



# Microbiota, Immune Subversion, and Chronic Inflammation

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Several host-adapted pathogens and commensals have evolved mechanisms to evade the host innate immune system inducing a state of low-grade inflammation. Epidemiological studies have also documented the association of a subset of these microorganisms with chronic inflammatory disorders. In this review, we summarize recent studies demonstrating the role of the microbiota in chronic inflammatory diseases and discuss how specific microorganisms subvert or inhibit protective signaling normally induced by toll-like receptors (TLRs). We highlight our work on the oral pathogen *Porphyromonas gingivalis* and discuss the role of microbial modulation of lipid A structures in evasion of TLR4 signaling and resulting systemic immunopathology associated with atherosclerosis. *P. gingivalis* intrinsically expresses underacylated lipid A moieties and can modify the phosphorylation of lipid A, leading to altered TLR4 signaling. Using *P. gingivalis* mutant strains expressing distinct lipid A moieties, we demonstrated that expression of antagonist lipid A was associated with *P. gingivalis*-mediated systemic inflammation and immunopathology, whereas strains expressing agonist lipid A exhibited modest systemic inflammation. Likewise, mice deficient in TLR4 were more susceptible to vascular inflammation after oral infection with *P. gingivalis* wild-type strain compared to mice possessing functional TLR4. Collectively, our studies support a role for *P. gingivalis*-mediated dysregulation of innate and adaptive responses resulting in immunopathology and systemic inflammation. We propose that anti-TLR4 interventions must be designed with caution, given the balance between the protective and destructive roles of TLR signaling in response to microbiota and associated immunopathologies.

**Keywords:** microbiota, inflammation, toll-like receptors, innate immunity, immune subversion, immune dysregulation, atherosclerosis

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## INTRODUCTION

Many inflammatory conditions and immunological disorders have been recently linked to the microbiota (1). Studies in humans have documented that both shifts in the microbiota (dysbiosis) and specific microorganisms are associated with these immunological disorders (2–4). A number of epidemiological studies have reported phylogenetic differences in the presence and relative abundance of specific microbial communities between subjects with a particular disease and “healthy” individuals (5–7). While overall shifts in biodiversity within (alpha diversity) or among (beta diversity) subject samples are often reported, more recent work has considered functional diversity elucidated by metagenomic analyses (8, 9). The most well-studied microbial dysbiosis is that of the gut microbiota, which is associated with inflammatory bowel diseases (IBD) and colorectal cancer

(7, 9–11). Dysbiosis of the oral microbiota has been associated with oral squamous cell carcinoma (OSCC), and dysbiosis of the lung microbiota has been associated with cystic fibrosis (CF) (12–15). However, gut microbiota dysbiosis has also been associated with non-intestinal diseases including obesity, type 1 diabetes (T1D), rheumatoid arthritis (RA), and atherosclerosis (16, 17). Likewise, dysbiosis of the oral microbiota has been associated with diseases occurring outside of the oral cavity, such as lung and pancreatic cancers as well as atherosclerosis and RA (18–21).

In addition to dysbiosis, the presence of specific microorganisms has also been associated with cancer, atherosclerosis, autoimmune disorders, and RA (22–40). Indeed, much of the experimental evidence aimed at defining mechanistic links between the microbiota and systemic inflammatory conditions has focused on metabolic and immunological pathways induced by specific microorganisms. In this review, we summarize recent studies aimed at defining immunological mechanisms that link specific microorganisms to low-grade chronic inflammation and immunopathology.

## MICROBIOTA AND CHRONIC INFLAMMATORY DISEASES

In June 2012, the Human Microbiome Project Consortium (HMP) reported on the “healthy” microbiome at 15–18 anatomic sites and provided a framework for future studies on defining the association of the microbiome with disease states. In the few years since the healthy baseline was established, a number of reports have defined altered microbiota that may contribute to disease. A common finding among studies investigating dysbiosis of the microbiota was a decrease in alpha diversity in diseased vs. healthy states (41–48). Many studies have also demonstrated that changes in the microbiome correlate with the pathogenesis of various systemic inflammatory diseases (17, 18, 47, 48). As might be expected, changes in the microbiota specific to a particular anatomic location have been associated with inflammatory diseases of that area as well as distant tissues or organs.

