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Understanding the role of endotoxin tolerance in chronic inflammatory conditions and periodontal disease.

Running title: Endotoxin tolerance in periodontitis

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ABSTRACT

Objective: This review aims to present the current understanding of endotoxin tolerance (ET) in chronic inflammatory diseases and explores the potential connection with periodontitis.

Summary: Subsequent exposure to lipopolysaccharides (LPS) triggers ET, a phenomenon regulated by different mechanisms and pathways, including toll-like receptors (TLRs), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), apoptosis of immune cells, epigenetics, and microRNAs (miRNAs). These mechanisms interconnect ET with chronic inflammatory diseases that include periodontitis. While the direct correlation between ET and periodontal destruction has not been fully elucidated, emerging reports point towards the potential tolerization of human periodontal ligament cells (hPDLCs) and gingival tissues with a significant reduction of TLR levels.

Conclusions: There is a potential link between ET and periodontal diseases. Future studies should explore the crucial role of ET in the pathogenesis of periodontal diseases as evidence of a tolerized oral mucosa may represent an intrinsic mechanism capable of regulating the oral immune response. A clear understanding of this host immune regulatory mechanism might lead to effective and more predictable therapeutic strategies to treat chronic inflammatory diseases and periodontitis.

Keywords (MeSH): endotoxins, sepsis, endotoxin shock, inflammation, periodontal diseases

Clinical relevance (100 words)

Scientific rationale for the study: The mechanism of endotoxin tolerance (ET) could play a critical role in modulating the inflammatory response. Hereby, we discuss the potential involvement of ET in the development and progression of periodontitis disease.

Principal findings: Here, we present the current understanding of the mechanisms involved in the activation of ET and the emerging findings that demonstrate that low doses of LPS can induce ET leading to reduced production of inflammatory cytokines.

Practical implications: A clear understanding of ET might lead to innovative therapeutic strategies to treat chronic inflammatory diseases and periodontitis.

INTRODUCTION

The innate immune response is the first line of defense against pathogens. When the innate immune response detects pathogens through the pattern recognition receptors (PRRs), a fast, non-specific response is mounted, triggering an inflammatory response. Unregulated responses can lead to uncontrolled inflammation that can be localized or systemic, leading to septic shock or the development of autoimmune diseases (Lopez-Collazo & del Fresno, 2013). Nonetheless, the immune system is endowed with adaptive capabilities mainly through long-term immunological memory. Similar to the adaptive properties of the immunological memory, emerging evidence has pointed towards the ability of the innate immune system to adapt its immune response through a process known as trained immunity (Conrath, Beckers, Langenbach, & Jaskiewicz, 2015; Kurtz, 2005). Trained immunity is defined as a higher degree of the immune response following a second pathogen challenge when compared to the first response. Such lasting reprogramming of innate immune cells is mediated by epigenetic events rather than a specific transcriptional program (Netea, Schlitzer, Placek, Joosten, & Schultze, 2019). It is important to note that erroneous trained immunity can lead to disease progression spanning from inflammatory response to a continuous process of immunotolerance (Netea et al., 2020).

Endotoxin tolerance (ET) constitutes a functional program from the innate immunity capable of damping the inflammatory reaction to subsequent LPS stimuli (del Fresno et al., 2009; Draisma, Pickkers, Bouw, & van der Hoeven, 2009; Foster, Hargreaves, & Medzhitov, 2007; Novakovic et al., 2016; Vergadi, Vaporidi, & Tsatsanis, 2018). ET is described as a cell's reduced capacity to respond to a second endotoxin challenge (D. Liu, Cao, Zhou, & Xiong, 2019). This is the case of innate immune cells like monocytes and macrophages that, upon exposure to low doses of bacterial lipopolysaccharide (LPS), produce lower amounts of pro-inflammatory cytokines while sustaining the production of anti-inflammatory cytokine (Kopanakis et al., 2013).

Here, we present the current understanding of the mechanisms involved in the activation of ET in slow, long-term inflammation (chronic inflammatory diseases) and the development of septic shock. Furthermore, we discuss the potential involvement of ET in the development and progression of periodontitis disease.

UNDERSTANDING ENDOTOXIN TOLERANCE

What is endotoxin tolerance?

ET constitutes a reduced response to LPS stimuli (hypo-responsiveness) upon subsequent exposures of low levels of LPS, precluding excessive innate immune response when faced with a pathogenic threat (Bauer, Weis, Netea, & Wetzker, 2018) (Figure 1).

ET has been reported in many systemic inflammatory diseases, including sepsis, acting as a protective mechanism to prevent self-destruction by attenuating inflammatory pathways (tolerant response). Emerging evidence suggests that the mechanism of ET may play a crucial function as an immune cell adaptation to continuous bacterial infection (chronic LPS exposure). Nonetheless, ET can also result in harmful effects on tissue integrity as a hypo-responsiveness phase follows an acute pro-inflammatory response to bacterial insults as seen during sepsis (Rackov, Shokri, De Mon, Martinez, & Balomenos, 2017) (Figure 2).

