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The immune response to *Prevotella* bacteria in chronic inflammatory disease

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Summary

The microbiota plays a central role in human health and disease by shaping immune development, immune responses and metabolism, and by protecting from invading pathogens. Technical advances that allow comprehensive characterization of microbial communities by genetic sequencing have sparked the hunt for disease-modulating bacteria. Emerging studies in humans have linked the increased abundance of *Prevotella* species at mucosal sites to localized and systemic disease, including periodontitis, bacterial vaginosis, rheumatoid arthritis, metabolic disorders and low-grade systemic inflammation. Intriguingly, *Prevotella* abundance is reduced within the lung microbiota of patients with asthma and chronic obstructive pulmonary disease. Increased *Prevotella* abundance is associated with augmented T helper type 17 (Th17) -mediated mucosal inflammation, which is in line with the marked capacity of *Prevotella* in driving Th17 immune responses *in vitro*. Studies indicate that *Prevotella* predominantly activate Toll-like receptor 2, leading to production of Th17-polarizing cytokines by antigen-presenting cells, including interleukin-23 (IL-23) and IL-1. Furthermore, *Prevotella* stimulate epithelial cells to produce IL-8, IL-6 and CCL20, which can promote mucosal Th17 immune responses and neutrophil recruitment. *Prevotella*-mediated mucosal inflammation leads to systemic dissemination of inflammatory mediators, bacteria and bacterial products, which in turn may affect systemic disease outcomes. Studies in mice support a causal role of *Prevotella* as colonization experiments promote clinical and inflammatory features of human disease. When compared with strict commensal bacteria, *Prevotella* exhibit increased inflammatory properties, as demonstrated by augmented release of inflammatory mediators from immune cells and various stromal cells. These findings indicate that some *Prevotella* strains may be clinically important pathobionts that can participate in human disease by promoting chronic inflammation.

Keywords: cytokines; inflammation; inflammatory disease; mucosa; *Prevotella*; T cells.

Abbreviations: APC, antigen-presenting cell; BV, bacterial vaginosis; CCL, C-C motif chemokine; CCR, chemokine receptor; CII, type II collagen; COPD, chronic obstructive pulmonary disease; CRA, chronic RA; IFN, Interferon; IL, interleukin; LPS, lipopolysaccharide; MIP-1α, macrophage inflammatory protein-1α; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NORA, new-onset RA; RA, rheumatoid arthritis; Th, helper T cell; TLR, Toll-like receptor; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin

Introduction

Studies in germ-free mice and the use of microbial reconstitution have underlined the importance of the commensal microbiota in shaping immune development and function, and hence the risk of inflammatory disease.[1] The advances in next-generation-sequencing have allowed in-depth characterization of non-culturable bacterial communities.[2] This has sparked significant interest in deciphering the health- and disease-related microbiotas to identify bacteria and mechanisms that could play a part in disease aetiology and progression. Characterization of the healthy human microbiota has revealed distinct bacterial communities at different body sites (gastrointestinal, urogenital, skin, lung, oral and nasal) supporting the notion of microbial communities adapting to different ecological environments in the body.[3] Interestingly, bacterial *Prevotella* species have been found to be prevalent commensal colonizers at mucosal sites; being the predominant genus in the respiratory system[4,5] and a central constituent in one of three gut bacterial enterotypes,[6] as well as present in saliva and several oral sites.[3]

Prevotella species are anaerobic Gram-negative bacteria of the *Bacteroidetes* phylum, which also includes the clinically important genera *Bacteroides* and *Porphyromonas*.[7] *Prevotella* strains are classically considered commensal bacteria due to their extensive presence in the healthy human body and their rare involvement in infections. Only a few strains have been reported to give rise to opportunistic endogenous infections, including chronic infections, abscesses and anaerobic pneumonia.[8–10] In light of the abundant *Prevotella* colonization and low pathogenicity it is likely that humans have co-evolved with *Prevotella*, giving rise to a mutualistic relationship. However, emerging studies have linked increased *Prevotella* abundance and specific strains to inflammatory disorders, suggesting that at least some strains exhibit pathobiontic properties. The present review addresses the interaction between *Prevotella* and the immune system, and how *Prevotella* may promote inflammatory disease. Many studies have addressed the link between bacteria and inflammatory diseases using various methods (e.g. culture techniques and quantitative PCR for specific stains, genera, or phyla); however, this review will mainly focus on recent studies employing genomics-based culture-independent methods of in-depth microbiota characterization (16S rRNA or metagenomic sequencing).

Periodontitis

The first link between *Prevotella* and chronic inflammatory disease was indicated as early as 1928 with the observation of black-pigmented Gram-negative anaerobes in periodontal disease.[11] Indeed, later studies confirmed the presence of *Prevotella* in biofilms of gingivitis and periodontitis.[12] It is well established that bacteria are a central driver of these diseases characterized by neutrophil recruitment, pro-inflammatory cytokines and metalloproteinase expression mediating destruction of connective tissues and alveolar bone.[13] Most mechanistic research has focused on the role of *Porphyromonas gingivalis* (a Gram-negative anaerobic member of the *Bacteroidetes* phyla, similarly to *Prevotella*), because this species was thought to be the main driver of disease. However, metagenomic studies have revealed a more diverse dysbiotic bacterial community that collectively may shape disease progression.[14] A recent study in mice has shown that *Prevotella nigrescens*, similarly to *P. gingivalis*, can drive periodontal disease – as demonstrated by maxillary alveolar bone loss following oral inoculation.[15] It was found that infection promoted immune responses characterized by increased T helper type 17 (Th17) [i.e. interleukin (IL-17)], suppressed Th2 (IL-4, IL-5 and IL-9), and similar Th1 [interferon-γ (IFN-γ)] cytokine production by lymph node T cells compared with uninfected mice. *Prevotella nigrescens* was found to drive Th17 responses *in vitro* through the production of IL-1 by bone-marrow-derived dendritic cells in a Toll-like receptor 2 (TLR2) -dependent manner (Fig. 1). The role of *Prevotella* in driving Th17-mediated immune responses in periodontitis is supported by studies linking IL-1α and IL-1β levels in crevicular fluid to *Prevotella* colonization.[16]