### Association of Microbiota with Local Inflammation

Several studies have linked IBD with dysbiosis of the gut microbiota characterized by decreases in *Bifidobacterium*, *Clostridium*, and *Faecalibacterium prausnitzii* and increases in *Ruminococcus gnavus* and adherent-invasive *Escherichia coli* (46, 49–51). In subjects with IBD, there was also a decrease in the genus *Roseburia*; interestingly, this decrease was observed in healthy subjects with a high genetic risk for IBD (52–55). Not only was dysbiosis of the gut microbiota associated with IBD but distinct shifts in the gut microbiota and a decrease in alpha diversity were also shown to distinguish ileal vs. colonic Crohn’s disease (55). Significant phylogenetic differences were found between patients who respond to treatment for ulcerative colitis vs. those who do not respond to treatment (53).

Many cancers have now been linked to dysbiosis of the local microbiota. The gut microbiome of colorectal cancer patients includes significantly higher populations of *Enterococcus faecalis*,

*Streptococcus bovis*, and *Fusobacterium* than the microbiota of healthy controls (56). The oral cancer OSCC is associated with a shift in the oral microbiome. *Streptococcus* species dominate the salivary microbiota within OSCC tumor sites compared to non-tumor sites within the same individual (57). There also is significant enrichment of Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, *Porphyromonas*, and *Treponema* in the uterine microbiota of individuals with endometrial cancer (58).

### Association of Microbiota with Systemic Inflammation at Sites Distant from Infection

Changes in the gut microflora have also been associated with metabolic and inflammatory conditions distant from infection including obesity, diabetes, and autoimmune diseases. Studies have reported that the relative proportion of Actinobacteria and Bacteroidetes was increased and decreased, respectively, in the gut microbiota of obese individuals compared to lean individuals (43, 59). An increased abundance of Bacteroidetes and *Bacteroides* and a decreased abundance of Firmicutes and *Bifidobacterium* and *Prevotella* were also reported to distinguish the intestinal microbiome of children with T1D from that of age-matched healthy controls (45, 60–62).

An increased abundance of *Prevotella* and a decreased abundance of *Bacteroides* were reported in stool samples from subjects with new-onset RA compared to healthy controls (63). A separate study characterized an RA-associated fecal microbiome as one in which Actinobacteria and *Collinsella* and *Eggerthella* were consistently expanded while *Faecalibacterium* was notably decreased compared to healthy controls (47). A recent study examined both the gut and oral microbiota of subjects with RA and reported increased levels of *Lactobacillus salivarius* and decreased levels of *Haemophilus* spp. compared to healthy controls in both sites (64). Shifts in the oral microbiome have been reported in subjects with RA, including lower levels of *Corynebacterium* and *Streptococcus* compared to healthy controls (18). Subjects with new-onset RA also have shifts in the oral microbiota with increases in *Prevotella* and *Leptotrichia*; these organisms were absent in the oral microbiota of healthy controls (18). Bronchoalveolar lavage fluid from subjects with early RA revealed dysbiosis of the lung microbiome that was attributed to a significant decrease in *Actinomyces*, *Burkholderia*, *Porphyromonas*, *Prevotella*, and *Treponema* compared to healthy controls (48).

Some cancers have been associated with oral microbiota changes at sites distant from the primary tumor. In saliva samples from subjects with lung cancer, *Capnocytophaga*, *Selenomonas*, and *Veillonella* were more abundant and *Neisseria* was less abundant compared to healthy controls (19). The presence of the oral pathogens *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* in oral wash samples were associated with a higher risk of pancreatic cancer, while presence of *Fusobacteria* and *Leptotrichia* in oral wash samples was associated with a lower risk of pancreatic cancer risk (65).

Shifts in the gut and oral microbiota have also been associated with symptomatic atherosclerosis patients. The gut microbiota of subjects with atherosclerosis had increased levels of *Desulfovibrio*,

*Enterobacter*, *Megasphaera*, and *Oscillibacter* and less *Bacteroides*, *Faecalibacterium*, and *Prevotella* compared to asymptomatic subjects (66). Subjects with symptomatic atherosclerosis have elevated levels of several genera of bacteria in the oral cavity, including *Anaeroglobus* and *Porphyromonas* (20).