The mechanism of ET was first described in the early 1900s when the injection of fever-inducing LPS was employed in the treatment of tumors and various inflammatory diseases. Interestingly, continuous administration of LPS resulted in tissue tolerization, a condition in which a gradual increment in LPS dosage was required to obtain similar therapeutic results (Bennett & Nicastri, 1960). Similarly, rabbits receiving daily injections of bacteria demonstrated a progressive downregulation of the immune response that lasted for a short period (Beeson & Roberts, 1947a, 1947b; Van Epps, 2006). Following studies have shown a significant reduction in the mortality of hosts receiving a lethal dose of endotoxin after the activation of ET (Berry & Smythe, 1965; Greisman, Young, & Carozza, 1969; Milner, 1973; Neter, 1969). Furthermore, ET can develop after infection and/or tissue damage in which cells from the innate immune system undergo a refractory state (Lopez-Collazo & del Fresno, 2013). In vitro studies showed that exposure to low doses of LPS results in tolerized cells capable of down-regulating inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-12, IL-1β, and the mitogen-activated protein kinase kinase kinase (MAP3K). Interestingly, low doses of LPS resulted in the up-regulation of anti-inflammatory cytokines such as IL-10, transforming growth factor-beta (TGF-β) and IL-1 receptor antagonist (IL-1RA). This LPS tolerant phenotype is also

characterized by inhibiting MAPK activation and impaired nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) nuclear translocation (Kopanakis et al., 2013; West & Heagy, 2002).

ET also activates high phagocytosis levels, decreased antigen presentation, reduces expression of class II major histocompatibility complex, and impair microbial killing (Grondman et al., 2019; Landelle et al., 2010; Lopez-Collazo & del Fresno, 2013). Patients with acute ischemic stroke present monocytes exhibiting ET and enhanced risk for pathogen colonization (Hernandez-Jimenez et al., 2017). Reports suggest that around one-third of stroke patients die due to the potential impairment of the immune defense.

LPS from various bacteria do not have the same ability to activate toll-like receptors (TLR) and trigger an immunological response. Thus, not all LPS have the same capacity to induce ET. When comparing LPS from *Escherichia coli* and *Porphyromonas gingivalis*, higher concentrations of *P. gingivalis* LPS are needed to induce ET (Martin, Katz, Vogel, & Michalek, 2001). Macrophages also respond differently to ET signals. Cellular polarization dictates the pro-inflammatory (M1 polarization) or anti-inflammatory (M2 polarization) differentiation of macrophages and consequently its response to ET (Das et al., 2015). Macrophages of different phenotype seem to respond differently to ET; for instance, M1 macrophages exposed to *P. gingivalis* LPS appears to be less sensitive to ET than M2 macrophages. As a matter of fact, LPS from *P. gingivalis* suppresses the expression of TNF-α, IL-1β, IL-6, and NFκB factors from M2 macrophages (Al-Shaghdali, Durante, Hayward, Beal, & Foey, 2019; Foey & Crean, 2013). However, induction of ET using *E. coli* LPS does not interfere with TNF-α production while IL-10 and IL-6 are suppressed in M1, but not in M2 macrophages (Al-Shaghdali et al., 2019).

Molecular mechanisms and regulation of endotoxin tolerance

TLR-4 is a vital factor in the regulation of ET (D. Liu et al., 2019). LPS binds to PRRs, such as TLR-4, and triggers an innate immune response, leading to the production of inflammatory cytokines (Takeda & Akira, 2015). The binding of LPS activates two signaling pathways by inducing the formation of a dimer consisting of TLR-4/MyD88 and TLR-4/TIR domain-containing adapter-inducing

interferon-β (TRIF), respectively. In turn, MyD88 causes inflammatory cytokines production through the NFκB signaling pathway, while the TRIF pathway induces interferon (IFN) type 1 via interferon regulatory factor 3 (IRF3) activation. On the other hand, NFκB can also be activated by the TRIF/ MAP3K7/ inhibitor of the NFκB kinase subunit beta (IKKB) signaling pathway (D. Liu et al., 2019; Takaesu et al., 2003; Takeda & Akira, 2015). Other molecules like Src homology two domain-containing inositol polyphosphate 5-phosphatase 1 (SHIP1), tumor necrosis factor-alpha-induced protein 3 (TNFAIP3), and interleukin-1 receptor-associated kinase-3 (IRAK3) also play a critical role in the activation of ET in different cell types (Lopez-Collazo & del Fresno, 2013; Lyroni et al., 2017). Figure 3 depicts currently known molecular and regulatory mechanisms related to ET.

Apoptosis of immune cells has recently been suggested to be an important indicator of ET in sepsis as the presence of bacterial endotoxin can trigger immune cells apoptosis (Rimmele et al., 2016; van der Flier et al., 2013). This is the case of macrophages that undergo apoptosis due to the increased production of nitric oxide (NO) mediated by the presence of endotoxin in the culture media (Yamamoto, He, Klein, & Friedman, 1994). Furthermore, TNF-α is a cytokine suggested as an ET indicator due to its ability to induce tissue damage and cell death during sepsis. Interestingly, TNF-α has been found to activate inflammatory response due to cellular exposure to LPS and also, able to induce a tolerance-like state (Loosbroock & Hunter, 2014). Anti-inflammatory cytokines, such as IL-10 and TGF-β, can negatively regulate monocytes and activate macrophages through IRAK3 and suppressor of cytokine signaling 1 (SOCS1) signaling pathways. SOCS1 has been found to downregulate NFκB signaling pathway after LPS treatment (Nakagawa et al., 2002).