Only a few studies have compared the immunological properties of *Prevotella* to innocuous oral commensal bacteria, like *Streptococcus* and *Lactobacillus* species.[17] *In vitro* studies using human monocyte-derived dendritic cells,[18] odontoblast-like cell clones[19] and a gingival epithelial cell line[20] suggest that *Prevotella* exhibit an enhanced capacity to induce inflammatory mediators [IL-6, IL-8 and tumour necrosis factor-α (TNF-α)] when compared with strict commensal oral bacteria and even *P. gingivalis*. Interestingly, a murine study using a subcutaneous chamber model found that oral commensal *Streptococcus mitis* infection could readily be cleared, whereas infection with *Prevotella intermedia* was uncontrolled for more than 7 days.[21] The *Prevotella intermedia* infection was found to induce increased host cell infiltration compared with *S. mitis*; however the infiltrating neutrophils were defective in terms of phagocytosis and reactive oxygen species production, and exhibited a necrotic morphology. Interestingly, neutrophil dysfunction is a prominent feature of periodontal disease.[22] Combined, the studies suggest that *Prevotella* can promote periodontitis by driving neutrophil recruitment via Th17 immune responses. Chronic activation of the Th17 pathway may mediate tissue and bone destruction because recruited neutrophils are unable to clear the bacteria and promote resolution of tissue inflammation.

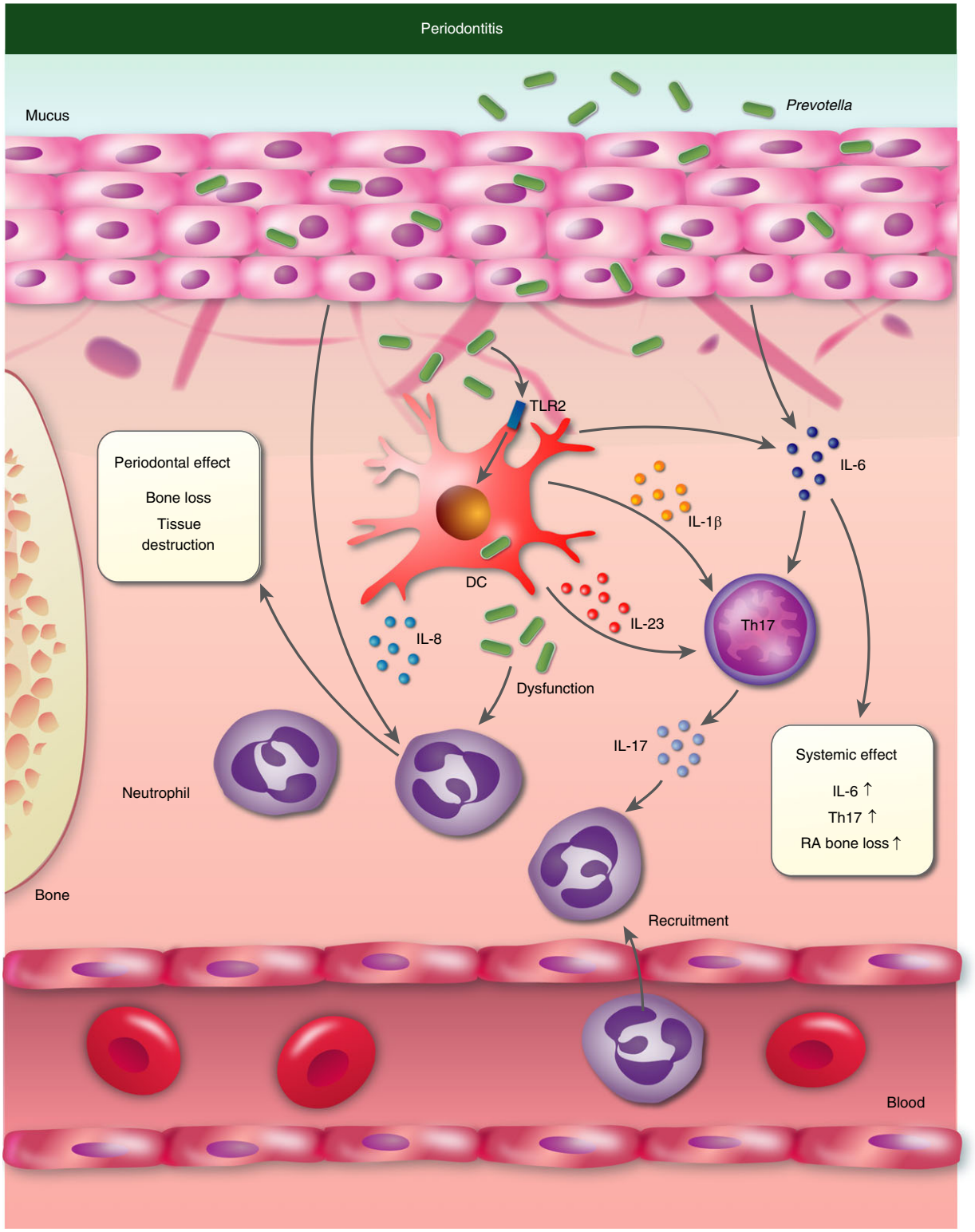

Figure 1. *Prevotella*-mediated inflammation in periodontitis. *Prevotella* stimulate the release of interleukin-1β (IL-1β), IL-6 and IL-23 by dendritic cells (DC) through Toll-like receptor 2 (TLR2), which in turn mediates IL-17 production by T helper 17 (Th17) cells that activate neutrophils. Epithelial cells contribute to neutrophil recruitment and Th17 cell activation through the production of IL-8 and IL-6, respectively. *Prevotella* directly induce dysfunction in recruited neutrophils. Chronic inflammation, characterized Th17 immune responses and recruitment of neutrophils, leads to localized bone loss and tissue destruction characteristic of periodontitis. Local inflammation disseminates and affects systemic disease, including bone loss in rheumatoid arthritis (RA).