## INNATE IMMUNE MECHANISMS LINKING SPECIFIC MICROORGANISMS TO CHRONIC INFLAMMATION AND IMMUNOPATHOLOGY

A number of studies have examined the ability of pathogens to induce systemic inflammation and immunopathology at sites distant from infection. Well-defined animal models of RA, cancer, and atherosclerosis have been utilized to demonstrate a link between infection with specific pathogens and acceleration of disease (67–75). Many of these studies have focused on the role of the innate immune system and in particular toll-like receptors (TLRs) in microbial-induced immunopathology and disease (70, 73, 76–82).

Toll-like receptors detect conserved microbial products and play a central role in the activation of innate and adaptive immune pathways (83, 84). TLR2 and TLR4 are two of the most well-characterized TLRs that respond to microbial membrane components. TLR2 is a cell-surface receptor that recognizes pathogen-associated molecular patterns (PAMPs) that are typically associated with both Gram-positive and Gram-negative bacteria, such as lipoproteins, lipoteichoic acid, peptidoglycan, zymosan, and porins (85–89). TLR4 is a cell-surface receptor that recognizes lipopolysaccharide (LPS) from Gram-negative bacteria (90–92). Signal transduction following recognition of LPS by the TLR4 complex (CD14–TLR4–MD2) is an essential component of host immunity to Gram-negative bacterial infection (93). TLR4 signals through MyD88- and TRIF-dependent pathways to promote proinflammatory cytokine production and type I IFN (IFN $\beta$ ) responses, respectively (94, 95). In addition to PAMPs, various endogenous “danger” molecules released from damaged host cells activate TLRs; these molecules are known as danger-associated molecular patterns and include heat-shock proteins, hyaluronic acid, and oxidized low-density lipoprotein (96).

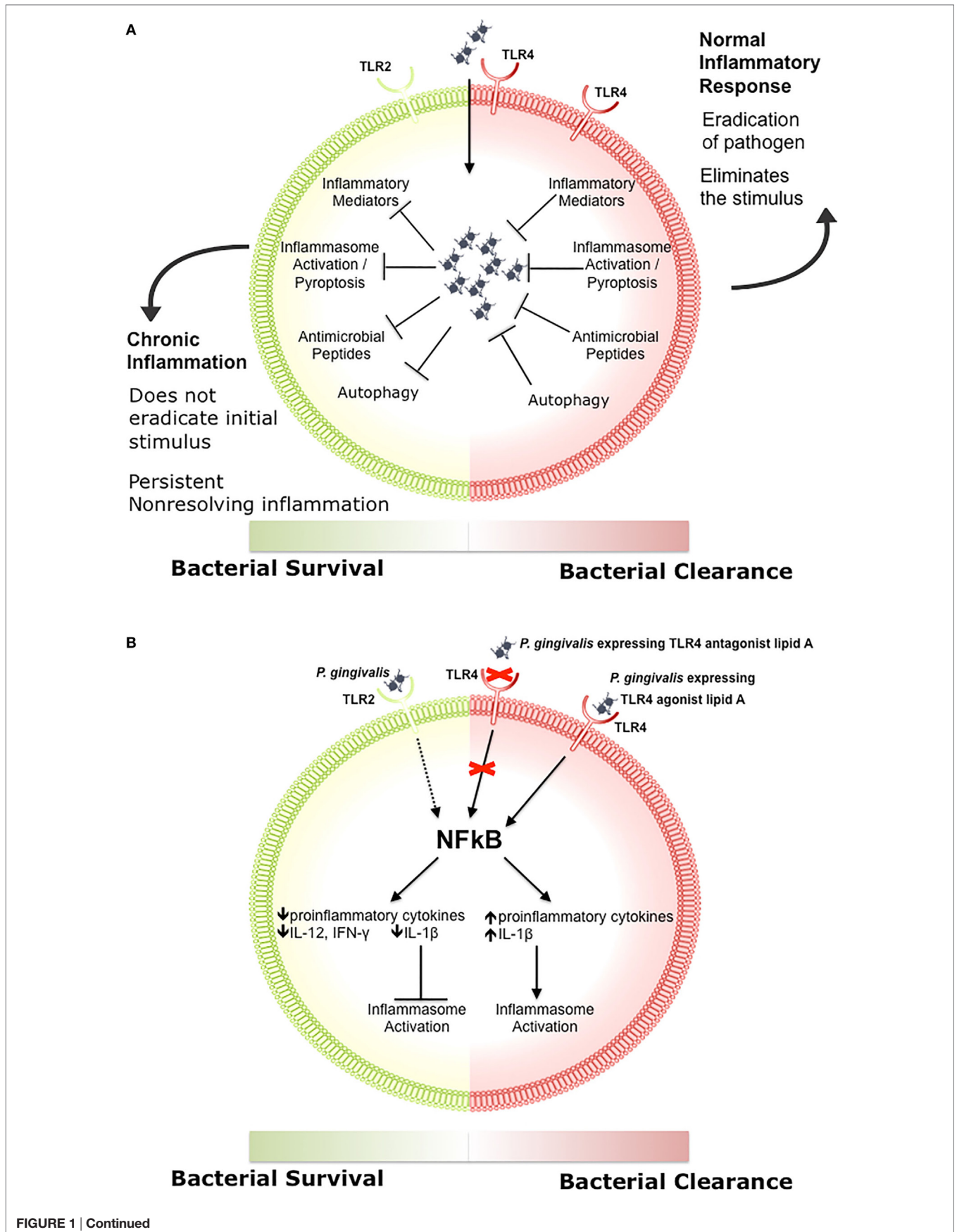
Engagement of TLR signaling triggers an inflammatory response that is primarily aimed at eliminating the invading organisms, initiating repair to damaged tissues, and initiating the adaptive immune response (83, 84, 86, 97–99). When well controlled, this beneficial inflammatory response manages a delicate balance between the clearance of pathogens and damage to the host through feedback loops and negative regulation, resolving once the stimulus has been removed (Figure 1A) (100–102). Clearance of pathogens is orchestrated through a combination of antimicrobial peptides, inflammatory mediators, phagocytosis, autophagy, and inflammasome activation (Figure 1A) (97, 103, 104). Chronic inflammation occurs when there is a breakdown in the regulation of these processes, which disrupts host cells locally and systemically, and it is increasingly associated with chronic conditions such as autoimmune diseases, cancer, IBD, arthritis, and atherosclerosis (105–107).