Recently, epigenetic modifications and microRNAs (miRNAs) have emerged as novel critical regulatory of ET (Seeley & Ghosh, 2017; Vergadi et al., 2018). Epigenetic changes refer to shifts in gene expression that are not encoded in the DNA sequence, including chemical alterations of the DNA and associated proteins (i.e., histones). As a result, chromatin remodeling leads to the activation or inactivation of genes (Larsson, Castilho, & Giannobile, 2015). Specific epigenetic changes, such as histone modifications, are known to lead to chromatin re-organization and the control of several genes related to ET (Y. Liu et al., 2019). One specific gene regulated by the methylation of histone H3 is

IRAK3. The trimethylation of the IRAK3 promoter gene at histone H3 lysine 27 (H3K27me3) is observed in unstimulated macrophages. However, upon LPS stimuli, this silencing marker is removed, resulting in recruitment of the CCAAT/enhancer-binding protein-beta (C/EBP-β) to the promoter of IRAK-M and gene transcription (Lyroni et al., 2017). Most recently, ex-vivo treatment with β-glucan has been shown to reverse the epigenetic immune tolerance of human macrophages while reinstating normal cytokine release levels (Novakovic et al., 2016).

Additionally, miRNAs regulate gene expression through post-transcriptional modifications. These molecules represent a group of small non-coding RNAs of about 22 bp in length that binds to the target gene's mRNA. miRNAs lead to the suppression of gene expression either by the degradation of a target mRNA or by preventing translation (Asa'ad, Monje, & Larsson, 2019). Further, the presence of differentially expressed miRNAs in periodontal diseases is known to impact the expression of TLR (Asa'ad, Garaicoa-Pazmino, Dahlin, & Larsson, 2020) and potentially influence the activation of ET. Both non-coding RNAs (e.g., miRNAs) and long non-coding RNAs (lncRNAs) are vital regulators of ET through the TLR pathway (Vergadi et al., 2018). The LPS inducible miRNA-155 and miRNA-146α have been identified to be coordinately regulated via gene colocalization and transcription factor binding (Doxaki, Kampranis, Eliopoulos, Spilianakis, & Tsatsanis, 2015). While miRNA-146α targets IRAK1 and TNF receptor-associated factor-6 (TRAF6), miRNA-155 inhibits SHIP1 and SOCS1 (Nahid, Satoh, & Chan, 2011; X. Sun et al., 2018). Thus, these miRNAs influence critical components of TLR signaling. Additionally, several TLR-inducible lncRNAs can limit the excessive inflammatory responses by negatively regulating TLR signaling (Vergadi et al., 2018). Myocardial infarction associated transcript 2 (Mirt2) (Du et al., 2017), TNF-α heterogenous nuclear ribonucleoprotein L (THRIL) (Li et al., 2014), metastasis associated lung adenocarcinoma transcript 1 (MALAT1) (Zhao, Su, Song, Mao, & Mao, 2016), NFκB interacting LncRNA (NKILA) (B. Liu et al., 2015), long intergenic noncoding RNA p21 (lincRNA-21) (Zhou, Wang, Shao, & Wang, 2016), and Lymphotoxin/TNF-α locus lncRNA "Sense Transcript" (SeT) (Stratigi et al., 2015) are responsible molecules to suppress the expression of pro-inflammatory mediation, such as TNF-α, and thus, contributing to ET.

Endotoxin tolerance and sepsis

Perhaps one of the most notable examples of ET is sepsis. Sepsis is a systemic inflammatory response to an infection that can trigger a cascade of events leading to multi-organ dysfunction and organ failure. A recent definition of sepsis is considered a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). Moreover, a septic shock is caused by gram-negative bacteria in about 70% of cases, with a mortality rate of approximately 40% (Hernandez et al., 2019; Touyz, 2013).

During sepsis, there is a deregulation of the innate immune response upon systemic bacterial infection leading to LPS-mediated stimulation of TLR and activation of ET. The following features have been proposed as hallmarks of sepsis, including 1) a decrease in lymphocyte proliferation in response to antigen stimulation, 2) decreased in production of IL-2 and IFN-γ by peripheral blood mononuclear cells, 3) a diminished monocyte expression of human leukocyte antigen-DR isotype (HLA-DR) and CD86; and of lymphocyte CD28, 4) an increase in cytotoxic T lymphocyte antigen-4 (CTLA4) expression, and 5) a decreased monocyte capacity to release pro-inflammatory cytokines in response to LPS (Monneret, Venet, Pachot, & Lepape, 2008). Furthermore, ET's clinical features during sepsis comprise two main phases; an inflammatory stage followed by a tolerance phenotype phase of the host immune cells. This results in a high risk of secondary infections leading to an increased mortality rate (Lopez-Collazo & del Fresno, 2013). ET is often correlated with the late stage of sepsis when there is a pronounced hypo-inflammatory response due to decreased TLR-4/MyD88 signaling and M2 macrophage polarization. Hence, explain the greater risk of secondary infections (Monneret et al., 2008).

