.4 g/day): 8 - Lacto-ovo-vegetarian diet containing insulin and resistant starch-rich foods: 9	To evaluate before and after dietary interventions (at month 0 and at month 3) the effect of 2 butyrate-enriched diets on blood redox status and fibrin degradation in clinical variables of BD and gut microbiota.	In both groups, month 0 *vs* month 3: - Reduction in leukocyte ROS production . Improvement in fibrin susceptibility to plasmin-induced lysis - Enrichment in *Clostridium XIVa*, *Romboutsia,* and *Eggerthella* genera - Improvement of BD symptoms, reduction of corticosteroids use - No change in blood inflammatory parameters.	These results suggest benefits of butyrate dietary enrichment in BD pathophysiology, especially for cardiovascular prevention. Of note, SCFA faecal concentrations were unchanged.
Pagliai *et al*., 2020 (69) *	**Italian** BD = 90 3 groups: - Lacto-ovo-vegetarian diet: 30 - Mediterranean diet: 30 - Mediterranean diet supplemented with butyrate: 30	To investigate whether 3 months of dietary intervention could ameliorate the clinical manifestations and modulate the gut microbiota of BD patients.	Study in progress: results not available.	
Yay *et al*., 2019 (78)	**Turkish** BD = 194 (81 male, 113 female) mean age: 37.8	To examine whether oral health (*i.e*. presence of an infection site) influences disease course in BD patients.	During follow-up, patients having had tooth extraction at their last dental visit and patients with dental caries had a higher disease severity score than others.	Male sex is also associated with a more severe disease course in BD. Limits: retrospective study.
Senusi *et al*., 2020 (72)*	**UK** BD = 261 (120 male, 141 female) mean age: 40.5 3 groups: - Antibiotics + betamethasone mouthwash: 95 - Betamethasone mouthwash: 81 - No mouthwash: 85	To evaluate the effectiveness of antibiotic supplementation in betamethasone mouthwash on oral ulcerations, oral health, quality of life and Behçet's current activity form after 3 and 6 months.	Oral ulcer severity score is decreased in antibiotic-recipient patients in comparison with control groups. The antibiotic-enriched mouthwash recipient patients were significantly more satisfied than the betamethasone mouthwash recipient patients.	Improvement of oral ulcers severity under antibiotics treatment underlines the role of oral microorganisms in BD pathophysiology.

BD: Behçet's disease; ROS: reactive oxygen species; SCFA: short-chain fatty acid.
*interventional study.

Although the mechanisms have not been elucidated, decrease in butyrate production is clearly associated with immune dysregulation in BD.

Discussion

This review on the microbiome and BD underlines the presence of gut dysbiosis in patients. Regarding gut microbiota, pro-inflammatory alterations have been reported in EAU and EAE mouse models, although their causal role in BD has not been fully demonstrated. Butyrate-producing bacteria are decreased in BD patients, in line with the regulatory role of this SCFA in immune and especially T cell homeostasis. The importance of butyrate in pathogenesis is further emphasised by the improvement of clinical scores following administration of butyrate-producing bacteria in the HSV-1 mouse model of BD. Tryptophan-metabolising bacteria also seem under-represented in BD patients. Considering the elevated IL-22 levels in these patients, the role of tryptophan metabolites and downstream pathways in BD deserves attention. In other MHC-I-opathies [ankylosing spondylarthritis (83), psoriasis (84, 85)], dysbiosis has also been reported and microbiota profiles may share some similarities with Crohn's disease (86, 87).Various microbiota-driven mechanisms could thus play a role in BD aetiopathogenesis, as already reported in these diseases (39, 88, 89).

The crucial role of oral microbiota in BD is supported by the association of dentistry needs with a more severe disease course, and by the beneficial effect of antibiotic-containing mouthwash. It is of interest that *Streptococcus sanguinis* is preferentially associated with oral aphthous ulceration in BD patients, in line with its suspected involvement in pathogenesis through molecular mimicry. However, this finding does not exclude a contribution of other microbiota members, nor does it prove the effectiveness of this mechanism. All these microbiota studies have obvious limitations because results were not adjusted for treatment and environmental factors such as tobacco consumption, diet, recent antibiotic treatment or delivery birth mode. Only the V_3 and V_4 regions of 16S rRNA were sequenced, whereas the V_1 region is preferable for differentiating oral *Streptococci*. Further work will thus be needed to elucidate the role of *Streptococcus* in BD and validate past studies with current technologies. Interestingly, a recent case report showed an improvement of uveitis symptoms and inflammation after symbiotic treatment with fructo-oligosaccharides associated with living *Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium longum*, and *Lactobacillus bulgaricus* (90). Microbiota-targeting approaches could thus be useful for BD treatment.