We recently demonstrated that differential TLR signaling by variant *P. gingivalis* lipid A moieties was associated with the production of proinflammatory mediators and bacterial survival in macrophages (Figure 1B) (108).

A number of studies have examined the role of TLR signaling in microbial-induced chronic inflammation and immunopathology (70, 73, 76, 82). *Mycobacterium tuberculosis* and commensal gut microbiota have been shown to induce autoimmune arthritis through TLR signaling (73, 76). *E. coli* has been shown to increase non-small cell lung cancer metastasis in a TLR4-dependent manner (82). *Chlamydia pneumoniae* accelerates atherosclerosis through a TLR/MyD88-dependent mechanism (70). Our work has focused on defining the role of TLR2 and TLR4 signaling in *P. gingivalis*-mediated inflammatory atherosclerosis using a well-defined hyperlipidemic mouse model (ApoE<sup>-/-</sup>) (77–81). Numerous studies have documented a role for TLR signaling in lipid-induced atherosclerosis progression (110–113). Oxidized LDL particles are recruited to the atheroma and trigger TLR signaling in macrophages and endothelial cells. This results in foam cell formation and the production of proinflammatory cytokines and other proinflammatory mediators, such as IL-1, TNF $\alpha$ , and macrophage colony-stimulating factor, which perpetuate inflammation within the vasculature (109, 114, 115). It has been postulated that the association between microbial infection and atherosclerosis involves common mechanisms of signaling via TLR2 and TLR4. Some investigators have proposed that TLR signaling induced by multiple pathogens and endogenous ligands may explain the link to atherosclerosis and that therapeutic TLR antagonism could prove beneficial in the treatment of chronic atherosclerosis (13, 14, 116–118). However, our work revealed that *P. gingivalis*-mediated TLR4 signaling protects from atherosclerosis, suggesting that effects are pathogen specific.