ENDOTOXIN TOLERANCE AND CHRONIC INFLAMMATION

The interrelationship between endotoxin tolerance and chronic inflammatory diseases

The presence and activation of ET have been reported in diseases and conditions such as trauma, surgery, pancreatitis, and cystic fibrosis. Of interest, cystic fibrosis is characterized by repeated infections leading to the inflammation of the lungs and progressive tissue destruction that result in a

compensatory anti-inflammatory response syndrome (CARS), a condition often referred to as the clinical manifestation of ET (Biswas & Lopez-Collazo, 2009; Lopez-Collazo & del Fresno, 2013). CARS is an autoimmune suppression state observed during significant insults to the human body, such as extensive burns, large tissue injuries, and sepsis (Ward, Casserly, & Ayala, 2008). Like CARS, ET is defined as a hypo-responsiveness disease presenting a reduced response of myeloid cells to inflammatory stimuli, particularly those initiated by bacterial LPS (Vergadi et al., 2018). Compromised epithelial barrier has a severe impact on the maintenance of chronic inflammatory diseases. Loss of epithelial protection often results in endotoxemia, that is, the accumulation of LPS in the blood, leading to chronic stimulation of myeloid cells and potential association with ET (Carron et al., 2019). One example of endotoxemia is found during chronic inflammation of end-stage renal disease (ESRD), where the loss of intestinal epithelium integrity and consequent bacterial presence in the bloodstream results in binding of LPS to LPS-binding proteins (LBP), activation of MyD88 and TRIF, and the production of pro-inflammatory cytokines, leading to chronic inflammation (Carron et al., 2019). The connection between endotoxemia and the development of ET was demonstrated in patients with acute respiratory distress syndrome (ARDS). The development and severity of ARDS are associated with the endotoxemia levels and the development of tolerance as judged by the reduced ability of monocytes to release cytokines (Buttenschoen et al., 2008).

The role of circulating LPS and their influence in ET and chronic systemic diseases has been discussed (van Lier, Geven, Leijte, & Pickkers, 2019). From experimental human models of endotoxemia, intravenous administration of LPS results in neutrophilia, mono- and lymphocytopenia after 2 to 4 hours, associated with a marked upregulation of reactive oxygen species (ROS) (Kiers et al., 2017; Pillay et al., 2010). Plasma cytokine levels (e.g., TNF-α, IL-1β, IL-6, IL-10) have a distinct, dose-dependent, and highly reproducible time course that returns to baselines values 6-8 hours after the LPS challenge (Kiers et al., 2017). TNF-α leads the LPS-induced inflammatory response by peaking at 90-120 minutes followed by IL-1β, IL-6, and IL-10 and reaching maximum plasma concentrations at approximately 3 hours (van Lier et al., 2019). This experimental model has been used to induce short-lived, well-tolerated, and controlled inflammatory responses, like those observed in sepsis, to explore

the role of endotoxemia-induced organ-specific effects in the immune system, serum, cardiovascular system, kidneys, lungs, respiratory muscle, and gastrointestinal tract.

Conversely, subclinical serum LPS may be a risk factor for conditions characterized by a non-resolving inflammation as observed in atherosclerosis (Geng et al., 2016). Interestingly, epithelial cells of the nasal cavity and airway present a tolerant phenotype in healthy individuals. A second signal stimulating the Fc-gamma receptor III (FcγRIII) and LPS stimulation of TLR-4 was needed in these cells to break this immune tolerance (Golebski et al., 2019).

ENDOTOXIN TOLERANCE AND PERIODONTAL DISEASE

Periodontal disease is a form of chronic inflammatory disease

Periodontal disease development is an intricate relationship between disease susceptibility, bacterial colonization, and host response. Although oral hygiene and the microbial community are critical factors in developing periodontitis, the imbalance in the host susceptibility is the final element for the disease. Recent findings suggest that the host immune response may play an integral role in chronic periodontitis susceptibility (Hajishengallis, 2015). Whole blood cell cultures obtained from periodontitis-affected and non-periodontitis patients were stimulated with *P. gingivalis* LPS, revealing increased IFN-γ levels only in periodontitis (Nogueira-Filho et al., 2014). These data support the notion that periodontitis alters the systemic immune cell response. LPS from other periodontal pathogens are also known to show similar effects. The low dose of bloodstream LPS (endotoxemia), similar to that found in periodontitis patients, appears to be sufficient to prime monocytes capable of affecting immune and inflammatory cells (Nakamura, Nitta, & Ishikawa, 2004). This study involved pretreating blood samples obtained from seven systemically and periodontally healthy individuals with or without *Aggregatibacter actinomycetemcomitans* LPS, followed by further LPS stimulation. The pre-treatment with *A. actinomycetemcomitans* LPS significantly enhanced the production of IL-1β and IL-6 from whole blood, suggesting the role of trained innate immunity (Nakamura et al., 2004).

The link between endotoxin tolerance and periodontal disease

For years, it was suspected that LPS led to the exaggerated innate immune response resulting in

tissue destruction observed with periodontitis (Bainbridge, Coats, & Darveau, 2002). Currently, little is known on the mechanism by which ET regulates periodontal homeostasis by providing sufficient tolerance to the presence of a healthy and balanced oral microbiota (Figure 4). However, the unbalance of the protective effects of ET poses a question on the potential mechanisms by which ET becomes dysfunctional. First, the presence of a dysbiotic oral microbiota may fail to activate the mechanism of ET, leading to the exacerbation of the inflammatory response and consequently tissue destruction. Conversely, excessive activation of ET mediated by a dysbiotic microbiota or the loss of epithelial barrier and consequently the development of endotoxemia may either prevent the recruitment of inflammatory cells or reduce the ability of monocytes to release cytokines resulting in uncontrolled bacteria-induced tissue destruction (Figure 4). It remains unknown whether the progression from a healthy periodontium to periodontitis is driven by an overly robust or insufficient immune response. Figure 4 illustrates our view of the potential role of ET in the pathogenesis of periodontal disease.