As genital ulcers are typical manifestations of BD, genital microbiota profiling of patients could be of interest. To our knowledge, this has never been attempted. Bacterial vaginosis (BV), or vaginal dysbiosis, is a common vaginal condition associated with aberrant changes in the vaginal microbiome. Bacterial vaginosis is characterised by a reduction of the resident lactic acid-producing *Lactobacillus* spp. and an overgrowth of anaerobic bacteria. No study has described a link between BD

Table IV. Transplantation of BD patients' microbiota into animal models.

Authors	Animal model	Main goals	Main results
Ye *et al*. (2018) [40]	Fecal transplantation of BD or HC gut microbiota EAU model	To investigate the impact of BD patients' microbiota on development and severity of uveitis	Severe uveitis observed in BD recipient mice *vs*. healthy controls (HC) recipient mice In BD- *vs*. HC-recipient mice: clinical manifestations are more severe and IL-17 and IFN-γ mRNA are up-regulated in spleen.
Wang *et al*. (2021) [74]	Faecal transplantation of BD or HC gut microbiota EAU model EAE model	To investigate whether BD gut microbiota contribute to inflammation and immune dysregulation in EAU and EAE	Following BD *vs*. HC fecal transfer: Increased intestinal permeability, neutrophil activation with NETosis, increased Th1 and Th17 responses EAU model, in BD- *vs*. HC-recipient mice: - uveitis is exacerbated (clinical and histological evaluations) - up-regulation of IFN-γ and IL-17 expression associated with higher counts of Th1 and Th17 in MLN, spleen, retina - neutrophil activation with increased MPO and NE levels EAE model, in BD- *vs*. HC-recipient mice: - encephalomyelitis is exacerbated (clinical evaluation) - up-regulation of IFN-γ, IL-17 and MCP-1 mRNA in lymphocytes, down-regulation of IL-10
Islam *et al*. (2021) [75]	ICR mice infected with HSV-1: 90% of normal healthy mice, 10% of BD-like mice Normal healthy mice, 3 groups: - PBS-treated mice - vehicle-treated mice - *E. rectale*-treated mice BD-like mice, 5 groups: - PBS-treated BD mice - Butyrate-treated BD mice - *E. rectale*-treated BD mice - Colchicine-treated BD mice - Colchicine- and *E. rectale*-treated mice	To investigate whether administration of butyrate or of butyrate-producing *Eubacterium rectale* improve immune variables and BD symptoms	In normal *vs*. BD-like mice: modification of microbial diversity (increased OTU number and Shannon index), altered gut microbiota composition In BD-like mice: - butyrate treatment improved BD symptoms compared to PBS treated mice - compared to culture media (vehicle), administration of *E. rectale* improved disease severity score and immune v (DC maturation, Treg activation, NK cell frequency, serum IL-17 level)

BD: Behçet's disease; DC: dendritic cells; EAU: experimental autoimmune uveitis; EAE: experimental autoimmune encephalomyelitis; *E. rectale: Eubacterium rectale*; HC: healthy controls; HSV-1: herpes virus simplex 1; ICR: Institute of Cancer Research; IL-17: interleukin-17; IL-10: interleukin 10; IFN-γ: interferon γ; MCP: monocytes chemoattractant protein-1; MPO: myeloperoxidase; NE: neutrophil elastase; NETs: neutrophil extracellular traps; NK: natural killer; OUT: operational taxonomic unit; PBS: phosphate-buffered saline.