We established that oral infection with *P. gingivalis* is associated with lipid accumulation and macrophage infiltration in the aortic sinus and innominate artery of ApoE<sup>-/-</sup> mice (69, 71). *P. gingivalis* oral infection induced the expression of inflammatory mediators and proinflammatory cytokines such as IFN- $\gamma$ , IL-1 $\beta$ , interleukin-6 (IL-6), and TNF $\alpha$  in the atherosclerotic lesions of ApoE<sup>-/-</sup> mice, which was significantly reduced in the atherosclerotic lesions of *P. gingivalis*-infected ApoE<sup>-/-</sup>TLR2<sup>-/-</sup> mice (77–79, 81). In contrast to the effect of TLR2-deficiency on *P. gingivalis*-induced atherosclerosis, we demonstrate that TLR4-deficiency leads to increased disease severity, indicating a protective role for TLR4 signaling in *P. gingivalis*-induced atherosclerosis (80). *P. gingivalis*-infected TLR4-deficient mice had increased vascular inflammation characterized by enhanced lesion progression and increased macrophage accumulation compared to infected TLR4-sufficient mice (80). In contrast to what was observed with *P. gingivalis*, other reports have documented that TLR4-deficient mice infected intranasally with *C. pneumoniae* had diminished vascular inflammation compared to infected TLR4<sup>+/+</sup> mice. These results suggest that the role of TLR4 signaling in atherosclerosis is pathogen specific.

*Porphyromonas gingivalis*-infected TLR4-deficient mice also had increased CD4/CD8 T cells, decreased regulatory T cell infiltration, and impaired Th1 immunity, implicating modulation of the adaptive immune response (80). Our results suggest that this



**FIGURE 1 | Continued**

**The role of toll-like receptor (TLR) signaling in chronic inflammation. (A)** During a normal inflammatory response, activation of TLR signaling results in an increase in proinflammatory mediators and antimicrobial peptides, activation of the inflammasome, and clearance of the pathogen (97, 103, 104). Eradication of the stimulus results in resolution of inflammation (100–102). Some bacteria inhibit one or more of these responses, preventing the resolution of inflammation. **(B)** *Porphyromonas gingivalis* activation of TLR2 results in decreased production of proinflammatory cytokines such as IL-12 and IFN- $\gamma$ , impairing bacterial clearance (109). *P. gingivalis* expressing a TLR4 antagonist lipid A moiety produces low levels of IL-1 $\beta$  and prevents activation of the non-canonical inflammasome, which also impairs bacterial clearance (108). In contrast, *P. gingivalis* expressing a TLR4 agonist lipid A moiety produces high levels of IL-1 $\beta$  and activates the inflammasome (108).

**TABLE 1 | Gram-negative bacteria that express divergent lipid A structures.**

Bacterial strain	Acylation/phosphorylation	Toll-like receptor 4 activation	Outcomes	Reference
<i>Helicobacter pylori</i>	Mono-phosphorylated, tetra-acylated	Weak agonist	Bacterial survival	(119, 127)
<i>Pseudomonas aeruginosa</i>	Hepta-acylated	Strong agonist	Severe cystic fibrosis (CF), neutrophil survival	(120)
	Penta-acylated	Weak agonist	Decreased IL-8, CF	
<i>Bacteroides thetaiotaomicron</i>	Under-phosphorylated, penta-acylated	Weak agonist	Bacterial survival	(121, 128)
<i>Porphyromonas gingivalis</i>	Di-phosphorylated, penta-acylated	Agonist	Modest inflammation, decreased atherosclerosis	(108, 122)
	Mono-phosphorylated, tetra-acetylated	Antagonist	Systemic inflammation, increased atherosclerosis	
<i>S. flexneri</i>	Tri- or tetra-acylated	Weak agonist	Low cytokine production, inflammasome inhibition	(123)
<i>Neisseria meningitidis</i>	Penta-acylated	Non-activating	Low NF $\kappa$ B activation	(124)
<i>Yersinia pestis</i>	Hexa-acylated	Strong agonist	Bacterial clearance, no systemic disease	(125, 129, 130)
	Tetra-acylated (37°C)	Weak agonist	Systemic disease	
<i>Francisella tularensis</i>	Mono-phosphorylated, tetra-acetylated	Weak agonist	Decreased TNF $\alpha$ , bacterial survival	(126, 131, 132)

protective role of TLR4 signaling may be orchestrated by dendritic cell (DC) IL-10 and IL-12 as well as by the induction of regulatory T cells (80). Collectively, our studies indicate that *P. gingivalis*-mediated dysregulation of innate and adaptive responses results in systemic inflammation and immunopathology.