Despite the fact that the clearance of periodontal pathogens through an inflammatory response relies heavily on the recognition of LPS, it has been shown that low doses of LPS can induce ET leading to reduced production of inflammatory cytokines (Rios, de Lima, Moretti, & Soriano, 2016; Seeley & Ghosh, 2017). In periodontitis, biofilm can disrupt the gingival tissues, resulting in a compromised epithelial barrier, and periodontal pockets followed by colonization with gram-negative bacteria (Touyz, 2013). Subsequently, this can further lead to endothelial damage and a leak of bacterial products into the bloodstream (endotoxemia), which supports the link between periodontitis and septic shock syndrome (Touyz, 2013).

As noted in vitro, isolated gingival fibroblasts have demonstrated a marked difference in LPS tolerance between healthy and inflamed gingiva (Kang, Hu, & Ge, 2016). The induction of pro-inflammatory cytokines (i.e., IL-6, IL-8, IL-1β, and TNF-α) by *P. gingivalis* LPS occurred at significantly lower levels among fibroblasts isolated from inflamed gingiva affected with periodontitis compared with fibroblasts obtained from healthy patients, and thus, suggesting increased tolerance in disease. Conversely, monocytes exposed to either *P. gingivalis* or *E. coli* LPS are downregulated for TLR-2 and TLR-4 at the mRNA and protein levels and reduce IL-1β mRNA and TNF-α secretion (Muthukuru, Jotwani, & Cutler, 2005).

Monocyte cultures can be tolerized upon exposure to LPS from *P. gingivalis*. The conditioned media from tolerized monocytes have a direct negative impact on the migration and apoptotic process of neutrophils while simultaneously increasing the concentration levels of ROS (Zhu et al., 2016). As such, the role of ET becomes more crucial in periodontal diseases as neutrophil apoptosis has been suggested to influence the progression of periodontitis. Using an ET model, the exposure of human periodontal ligament cells (hPDLCs) to *P. gingivalis* LPS showed that they could not develop ET (Blufstein, Behm, Nguyen, Rausch-Fan, & Andrukhov, 2018). In contrast, a significant reduction of TLR-4 was observed in pretreated cells compared to non-pretreated cells. The pre-treatment with *P. gingivalis* LPS did not decrease IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1), thus indicating that these cells may play an important role in sustaining the inflammation in periodontal disease. Another study using a similar model reported that hPDLCs differ in pro-inflammatory cytokines and TLRs after the first and second dose of the same LPS (Wu, Zhang, Wang, Zhang, & Tan, 2015). The authors hypothesized that since the oral cavity is continuously exposed to a large amount and a diversity of periodontal pathogens, hPDLCs are exposed to a "negative regulation of immune response" due to this repeated exposure to pathogens. These findings indicate ET's induction by LPS, leading to down-regulation of TLRs and the production of inflammatory cytokines.

Moreover**,** periodontal disease is characterized by rampant tissue destruction mediated by matrix metallopeptidases (MMPs). A reduction in MMPs would be expected in LPS tolerized individuals; however, this is not the case as one study identified the secretion of MMP-9 was not affected by LPS tolerance (Muthukuru & Cutler, 2015). Conversely, tissue inhibitor of metallopeptidase-1 (TIMP-1), the main antagonist of MMP-9, is greatly upregulated in monocytes that have been induced to a tolerant state.

Compared to LPS from other gram-negative bacteria such as *E. coli*, *P. gingivalis* LPS induces much lower levels of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Martin et al., 2001). *P. gingivalis* LPS is also known to trigger a state of imbalanced immune tolerance through continuous secretion of IL-8 and a decreased production of TNF-α (Zaric et al., 2010). These events can lead to enhanced migration of neutrophils to the site of infection and reduced apoptosis. The low toxicity of *P. gingivalis* LPS compared to other gram-negative bacteria and the pattern of pro-inflammatory

cytokines induced after primary and secondary stimulation play a critical role in the chronic inflammatory state seen in periodontal disease.

In addition to the downregulation of host response, modifications of LPS signaling by *P. gingivalis* may affect the immune response. Patients with chronic forms of periodontitis are well known to have increased TLR-2 and TLR-4 positive cells and IL-1β levels within the inflamed gingival tissues. Interestingly, the numbers of TLR-2 cells increase linearly with inflammation. In a study using peripheral blood monocytes, the first stimuli of LPS resulted in the upregulation of TLR-2 and TLR-4 mRNA. In contrast, a second stimulus downregulated both receptors at the mRNA and protein levels.