Rheumatoid arthritis

Epidemiological studies have linked periodontal disease to increased risk of systemic diseases, including rheumatoid arthritis (RA).[14] It has been speculated that periodontal pathogens drive systemic inflammation or disseminate to affected tissue, thereby promoting localized inflammation. Indeed, increased specific IgG to periodontal pathogens, including *Prevotella intermedia* and *P. gingivalis*, has been reported in RA.[23–27] Furthermore, the presence of DNA from periodontal pathogens has been found in serum and synovial fluid of patients with RA, indicating systemic dissemination of bacteria that directly promote localized synovial inflammation.[28–30] However, the effect-size and strength of association vary among bacterial species in these studies, suggesting a more complicated relationship that may depend on individual study design and clinical characteristics of the patient cohorts. Interestingly, a study employing 16S rRNA sequencing of the subgingival microbiota found that the presence of *P. gingivalis* correlated with periodontal disease but not RA, whereas *Prevotella* was associated with new-onset RA (NORA) but not chronic RA (CRA) independent of periodontal disease.[31] In mice, *Prevotella nigrescens* was found to induce periodontitis after oral inoculation.[15] The infected mice exhibited accelerated onset and severity of experimental arthritis compared with control mice when immunized with type II collagen (CII). Importantly, systemic administration of 100-fold heat-killed bacteria was unable to emulate this effect, suggesting that establishment of chronic oral infection and periodontitis by *Prevotella* is needed to promote RA. It was found that mice with *Prevotella*-mediated periodontitis promoted IL-17 but not IFN-γ production by CII-specific T cells. The enhanced IL-17 production by CII-specific T cells was found to correlate with arthritic bone erosion, indicating a central role of Th17 responses in promoting RA pathology. Collectively, the present studies suggest that *Prevotella*-mediated periodontitis can affect the progression of RA by modulating systemic immune responses; however, the relevance of oral *Prevotella* to human disease and specific patient groups requires further research.

Dysbiosis in the gut has been linked to RA, and suggested to be a risk factor responsible for the rise in disease incidence. Two recent studies performing 16S rRNA sequencing of faecal samples found dysbiosis associated with *Prevotella* strains closely related to *Prevotella copri* in patients with NORA.[32,33] Faecal matter from patients with RA and healthy controls was used to colonize the gut of arthritis-prone SKG mice.[33] The microbiota from patients with RA was found to induce increased numbers of intestinal IL-17$^+$ Th17 cells, but similar numbers of IFN-γ^+ Th1 and FoxP3$^+$ regulatory T cells compared with healthy microbiota. After triggering disease by zymosan administration, SKG mice colonized by the RA microbiota developed severe arthritis characterized by increased disease and histology scores, and increased serum rheumatoid factor levels. It was demonstrated that the severe arthritis was associated with increased IL-17 (Th17) and unchanged IFN-γ (Th1) production in response to the arthritis-related autoantigen RPL23A. Similarly, oral administration of *Prevotella melanogenica* in humanized HLA-DQ8 mice immunized with CII augmented RA onset and severity, which was associated with increased gut inflammation as demonstrated by shortening of intestinal villi and leucocyte infiltration.[34] Interestingly, the same study reported suppression of experimental RA by *Prevotella histicola* through the expansion of regulatory T cells in the gut and suppression of systemic CII-specific immune responses. These findings suggest that specific members of the *Prevotella* genus have different disease modulating properties, and highlight the importance of in-depth characterization of the microbiota in the study of inflammatory disease.

In vitro studies comparing *Prevotella copri* to the gut commensal bacteria *Bacteroides fragilis*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Escherichia coli* using bone-marrow-derived dendritic cells found that *Prevotella copri* was superior in inducing the Th17 driving cytokines IL-6 and IL-23.[33] Hence, *Prevotella copri*-stimulated bone-marrow-derived dendritic cells were able to prime naive Th cells to produce up to fivefold increased IL-17 levels compared with the commensal bacteria. These findings suggest that *Prevotella copri* exhibits intrinsic Th17 promoting capability, which when present in the gut microbiota can promote RA.

A recent study has shed light on the possible immune modulatory role of *Prevotella copri* in human RA. Of RA patients (including NORA and CRA), 32% were found to have serum IgA or IgG antibodies specific for *Prevotella copri*, which was almost absent in healthy controls and patients with other arthritic diseases.[35] In comparison, IgA or IgG antibodies specific for *P. gingivalis* were present at similar frequencies and levels in all patient groups and healthy controls, whereas antibodies to the gut commensals *Bacteroides fragilis* and *Escherichia coli* were largely absent. These findings indicate that the immune responses to *Prevotella copri* exclusively develop in RA patients and may contribute to disease initiation or progression in some patients. Interestingly, *Prevotella copri*-specific IgA but not IgG levels were associated with systemic levels of innate [macrophage inflammatory protein 1α (MIP-1α) and MIP-1β], Th1 (IFN-γ and IL-12), and Th17 (IL-23, IL-22, IL-17A, IL-17E and IL-17F) cytokines.[35] Patients with RA had either IgA, IgG or no specific antibodies, indicating that different immune responses to *Prevotella copri* can develop within the individual patient, which in turn may have implications for disease risk and outcomes.