and BV, but an American nationwide survey showed an association of BV with periodontitis. In women with BV, periodontitis was associated with higher inflammation than in women without BV. Models fully adjusted for age, smoking, body mass index, diabetes mellitus, and number of systemic conditions strengthened this association (91). In men, the microbiota of the penis has been studied mostly in connection with circumcision, HIV risk and female partner BV (92). It would be of interest to study vaginal and penile microbiota composition in relation to BD inflammation and the impact of circumcision on the frequency of penile aphthous ulceration. In the same line, human hair follicles carry complex microbial communities that differ from the skin microbiota. This likely reflects the moist, less acidic, and relatively ultraviolet light-protected environment of the hair follicle epithelium, part of which is immune-privileged, thus facilitating microbial survival (93). In hidradenitis suppurativa, a disease in which non-infectious genital ulcers are frequent like in BD (94), bacterial over-colonisation and specific bacterial changes have been described (93). It would therefore be useful to study the hair follicle microbiome in BD.

Finally, early manifestations of BD and how to treat them remain open essential questions to improve patients' quality of life. In particular, the link between recurrent aphthous stomatitis (RAS) and possibly subsequent onset of BD has been recently debated. RAS shares clinical features with BD and responds to the same treatments. Conflicting results according to the geographic origin of patients have been reported: RAS patients from Middle East and Asia rarely develop BD compared to those from Western Europe and North America (95). This strongly suggests that environmental factors may play a significant role in BD clinical evolution, and those factors associated to western lifestyle could be the same as those recognised for their detrimental impact on microbiota. Thus, future studies considering those factors and enrolling not only BD but also RAS patients would be of high interest to better understand BD clinical evolution and characterise a "pre-BD" clinical state, as in rheumatoid arthritis.

Conclusion

In summary, microbiome alteration may provide a receptive setting for the onset of BD through a variety of immunomodulatory mechanisms (Fig. 2). Future biobanking will enable the study of gut, oral, genital, and hair follicle microbiota in BD patients versus healthy controls in larger studies. Sequencing of the whole 16S rRNA gene will be required for *Streptococci*