Along with the TLR receptor, mRNA levels of IL-1β were also found to be reduced and TNF-α secretion levels were decreased by 10-folds. However, cytokines such as IL-6, IL-8, and IL-10 were also negatively impacted by the re-stimulation at a lesser degree than TNF-α (Muthukuru et al., 2005). Additionally, TLR-4 response to LPS varies significantly depending on phosphorylation patterns of the lipid A component. A clear example, 1'-phosphorylated lipid A demonstrated more pronounced TLR-4 activation than 4'-phosphorylated lipid A (Coats, To, Jain, Braham, & Darveau, 2009).

The exposure of THP-1 cells to *P. gingivalis* LPS stimulated NFκB activation and TNF-α release upon re-stimulation with the same LPS (Hajishengallis, Martin, Schifferle, & Genco, 2002). Conversely, NFκB-dependent transcription inhibition was observed in pretreated cell lines with *E.coli* LPS and restimulated with *P. gingivalis* LPS. Although *P. gingivalis* LPS treated cells could not become tolerant to subsequent exposure to *E. coli* LPS, *P. gingivalis* LPS inhibited the *E. coli* LPS-induced TNF-α and IL-6 release when added simultaneously into the same cell line. The re-stimulation of THP-1 monocyte cells using *P. gingivalis* and *E. coli* LPS resulted in decreased TNF-α and IL-1β but increased IL-10 levels (Y. Sun et al., 2014). Cells treated with LPS following retreatment with *P. gingivalis* LPS showed downregulation of TLR-2. In contrast, when restimulated with *E. coli* LPS, a decrease in TLR-4 was observed and associated with the irregular expression of IRAK3 and SOCS1 genes. In a similar study, the pre-treatment of THP-1 cells with *E. coli* LPS resulted in a decrease of TLR-4, TNF-α, IL-1β and IL-6 levels without changes in expression of CD14 (Martin et al., 2001). Nonetheless, cells pretreated with a similar concentration of *P. gingivalis* LPS displayed an increase in

CD14 and TLR-2 associated with a decrease in IL-1β. It is important to point out that *P. gingivalis* LPS suppressed *P. gingivalis* FimA protein-induced NFκB-dependent transcription in a 3E10/huTLR4 cell line not expressing TLR-2 (Hajishengallis et al., 2002). Thus, it suggests that *P. gingivalis* and *E.coli* LPS do not compete for common signaling intermediates and that *P. gingivalis* may target components in the TLR-4 receptor complex.

Observations from ligature-induced periodontitis models have shown that endotoxin-tolerant animals significantly reduced immune system protein mannose-binding lectin levels (Nowotny & Sanavi, 1983). While the direct correlation between ET and diminished periodontal destruction has not been extensively investigated, limited reports have explored the immunological response after exposure to bacteria. TLR-2 and TLR-4 positive cells were present in more significant numbers among gingival tissue biopsies from periodontitis patients (Muthukuru et al., 2005).

The question remains open as to how ET can drive periodontal disease progression while reducing the host pro-inflammatory response. The study from the Fujihashi's lab sheds light on this conundrum by demonstrating the presence of CD4+ T-cells in chronic periodontitis shifts from a pro-inflammatory state characterized by the production of IL-6 and TNF-α to an IL-10 producing phenotype in an experimental periodontitis model (Kobayashi et al., 2011).

CONCLUSIONS

There is still a lack of knowledge on the regulatory machinery involved in the activation of ET and a clear understanding of the role of tolerized cells in disease progression or tissue defense against bacterial insults. Future research should elucidate the crucial role of ET in the pathogenesis of periodontal diseases and the intricated process that balances the host-bacteria interactions during health. It is also important to determine the potential imbalance of the ET machinery in patients presenting increased susceptibility to periodontitis and unresponsive to traditional periodontal therapy. A clear understanding of this host immune regulatory mechanism can lead to the development of effective and more predictable therapeutic strategies to treat chronic inflammatory diseases and periodontitis.

FIGURES LEGENDS

Figure 1. Immune response and activation of tolerance to bacterial challenge. Graphical representation of LPS stimuli mediated by toll-like receptor (TLR) upon single stimuli or LPS rechallenge. LPS stimuli trigger NFκB signaling and enhanced inflammation (Inflammatory Phase). Sustained TLR activation mediated by rechallenge with LPS leads to the hypo-responsiveness of the innate immune system (Immunosuppression).

Figure 2. Innate immune activation of naïve and endotoxin-induced tolerant cells. Graphical representation of naïve response of T-cells upon first LPS stimuli and chronic LPS exposure resulting in a tolerant response and overall hypo-responsiveness of the innate immune. The successful outcome is a result of reduced tissue damage mediated by a tolerant response.

Figure 3. Current molecular and regulatory mechanisms associated with ET. Graphical representation of a cellular Naïve inflammatory response to LPS stimuli mediated by pattern recognition receptors (PRR) leading the TLR-4/MyD88 and TLR-4/TRIF signaling circuitry and activation of the NFκB pro-inflammatory signaling. Tolerant signaling prevents ubiquitination of IkB, resulting in the inability of p65 to migrate to the nucleus.

Figure 4. Graphical representation of ET in periodontal health and disease. ET plays a crucial role in maintaining the periodontium tissue integrity (Healthy Periodontium). Upon dysbiosis, LPS fails to trigger ET resulting in the increased inflammatory response and tissue destruction. Alternatively, dysbiosis led to excessive ET function and reduced inflammatory response along with enhanced bacteria-induced tissue destruction.