Bacterial vaginosis

Bacterial vaginosis (BV) is associated with poor health outcomes for women, including pre-term birth, and increased risk of acquiring HIV or other infections. The disease is characterized by the loss of a commensal *Lactobacillus*-rich microbiota and the blooming of anaerobic bacteria in the vaginal tract. Although earlier reports have implicated *Prevotella bivia* in BV,[36] a couple of very recent studies found that *Prevotella* abundance increased with severity of BV, which inversely correlated with the presence of *Lactobacillus*.[37–39] *Prevotella* in the vaginal microbiota was associated with increased innate (IL-1α, IL-1β, IL-8, and TNF-α) cytokines, and production of Th17 (IL-23 and IL-17) and Th1 (IL-12p70 and IFN-γ) related cytokines in cervicovaginal fluid.[37,38] These findings are in line with a previous study linking increased IL-1β and IL-8 levels in cervicovaginal fluid to *Prevotella bivia* colonization.[40] Increased numbers of activated $CCR5^+$ $HLA\text{-}DR^+$ $CD38^+$ Th cells were associated with the presence of *Prevotella* in the vaginal mucosa.[38] No apparent change in mucosal antigen-presenting cell (APC) numbers ($CD11c^+$ dendritic cells or $CD14^+$ monocytes/macrophages) was observed in the same study; however, APCs from *Lactobacillus*-rich compared with *Prevotella*-rich vaginal mucosas exhibited distinct transcriptional profiles.[37] *Prevotella*-rich mucosa APCs showed a profile similar to APCs activated by lipopolysaccharide (LPS), and expressed cytokine genes known to promote Th17 immune responses (IL23A, IL6, IL1A and IL1B). These findings indicate that *Prevotella* in the vaginal tract contributes to activation of a Th17 immune response via APCs, leading to the recruitment and activation of Th cells in the inflamed vaginal mucosa (Fig. 2). Important in relation to women's health, the recruitment of $CCR5^+$ Th cells into the vaginal mucosa may be a central underlying risk factor for increased HIV transmission in BV.[38]

Cervicovaginal epithelial cultures stimulated with *Prevotella* (*Prevotella bivia* or *Prevotella amnii*) compared with vaginal commensal *Lactobacillus* (*Lactobacillus crispatus*, *Lactobacillus iners* and/or *Lactobacillus acidophilus*) were reported to induce higher levels of IL-1α, IL-1β, IL-6, IL-8, CCL20, CXCL2, CXCL3 and CCL5.[37,38,41–43] This cytokine/chemokine profile suggests that *Prevotella* has a high intrinsic capacity to promote vaginal Th17-mediated immune responses and neutrophil recruitment through epithelial cells. The enhanced inflammatory property of *Prevotella* is supported by studies in mice, which found increased numbers of activated $CD44^+$ and $CCR5^+$ Th cells in the vaginal tract of germ-free mice following inoculation with *Prevotella bivia* compared with *Lactobacillus crispatus*.[38]

One of the recent studies linking *Prevotella* to BV[39] addressed the influence of host genetics on the vaginal microbiota in a twin-family cohort. It was reported that microbiota composition and *Prevotella* abundance were largely determined by host genetics, but influenced by environmental factors, including menopause, hormone therapy, human papillomavirus infection and obesity. Gene candidate analysis found that the minor allele group with polymorphism in the IL-5 gene had increased abundance of *Prevotella melaninogenica*. To address a potential causal effect of obesity in driving vaginal dysbiosis, the microbiota of obese mice on a high-fat diet was compared with that of lean mice on a control diet.[39] Obesity in mice was found to induce vaginal dysbiosis linked to increased abundance of *Prevotella*, and vaginal transfer of the dysbiotic microbiota to lean mice increased plasma LPS levels in recipient mice. These findings underline that complex gene–environment interactions shape the risk of acquiring a *Prevotella*-rich vaginal microbiota. However, once acquired, *Prevotella* may drive chronic inflammation associated with BV that in turn has systemic effects on other diseases.

Gut dysbiosis triggered by HIV

Persistent chronic inflammation is a central hallmark of HIV infection even after successful antiviral therapy. Low-grade systemic inflammation characterized by activated T cells, inflammatory cytokines, endotoxaemia and gut bacteria translocation has been linked to poor disease outcomes and increased mortality.[44,45] Studies have demonstrated that HIV infection is associated with intestinal dysbiosis characterized by increased *Prevotella* and reduction in *Bacteroides*.[46–49] Recent studies suggest that increased *Prevotella* in HIV is a driver for persistent inflammation in the gut leading to mucosal dysfunction and systemic inflammation.[50–52] Colon biopsies from untreated HIV-infected individuals showed increased *Prevotella* colonization, and *Prevotella* abundance was specifically associated with the elevated numbers of activated $HLA\text{-}DR^+$ $CD38^+$ Th and Tc cells, as well as higher CD40 expression on $HLA\text{-}DR^+CD1c^{high}$ myeloid dendritic cells in colon mucosa.[50] A later study from the same group reported that colon dysbiosis (linked to *Prevotella copri* and *Prevotella stercorea*) in HIV-infected individuals was associated with elevated CD40 expression on a $CD1c^+$ subset of $HLA\text{-}DR^+$ $CD11c^{high}$ myeloid dendritic cells, which in turn correlated with systemic levels of activated $HLA\text{-}DR^+$ $CD38^+$ Th and Tc cells.[52] Furthermore, CD40 expression levels on $CD1c^+$ myeloid dendritic cells correlated with Th17 (IL-1β, IL-5, IL-23 and IL-17), Th1 (IFN-γ) and innate (TNF-α and IL-10) cytokines in colonic tissue. Another study confirmed the link between *Prevotella*-rich gut dysbiosis in HIV and increased systemic levels of activated T cells.[51] The study additionally reported correlations between dysbiosis and increased systemic high-sensitivity C-reactive protein and LPS levels. However, no specific correlations to gut *Prevotella*