## INNATE IMMUNE SUBVERSION

A common theme that has recently emerged is that pathogens that are associated with chronic low-grade inflammation have developed mechanisms of immune subversion that either alter or inhibit protective signaling normally induced by TLRs. Lipid A, the biologically active moiety of LPS, can be expressed in variant forms by many human pathogens, allowing for evasion of the host innate immune system and establishment of a chronic infection (119–126). **Table 1** summarizes the impact of impaired TLR4 signaling as a result of divergent lipid A of several immune subversive Gram-negative bacteria. Strikingly, several of these host-adapted Gram-negative bacteria that express immune-evasive lipid A are associated with increased risk of autoimmune disease, atherosclerosis, and cancer. Although *Helicobacter pylori* expresses a hexa-acylated species of lipid A, its predominant lipid A species is mono-phosphorylated and tetra-acylated; this combination of underphosphorylation and underacylation of lipid A likely explains the low endotoxic and biological activities of *H. pylori* LPS (119).

SenGupta et al. (120) reported that structural lipid A variants of *Pseudomonas aeruginosa* correlate with severity and progression of CF. Specifically, hepta-acylated lipid A is uniquely associated with severe late stage CF, and this variant acts as a strong TLR4 agonist, resulting in neutrophil survival and substantial production of IL-8 (120). Penta-acylated lipid A is found in patients with

early stage or less severe (CF), accompanied by lower levels of IL-8 compared to those with severe late stage CF (120).

*Bacteroides thetaiotaomicron* expresses a penta-acylated 4'-dephosphorylated lipid A structure and exhibits resistance to various inflammation-associated CAMPs (121, 128). Using wild-type and *lpxF* mutant strains of *B. thetaiotaomicron*, Cullen et al. (128) demonstrated that CAMP resistance is *lpxF*-dependent but is also inflammation-dependent as *lpxF* deletion mutants were outcompeted by wild-type bacteria in germ-free mice only in the presence of inflammatory *Citrobacter rodentium* infection. These authors noted that *lpxF* orthologs have been identified in all sequenced human-associated Bacteroidetes. In addition to *B. thetaiotaomicron*, four other human-associated *Bacteroides* (*Bacteroides fragilis*, *Bacteroides vulgatus*, *Prevotella salivae*, and *P. gingivalis*) produce an under-phosphorylated lipid A structure (128).

Several Gram-negative bacteria that express immune-evasive lipid A species are associated with an increased risk of atherosclerosis (133, 134), but the oral pathogen *P. gingivalis* is a striking example of how lipid A variants allow a bacterium to evade TLR4 and promote chronic inflammation through dysregulation of both innate and adaptive immune responses (71, 78–80, 135).

In response to environmental conditions, *P. gingivalis* expresses a variety of lipid A species that are described as TLR4 agonist, antagonist, or non-stimulating depending on their phosphorylation and acylation states and the resulting immunostimulatory activity (122, 136–140). The underacylated lipid A moieties are poorly recognized by TLR4, and the antagonist lipid A species inhibits activation of TLR4 by agonist lipid A species from *P. gingivalis* or other Gram-negative organisms (122, 139, 141, 142). We recently examined the role of these *P. gingivalis* lipid A variants *in vitro* and in a mouse model of vascular

inflammation. We demonstrated that the antagonist lipid A species enhanced bacterial survival in macrophages through inhibition of non-canonical inflammasome activation and decreased production of proinflammatory mediators such as IL-1 $\beta$  (Figure 1B) (108). In contrast, the agonist lipid A species was associated with decreased bacterial survival in macrophages and high levels of IL-1 $\beta$  (Figure 1B) (108). ApoE<sup>-/-</sup> mice orally infected with the *P. gingivalis* wild-type or antagonist strains had progressive vascular inflammation characterized by enhanced lesion progression and increased macrophage accumulation compared to either sham-infected ApoE<sup>-/-</sup> mice or ApoE<sup>-/-</sup> mice that were orally infected with the *P. gingivalis* agonist strain. These studies indicate that distinct lipid A moieties allow *P. gingivalis* to evade the host innate immune response resulting in vascular inflammation. In addition to facilitating bacterial survival, it is possible that distinct lipid A moieties dysregulate host adaptive immune responses through manipulation of DC activation and downstream T cell polarization leading to systemic inflammatory pathologies.