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Figure 1

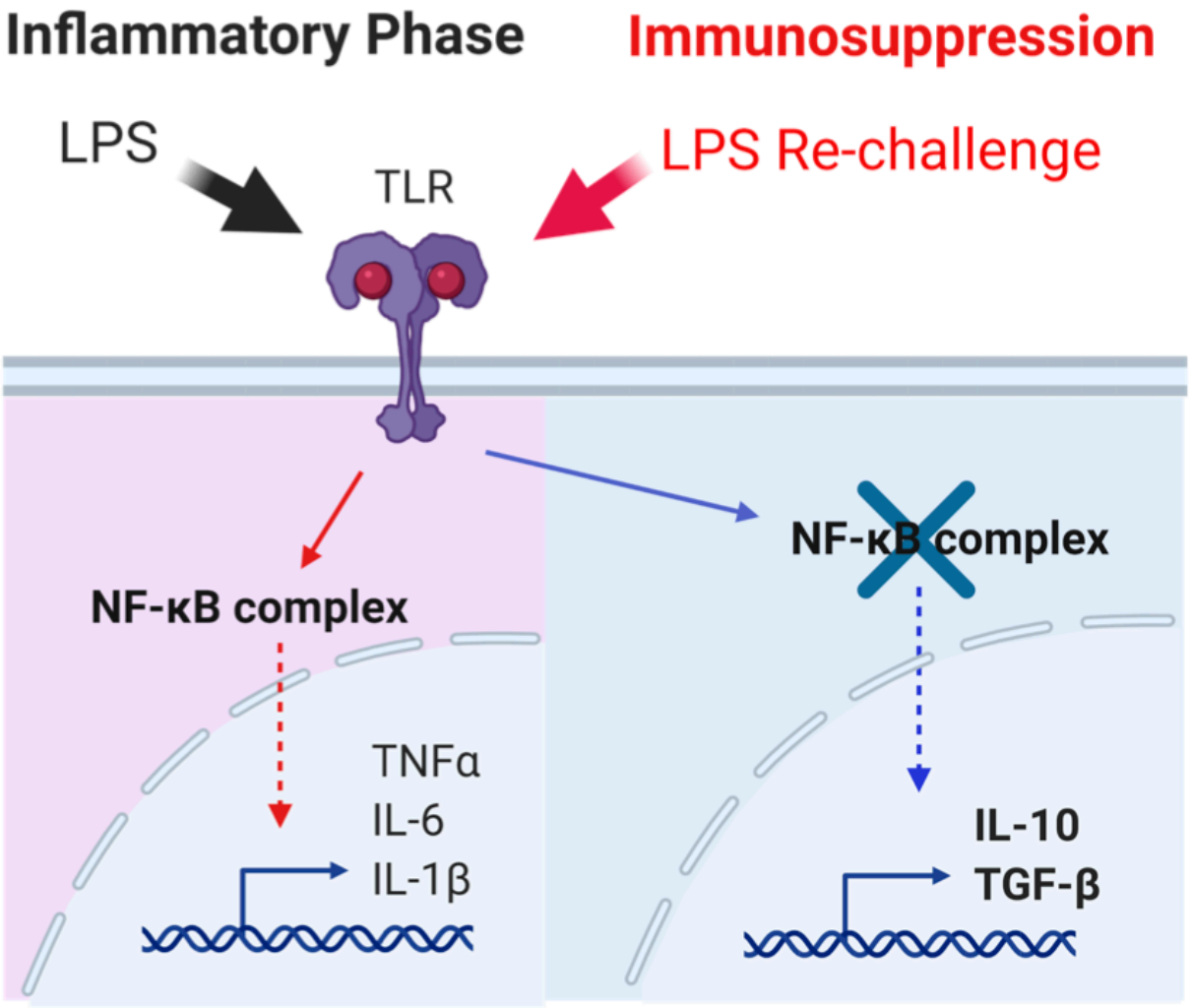