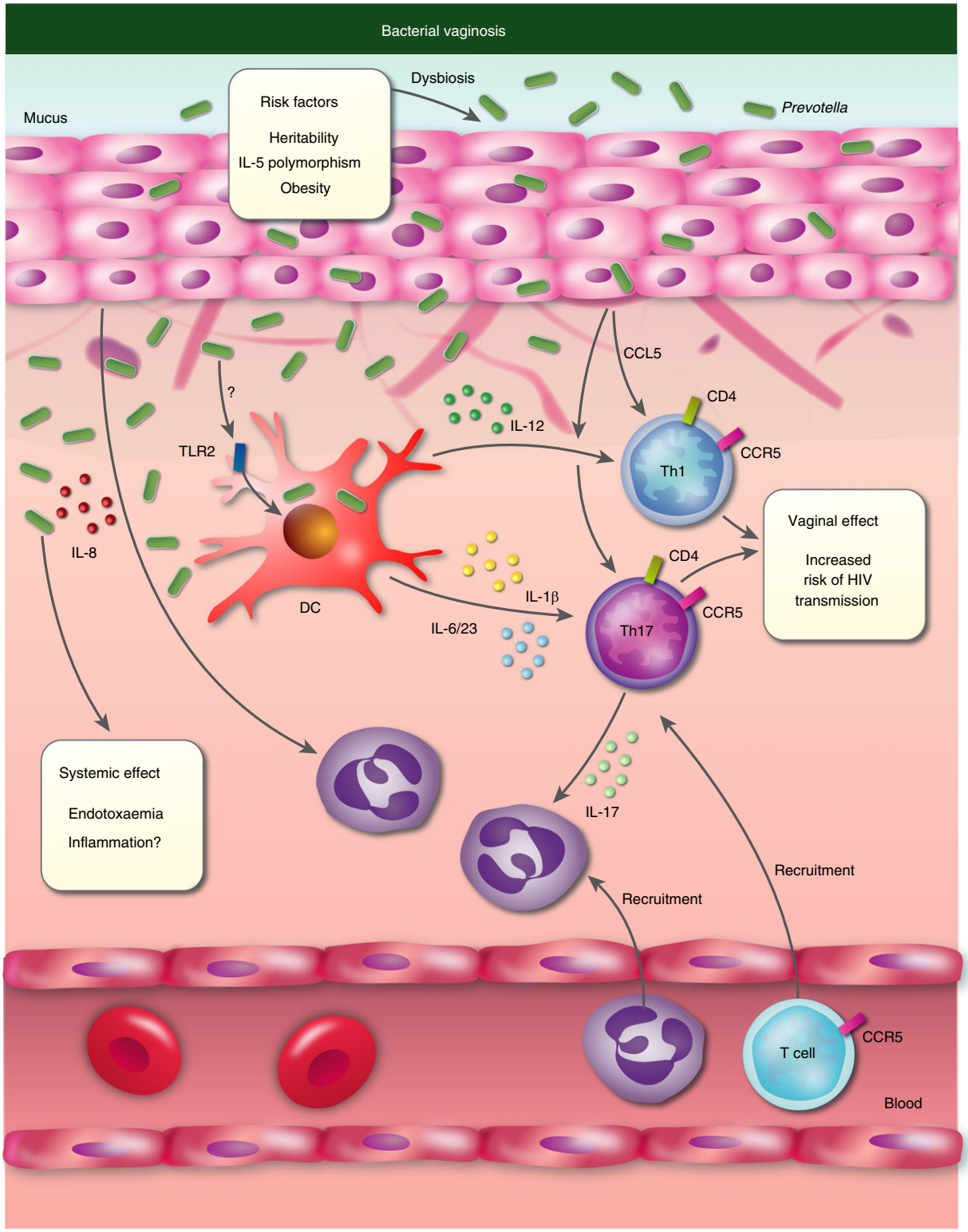

Figure 2. *Prevotella*-mediated inflammation in bacterial vaginosis. *Prevotella* stimulate release of interleukin-1β (IL-1β), IL-6 and IL-23 by dendritic cells (DC), which in turn mediates IL-17 production by T helper 17 (Th17) cells that activate neutrophils. DCs also produce IL-12, which mediates the activation of Th1 cells. Epithelial cells contribute to neutrophil and Th cell recruitment through the production of IL-8 and CCL5, respectively. Genetic background and obesity predispose to *Prevotella*-rich dysbiosis in bacterial vaginosis. Dysbiosis leads to systemic release of lipopolysaccharides (endotoxaemia), and possibly systemic inflammation. Increased numbers of mucosal CCR5-positive Th cells are associated with increased risk of HIV transmission.

abundance were investigated. Combined, these studies indicate that HIV-induced *Prevotella* accumulation leads to dysfunctional immune responses in the gut mucosa driving bacterial translocation, endotoxaemia and systemic inflammation (Fig. 3). A recent interventional study administering prebiotics to HIV patients found that short dietary supplementation attenuated gut dysbiosis and systemic inflammation.[53] However, longitudinal studies in humans and experimental work in mice are needed to delineate a causal relationship between HIV-induced *Prevotella*-rich dysbiosis, inflammation and adverse disease outcomes. Studies addressing *Prevotella* and gut dysbiosis independent of HIV are reviewed below.