## DYSREGULATION OF ADAPTIVE IMMUNITY AND CHRONIC INFLAMMATION

Dysregulation of host immunity has been proposed to contribute to chronic inflammation observed in systemic inflammatory diseases and typically involves modulation of DC responses, critical antigen-presenting cells that link innate and adaptive immunity. DCs play a key role in immune-modulatory functions and are often targeted by pathogens, resulting in altered T cell-mediated adaptive responses, immune dysregulation, and immunopathology. Immature and highly phagocytic DCs reside in the tissues and detect pathogens through PRRs including DC-SIGN, TLR2, TLR4, NOD2, and the mannose receptor (143). PRR ligation and pathogen phagocytosis initiate DC maturation, which is characterized by a decrease in phagocytic capability and an upregulation of co-stimulatory molecules that are involved in activating T cells. The type of T cell response is dictated by which PRR is activated and, consequently, which cytokines are produced (143). The induction of a CD4 T cell response is largely determined by pathogen detection by DCs that present antigens to naïve T cells to initiate an adaptive response. DCs can also be activated to efficiently cross-present extracellular antigens on MHC-I, leading to activation of CD8 T cells (144, 145).

Lipopolysaccharide isolated from the Gram-negative pathogens *E. coli* and *Salmonella enterica* serotype Typhimurium potently stimulate TLR4 on DCs leading to maturation and expression of proinflammatory cytokines that drive a Th1 response (143). The expression of immune-evasive LPS by host-adapted bacteria such as *P. gingivalis* may result in different DC responses leading to altered T cell responses. However, the impact of immune-evasive LPS on systemic immunopathology has not been explored. We postulate that altered DC activation by *P. gingivalis* lipid A mutant strains will result in dysregulation of adaptive immunity that leads to enhanced development and progression of atherosclerosis. Current studies are underway to define how modification of lipid A alters DC maturation and functional responses.

## IMPLICATIONS FOR FUTURE THERAPIES

Studies demonstrating a protective role for TLR deficiency in inflammation have prompted many to pursue therapeutic TLR antagonism for combating systemic inflammatory and autoimmune diseases. These TLR antagonists include structural analogs of agonists, anti-TLR antibodies, and small molecule antagonists. One example of a structural analog is eritoran, a TLR4 antagonist that inhibits LPS-induced inflammation and improves survival in a mouse model of sepsis (146). The small molecule inhibitor TAK-242 inhibits TLR4 signaling by binding selectively to TLR4 and disrupting interaction with its adaptor molecules (147). Wang et al. (148) demonstrated that TAK-242 diminished the accumulation of DCs, lymphocytes, macrophages, and neutrophils and enhanced production of IL-6, IL-8, and TNF- $\alpha$  in a mouse model of cigarette smoke-induced pulmonary inflammation. It has been suggested that therapy with eritoran or TAK-242 may be most efficacious against bacteria expressing hexa-acylated lipid A structures, which act as strong TLR4 agonists and elicit local inflammation but typically result in bacterial clearance and no systemic disease (149). In contrast, bacteria that do not produce hexa-acylated lipid A elicit little or no TLR4-mediated local inflammation, which permits bacterial survival and dissemination and contributes to the development and progression of systemic diseases. Anti-TLR4 therapy has been suggested as a prophylactic for necrotizing enterocolitis (NEC), but a recent study demonstrated that secretions from the probiotic *Bifidobacterium longum* subspecies *infantis*, often used to treat NEC, attenuate IL-1 $\beta$ -induced IL-6 in a TLR4-dependent manner in a human fetal small intestinal epithelial cell line (H4 cells) and primary NEC enterocytes (150). Despite the promising results of using TLR4 antagonists to prevent inflammation in mice, our results and others suggest that the role of TLR4 signaling in the pathogenesis of chronic inflammatory diseases is pathogen specific and that TLR4 antagonism could encourage systemic inflammation and dissemination of certain pathogens resulting in unintended outcomes (80, 108, 150). In addition, the presence of comorbid conditions and the bacterial characteristics of both local and distant microbiota create a complex environment in which chronic disease develops and progresses. For these reasons, we advocate caution in the development and testing of TLR4 antagonists for the treatment of chronic inflammatory diseases induced by the microbiota.

## AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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