Figure 2

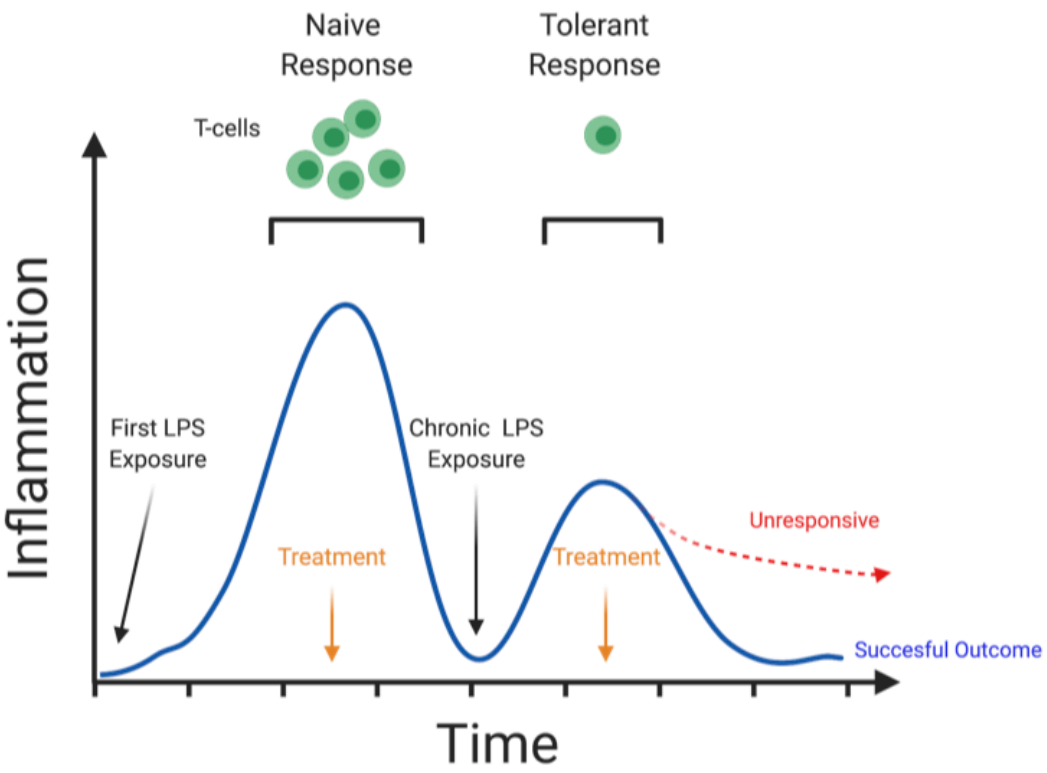

Figure 3

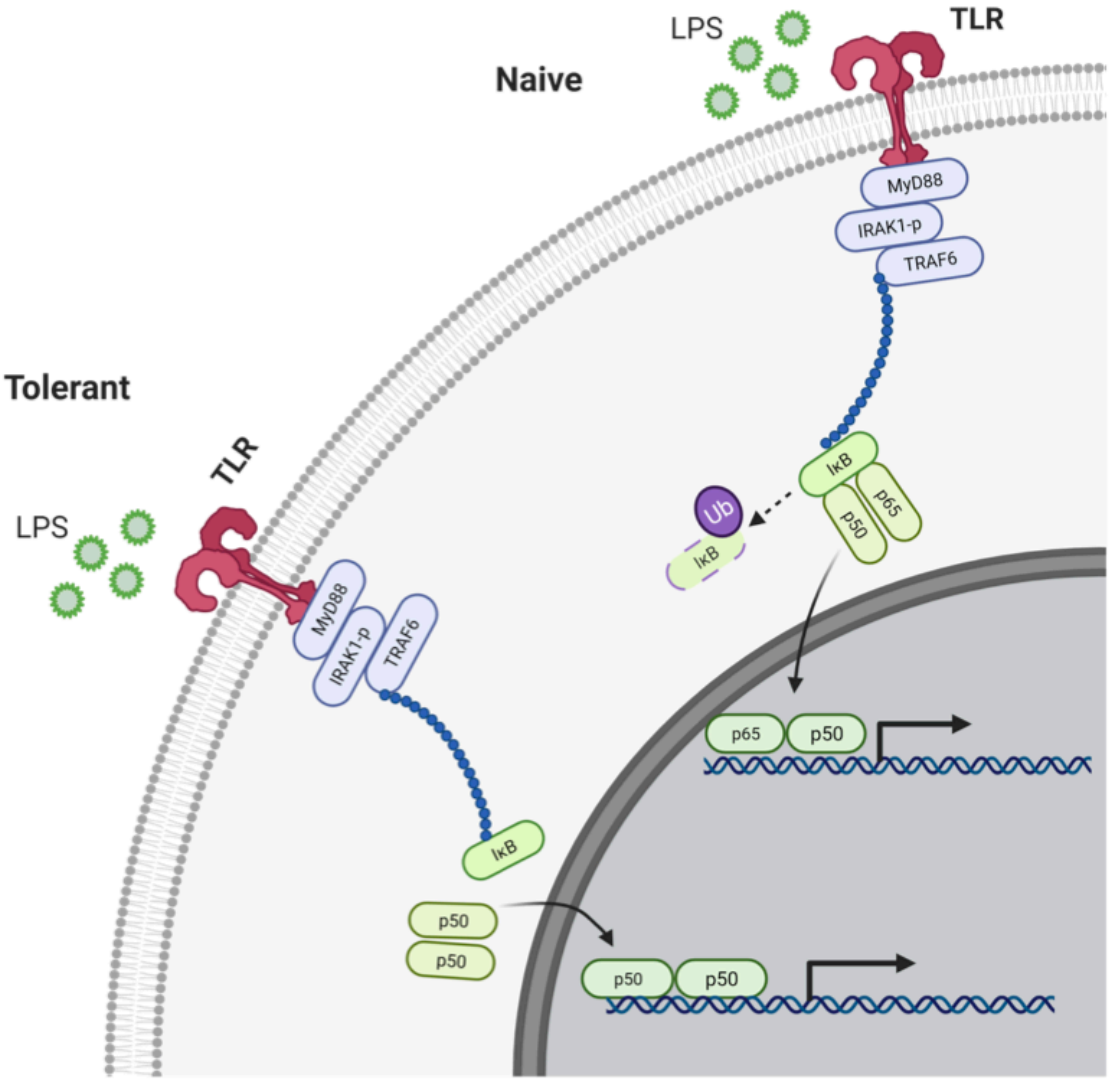

Figure 4