Gut dysbiosis and metabolic syndrome

Metabolic syndrome is a collection of risk factors (obesity, insulin resistance, high blood pressure and increased blood cholesterol/triglycerides) predisposing to the development of diabetes, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD). These diseases are highly interrelated, and a common feature is low-grade systemic inflammation. Gut dysbiosis is associated with disease, but reports on the involvement of *Prevotella* have been inconsistent. Increased abundance of *Prevotella* was found to be associated with insulin-resistance in a non-diabetic cohort,[54] in a cohort of morbidly obese patients,[55] and was linked to obesity,[56,57] hypertension,[58] and NAFLD[56,59] in case–control studies. Yet other studies found no such association in type 2 diabetes,[60–62] obesity,[59,63] and ischaemic stroke patients,[64] although a comprehensive study found an association with increased *Paraprevotella* (member of the Prevotellaceae family) in type 2 diabetes.[60] These discrepancies may be due to the complex interrelatedness of the diseases, making patient selection and stratification important study parameters. Furthermore, different bacterial species could be involved in expression of the same disease features. Indeed, increased abundance of both *Prevotella copri* and *Bacteroides vulgatus* was associated with insulin-resistance, but the species were mutually exclusive in the gut.[54] Adding further to the complexity, a study showed that individuals with improved glucose metabolism following a high-fibre dietary intervention had a *Prevotella*-rich gut microbiota,[65] suggesting interaction of diet on the outcome of specific disease features.

Studies in mice indicate that *Prevotella* can drive features of metabolic syndrome. Colonization of germ-free mice with a *Prevotella*-rich microbiota from patients with hypertension induced higher blood pressure compared with mice receiving microbiota from a normotensive donor.[58] *Prevotella copri* colonization in mice on a high-fat diet promoted increased insulin-resistance.[54] Furthermore, the role of *Prevotella*-rich dysbiosis in NAFLD and obesity was studied using the transfer of dysbiotic microbiota enriched with *Prevotella*, unknown *Prevotellaceae* and TM7 from mice with a deficient inflammasome pathway (Asc knockout or IL-18 knockout) to wild-type mice by co-housing.[66] The presence of a *Prevotella*-rich gut microbiota exacerbated methionine-choline-deficient diet-induced non-alcoholic steatohepatitis (NASH) characterized by increased liver steatosis and inflammation, and elevated liver-enzymes alanine aminotransferase and aspartate aminotransferase in blood. The study found that *Prevotella*-rich dysbiosis was associated with the presence of black-pigmented bacteria in colonic epithelial cells and macrophages. Furthermore, the study indicated that NASH disease propagation was driven by epithelium-derived CCL5-dependent intestinal inflammation giving rise to systemic release of bacterial TLR4 and TLR9 agonists promoting TNF-α-dependent inflammation and pathology in the liver. Additionally, transfer of *Prevotella*-rich microbiota from Asc knockout mice caused increased weight-gain in both wild-type mice on high-fat diet, and obesity-prone *ob*/*ob* mice.[66] However, the transfer did not affect insulin resistance, underlining a heterogenic effect of the microbiota in metabolic disease phenotypes. Although these findings are compelling, additional investigations of immune mechanisms in metabolic disease are needed in humans.

Gut dysbiosis and inflammatory bowel disease

An interesting line of research has demonstrated a central role of the Nucleotide-binding and oligomerization domain-Like Receptor P6 (NLRP6)-inflammasome in maintaining gut homeostasis and protection from *Prevotella*-rich dysbiosis, which can promote experimental colitis in mice. NLRP6-deficiency was found to cause goblet cell dysfunction and reduced mucus secretion on intestinal surfaces leading to increased susceptibility to *Citrobacter rodentium* infection.[67] The NLRP6-inflammasome in intestinal epithelial cells was found to sense microbiota-derived metabolites leading to IL-18-dependent production of antimicrobial peptides, which in turn shapes microbiota composition under homeostatic conditions.[68] Homeostatic epithelial cell-derived IL-18 has previously been shown to mediate $Foxp3^+$ regulatory T cell function, and directly suppress pathogenic Th17 cell function in the intestine.[69] This finding provides a possible model for immunological maintenance of gut homeostasis, where another layer of microbial protection involving regulatory T cell-stimulated IgA production could further shape the gut microbiota.[70,71] Indeed, gut bacteria known to promote colitis have been shown to be a target of T-cell-dependent IgA,[72] although a protective role of *Prevotella*-specific IgA remains to be formally demonstrated.

Transfer of *Prevotella*-rich dysbiotic gut microbiota from Asc knockout or NLRP6 knockout mice to wild-type mice was found to promote dextran sulphate