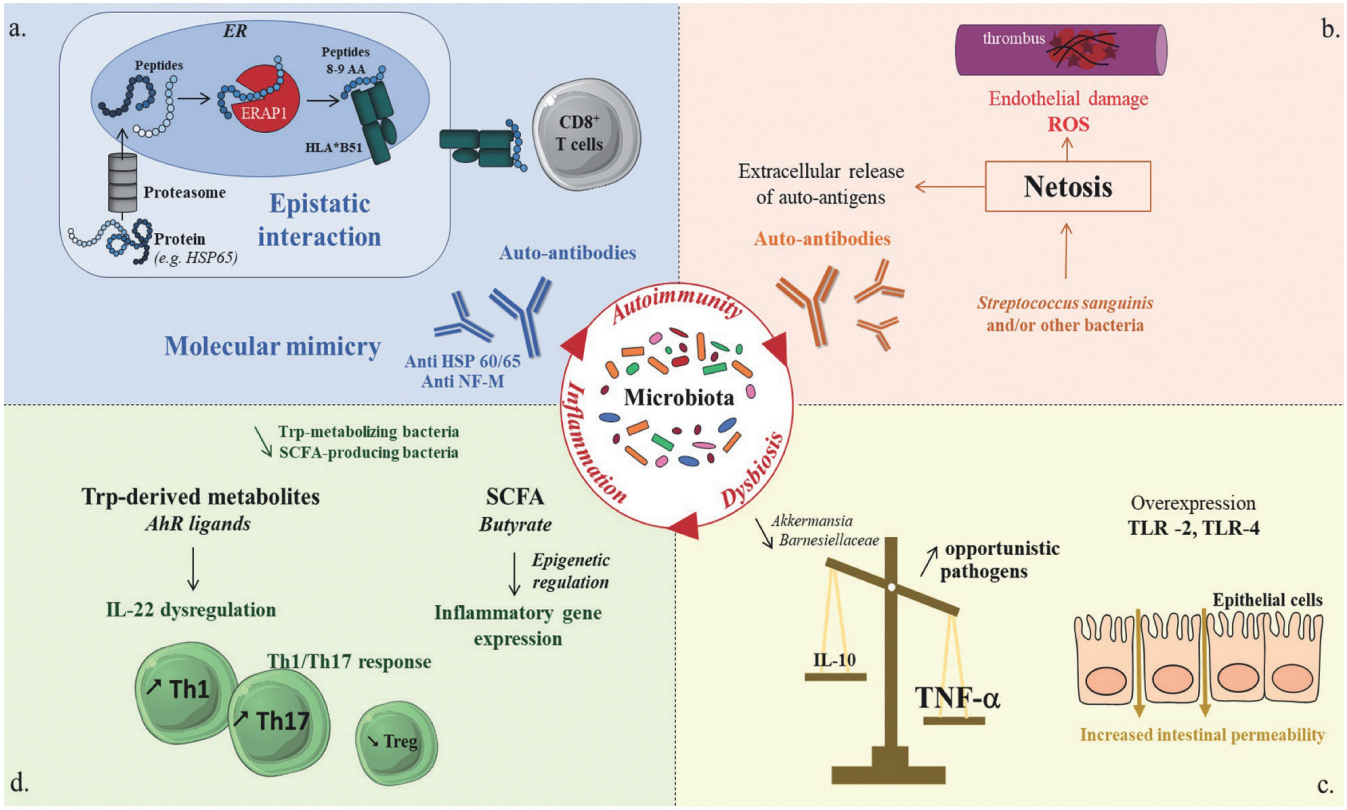

Fig. 2. Possible mechanisms of microbiota involvement in the pathogenesis of Behçet's disease.
a. Molecular mimicry between bacterial and human HSP and epistatic interaction between HLA-B*51 and ERAP1 could alter antigenic presentation to T cells.
b. Streptococcus sanguinis and other microbiota members activate NETosis, generating reactive oxygen species (ROS). NETosis exposes auto-antigens and favours auto-antibody responses against intracellular components.
c. Decreased Akkermansia and Barnesiellaceae abundances lead to decreased IL-10 and TNF-α, respectively. Enrichment in opportunistic pathogens leads to overexpression of TLR-4/TLR-2 due to intestinal epithelial barrier damage that facilitates entry of MAMPs into intestinal epithelial cells.
d. Decreased Bacteroides and Clostridia abundances in BD patients lead to decrease in indole metabolites, some of which acting as agonists for aryl hydrocarbon receptor (AhR) transcription factor. AhR exerts anti-inflammatory effects in intestinal mucosa through the regulation of IL-22. IL-22 influences T-cell responses and cytokine secretion. Decrease in SCFA-producing bacteria (particularly butyrate) in gut microbiota contributes to imbalance between Treg and Th1/Th17 and favours pro-inflammatory genes expression through epigenic modifications.
Ab: antibody; Ag: antigen; AhR: aryl hydrocarbon receptor; DNA: deoxyribonucleic acid; HLA: human leukocyte antigen; HSP: heat shock protein; IL-1: interleukin 1; IL-10: interleukin 10; IL-22: interleukin 22; MAMPs: microbe-associated molecular patterns; NF-M: median neurofilament; ROS: reactive oxygen species; SCFA: short-chain fatty acid; TLR-2: toll-like receptor 2; TLR-4: toll-like receptor 4; TNF-α: tumour necrosis factor α.

characterisation. Fungi, viruses, and archaea will also need to be studied. It will then be necessary to continue transplantation of patients' microbiota in animal models and/or inoculation of potential pathogens (*e.g. Streptococcus sanguinis* but also other bacteria) to better characterise the impact of BD-associated dysbiosis and confirm the involvement of specific microorganisms. Microbiota composition may change without necessarily affecting its functional continuity that is why we propose that microbiota-derived metabolites should also be fully characterised. The relationship between abnormalities of microbiota, epigenetic regulations, and BD pathogenesis may help gain a fuller understanding of susceptibility to this disease. Deeper insight into how host microbiota influence BD occurrence and disease course will pave the way for microbiota-targeting therapies in BD that could usefully add to existing treatments.

Take home messages

- BD-associated ulcers suggest a dysfunction of the host mucosa/microbiome interface.
- Gut dysbiosis and decreased abundance of SCFA-producing bacteria are reported in BD.
- Dysregulation of microbiota-derived metabolites could impact mucosal homeostasis.
- Molecular mimicry and NETosis could be implicated in BD aetiology.

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