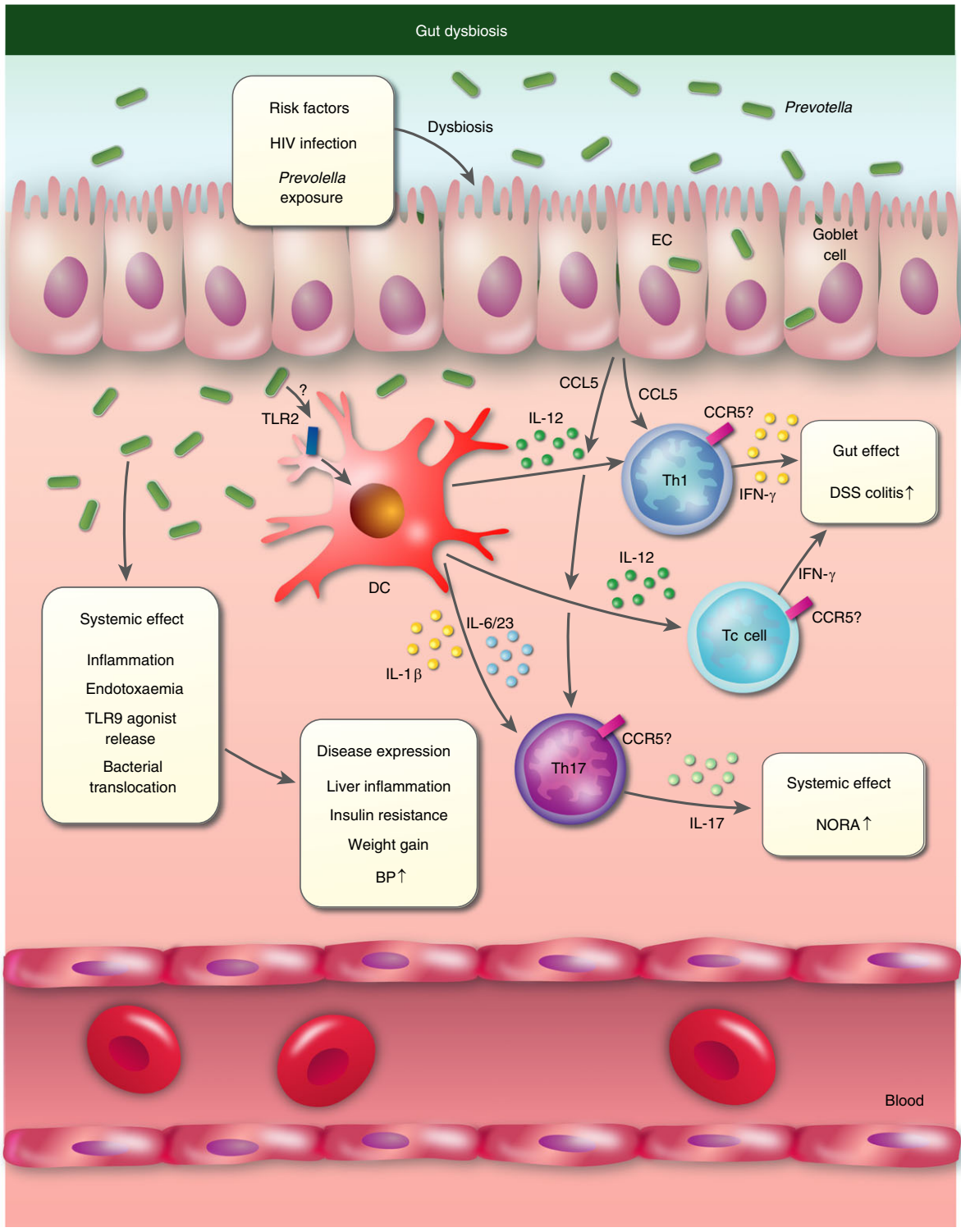

Figure 3. Gut inflammation associated *Prevotella*-rich dysbiosis. *Prevotella* stimulates release of interleukin-1β (IL-1β), IL-6 and IL-23 by dendritic cells (DC), which in turn mediate IL-17 production by T helper 17 (Th17) cells that activate neutrophils. DCs also produce IL-12, which mediates the activation of Th1 and cytotoxic T (Tc) cells. Epithelial cells may contribute to recruitment of CCR5-positive T cells through the production of CCL5. HIV infection and exposure to *Prevotella* are risk factors for *Prevotella*-rich dysbiosis in the gut. Dysbiosis leads to systemic release of inflammation, bacteria, lipopolysaccharides (endotoxaemia) and Toll-like receptor 9 (TLR9) agonists, which in turn mediates systemic disease expression, including liver inflammation, insulin resistance, weight gain and increased blood pressure (BP). Dysbiosis-associated increase in Th17 immune responses may affect new-onset rheumatoid arthritis (NORA). Dysbiosis increases Th1-mediated inflammation in dextran sulphate sodium (DSS)-induced experimental colitis.

sodium-induced experimental colitis characterized by increased weight loss, tissue pathology and death in recipient mice. Increased susceptibility to experimental colitis was dependent on CCL5, and increased CCL5 levels were associated with intestinal recruitment of conventional T cells, B cells and APCs in NLRP6 knockout mice.[73] Furthermore, the study indicated that *Prevotella*-rich dysbiosis drive decreased NLRP6-dependent IL-18 production in intestinal epithelial cells. Interestingly, this was supported by a later study demonstrating that transfer of *Prevotella*-rich dysbiotic microbiota would subvert IL-18-dependent antimicrobial peptide production through the production of metabolites antagonizing NLRP6 function, which in turn promoted establishment of the dysbiotic microbial community in the gut.[68] Colonization of antibiotic-treated C57BL/6 mice with *Prevotella copri* enhanced dextran sulphate sodium-induced colitis compared with control mice or mice colonized by commensal *Bacteroides thetaiotamicron*.[32] The enhanced colitis was associated with increased IFN-γ production by lamina propria Th cells from *Prevotella copri* colonized mice, suggesting that *Prevotella* promote Th1 immune responses in experimental colitis.

Although a role for *Prevotella* in inflammatory bowel disease is compelling from studies of experimental colitis in mice, currently no studies have provided an association between increased *Prevotella* abundance and disease in humans. In fact, a study indicated reduced *Prevotella* in paediatric Crohn's disease.[74] Furthermore, the most comprehensive study to date[75] found no association between *Prevotella* and new-onset Crohn's before treatment.[76] Rather, Crohn's disease was associated with outgrowth of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae* and *Fusobacteriaceae*, which is in line with earlier microbiome studies of Crohn's disease and ulcerative colitis.[77,78] The mechanisms by which *Prevotella* promote disease in mice (subversion of gut homeostasis and initiation of intestinal inflammation) may be shared with other bacterial species linked to human disease. Furthermore, human inflammatory bowel disease is highly heterogeneous and specific bacteria may be involved in different disease phenotypes and immune mechanisms,[79] suggesting a need for larger prospective cohort studies to delineate causal relationships.

Asthma and COPD

The healthy lung has traditionally been viewed as sterile due to the absence of culturable bacteria in the absence of clinical respiratory infection. However, a study in 2010[4] reported a low-density, but distinct microbial community dominated by *Prevotella* in the lung. This finding has subsequently been confirmed by later studies controlling for potential sources of contamination.[5,80–82] Intriguingly, *Prevotella* abundance was reported to be reduced in patients with asthma and with COPD, which instead presented with outgrowth of pathogenic proteobacteria.[4] Lung colonization by proteobacteria has previously been linked to increased risk of developing asthma in childhood,[83] exacerbation episodes,[84] as well as increased neutrophilia and IL-8 levels in patients with asthma.[85] Similarly, patients with COPD present with predominant proteobacterial colonization during both stable disease and excerbations.[86–88] This has led to speculation that proteobacteria take part in disease development and progression in COPD.[89] This hypothesis is supported by studies associating increased bacteria loads to increased airway inflammation[90,91] and accelerated decline in lung function.[92]

A study compared the inflammatory properties of *Prevotella* associated with healthy lungs (*Prevotella melaninogenica*, *Prevotella nanceiensis* and *Prevotella salivae*) with proteobacteria associated with asthma and COPD (*Haemophilus influenzae* B, non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis*).[93] *Prevotella* was found to induce similar levels of CD83, CD86 and CD40 activation-maker surface expression, but reduced production of IL-12p70, IL-23 and IL-10 cytokines in monocyte derived dendritic cells when compared with proteobacteria. This lower inflammatory capacity of *Prevotella* compared with proteobacteria was further demonstrated in mice reporting decreased MIP-2a (IL-8), TNF-α and thymic stromal lymphopoietin production by lung stromal cells, and decreased levels of TNF-α production by lung immune cells.[94] Titration experiments indicated that the lower stimulatory capacity of *Prevotella* was due to intrinsic differences in composition of pathogen-associated molecular patterns. It was hypothesized[93,94] that the difference could be ascribed to alternate LPS structures as *Prevotella* produce penta-acylated LPS whereas *Haemophilus influenzae* and *Moraxella catarrhalis* produce hexa-acylated and hepta-acylated LPS, respectively. Indeed, an analysis of the LPS synthesis pathway in publicly available genomes found that only gammaproteobacteria have the genetic capacity to produce hexa-acylated LPS (the prototypic LPS commonly isolated from *Escherichia coli*), which exhibit 100-fold stimulatory capacity on TLR4 compared with penta-acylated LPS.[95] Non-typeable *Haemophilus influenzae* was found to induce severe lung neutrophilia in mice accompanied by increased levels of MIP-2a (IL-8), CCL20 and IL-1β in lung tissue compared with *Prevotella nanceiensis*.[94] Furthermore, non-typeable *Haemophilus influenzae* induced severe immune pathology in lung tissue, whereas no pathology could be observed in response to *Prevotella nanceiensis* when compared with control mice. The diminished lung inflammatory capacity of *Prevotella nanceiensis* was dependent on TLR2, whereas the inflammation mediated by non-typeable *Haemophilus influenzae* was TLR2-independent. These findings support that *Prevotella* exhibit limited TLR4-stimulating capacity

as this genus cannot produce hexa-acylated LPS. Furthermore, proteobacteria may specifically participate in driving inflammatory features of asthma and COPD, whereas *Prevotella* in comparison may be well tolerated in the lung.

A possible homeostatic role for *Prevotella* in the healthy lung remains largely unknown. Induction of COPD-like lung inflammation and pathology in mice by LPS/elastase inhalation was found to decrease *Prevotella* abundance, and mediate *Pseudomonas* and *Lactobacillus* outgrowth.[96] This suggests that reduced *Prevotella* in human asthma and COPD[4] may not be a risk factor before disease, as disease-related inflammation may directly drive decreased *Prevotella* abundance by creating a microenvironment not suitable for survival. Studies suggest that the low-density lung microbiota is transmitted from the oral microbiota by microaspiration and continuously eliminated.[80,81] It could therefore be speculated that the limited immune stimulatory potential of *Prevotella* may drive its own elimination by mediating a low-grade inflammatory process, which in turn may protect from invading respiratory pathogens and chronic disease under homeostatic conditions.[80] Alternatively, a study found that early establishment of a *Bacteroidetes*-rich lung microbiota in neonatal mice drives expansion of regulatory T cells protecting from allergic airway disease in response to house-dust-mite.[97] Hence *Prevotella* (a member of the *Bacteroidetes* phyla) may participate in establishing tolerance in the lung, although a role for *Bacteroides* species with known immune regulatory properties[98] cannot be ruled out. Combined, these studies show that many speculations can be made as to the role of *Prevotella* in the healthy lung, and so there is a need for more experimental work for clarification.

Concluding remarks

Emerging studies are linking *Prevotella* abundance and specific strains to inflammatory disease mediated by Th17-related immune responses. Indeed, at least some *Prevotella* strains seem to be inflammophilic pathobionts that thrive in an inflammatory environment, and exhibit superior intrinsic capacity to stimulate Th17-mediated inflammation compared with strict commensal bacteria. There is compelling mechanistic and causal evidence in mice that *Prevotella* can promote inflammatory disease features. However, there is a need for more studies in humans to ascertain a causal and potential disease-triggering role for *Prevotella*. Inflammatory diseases are highly heterogeneous and develop through the complex interactions between host genetic risk factors and environmental exposures.[99] *Prevotella* may only play a part in certain disease endotypes, and larger cohort studies are needed to delineate causal relationships. Additionally, *Prevotella* may not be the only genus participating in inflammatory disease, and specific *Prevotella* species may exhibit different properties. A recent comprehensive study comparing several bacterial species suggests that membership of a specific phylum does not predict immunological properties, underlining the importance of characterizing properties at species level.[100] Furthermore, studies indicate that *Prevotella* is a genus with high genetic diversity within and between species.[101,102] This may explain why *Prevotella* is abundant in the healthy microbiota, and suggests that only certain strains may exhibit pathobiontic properties. Species heterogeneity also underlines the need to continue in-depth metagenomic characterization of the microbiota in inflammatory disease to reveal disease-modulating properties. Intriguingly, some *Prevotella* species could have evolved immune escape mechanisms, including induction of neutrophil dysfunction,[21], that may lead to chronic inflammation due to defective clearance. Deciphering the genetic and mechanistic basis of immune escape by *Prevotella* may in the future reveal disease-modifying drug targets.

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The author declares that there is no conflict of interest.

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