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REVIEW

Periodontal innate immune mechanisms relevant to obesity

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SUMMARY

Obesity affects over 35% of the adult population of the USA, and obesity-related illnesses have emerged as the leading cause of preventable death worldwide, according to the World Health Organization. Obesity's secondary morbidities include increased risk of cardiovascular disease, type II diabetes, and cancer, in addition to increased occurrence and severity of infections. Sedentary lifestyle and weight gain caused by consumption of a high-fat diet contribute to the development of obesity, with individuals having a body mass index (BMI) score > 30 being considered obese. Genetic models of obesity (*ob*/*ob* mice, *db*/*db* mice, and *fa*/*fa* rats) have been insufficient to study human obesity because of the overall lack of genetic causes for obesity in human populations. To date, the diet-induced obese (DIO) mouse model best serves research studies relevant to human health. Periodontal disease presents with a wide range of clinical variability and severity. Research in the past decade has shed substantial light on both the initiating infectious agents and host immunological responses in periodontal disease. Up to 46% of the general population harbors the microorganism(s) associated with periodontal disease, although many are able to limit the progression of periodontal disease or even clear the organism(s) if infected. In the last decade, several epidemiological studies have found an association between obesity and increased incidence of periodontal disease. This review focuses on exploring the immunological consequences of obesity that exacerbate effects of infection by pathogens, with focus on infection by the periodontal bacterium *Porphyromonas gingivalis* as a running example.

INTRODUCTION

Obesity affects over 35% of the adult population of the USA, and obesity-related illnesses have emerged as the leading cause of preventable death worldwide, according to the World Health Organization. Obesity's secondary morbidities include increased risk of cardiovascular disease, type II diabetes and cancer, in addition to increased occurrence and severity of infections (both community-acquired and nosocomial) (Canturk *et al*., 2003). Sedentary lifestyle and weight gain caused by consumption of a high-fat diet contribute to the development of obesity, with individuals having a body mass index (BMI) score ≥30 being considered obese. Rates of obesity worldwide have more than doubled since 1980, underscoring the need for additional research on its molecular biology. Genetic models of obesity (*ob*/*ob* mice, *db*/*db* mice, and *fa*/*fa* rats) have been insufficient to study human obesity, because of the overall lack of genetic causes for obesity in human populations. To date, the diet-induced obese (DIO) mouse model best serves

research studies relevant to human health (Lawrence *et al*., 2012).

Periodontal disease presents with a wide range of clinical variability and severity. Research in the past decade has shed substantial light on both the initiating infectious agents and host immunological responses in periodontal disease, both of which have been shown to modify the disease progression. Cullinan *et al.* (2003) have shown that up to 46% of the general population harbor the causative organism(s) of periodontal disease, although many are able to limit the progression of periodontal disease or even clear the organism(s) if infected. In the last decade, several epidemiological studies have found an association between obesity and increased incidence of periodontal disease (Saito *et al*., 1998, 2001, 2005; Genco *et al*., 2005; Saito & Shimazaki, 2007).

This review focuses on exploring the immunological consequences of obesity that exacerbate the effects of infection by pathogens, with focus on infection by the periodontal bacterium *Porphyromonas gingivalis* as a running example.

OBESITY AND PERIODONTAL INFECTION IN THE DYSREGULATED IMMUNE SYSTEM

The characteristics of an individual's immune response to infectious microbes greatly determine the severity of precipitating periodontal disease (Van Dyke & Sheilesh, 2005). Michalowicz *et al.* (2000) used a twin study to show that genetic hereditability appears to contribute approximately 50% to the clinical susceptibility of periodontal disease. In addition, previous studies have suggested that dysregulation of innate immunity plays a key role in the progression of periodontal disease (Slots & Genco, 1984). Specifically, the host inflammatory response is suppressed upon low-level stimulation of critical pattern recognition receptors (PRRs), leading to a muted local immune response, so enabling periodontal disease-associated bacteria such as *P. gingivalis* to evade the host immune system (Muthukuru *et al*., 2005; Tanabe & Grenier, 2008). *Porphyromonas gingivalis* belongs to the 'red bacteria' complex and as such is considered a major pathogen in the onset of chronic adult periodontitis.(Hajishengallis, 2009) Although found in low abundance in the oral cavity it is responsible for a microbial shift of the oral cavity allowing for excessive growth of the commensal pathogen. *Porphyromonas gingivalis* has been associated with increasing the virulence of other commensal bacterium. Most notably, whereas inoculation of germ-free mice with *P. gingivalis* mono-infection causes no bone loss; in contrast specific pathogen-free mouse models of periodontal infections that include not only inoculation with *P. gingivalis* but also commensal bacteria show significant bone loss – a hallmark of the disease. This clearly indicates that *P. gingivalis* alone cannot induce periodontitis (Hajishengallis *et al*., 2011).

Several mechanisms have been proposed to explain how *P. gingivalis* evades host immune responses. These mechanisms affect *P. gingivalis* virulence and include gingipain proteases, a capsular polysaccharide, induction of host cell proliferation, and the cleavage of chemokines responsible for neutrophil recruitment. (Hajishengallis *et al*., 2011; Vincents *et al*., 2011). Furthermore, leukocyte recruitment is modulated by virulent *P. gingivalis* via proteolysis of cytokines and chemokines secreted by the host cells with Arg-gingipain and Lys-gingipains mainly responsible for this proteolysis. *Porphyromonas gingivalis* was found to downregulate interleukin-8 (IL-8) induction causing delayed neutrophil recruitment, which in turn inhibits the clearance of the microorganism from the site of infection allowing excessive colonization. In addition, opsonization-mediated phagocytosis of polymorphonuclear leukocytes, an important innate immune defense mechanism, was significantly altered of *P. gingivalis* using Gingipain K (Kgp) to cleave immunoglobulin G1 and immunoglobulin G3, further impairing signaling (Vincents *et al*., 2011). Recent studies have reported that that *P. gingivalis* can undermine the complement pathway (C5αR and C3αR), affecting the killing capacity of leukocytes and permitting uncontrolled bacterial growth. (Hajishengallis *et al*., 2011; Liang *et al*., 2011) Proinflammatory and antimicrobial responses were found inhibited by *P. gingivalis* in human monocytes and mouse macrophages. This mechanism involved binding of *P. gingivalis* fimbria to CXCR4, inhibiting the Toll-like receptor 2 (TLR2) -mediated immune response (Hajishengallis *et al*., 2008). Cell invasion by *P. gingivalis* involves inhibiting apoptosis by modulating the JAK/Stat pathway, which controls mitochondrial apoptotic pathways (Mao *et al*., 2007; Kuboniwa *et al*., 2008) A proliferative phenotype may be beneficial to the bacterium as it provides nutrients, impairs

host cell signaling, and compromises the integrity of the epithelial cell layer allowing for invasion and colonization.(Kuboniwa *et al.*, 2008). Periodontitis can now emerge through the disruption of the host tissue homeostasis and immune response (Darveau *et al.*, 2012).

Obesity has been shown to contribute to an individual's immune response to many pathogens (Genco *et al.*, 2005). The effect of obesity on an individual's immune response was further characterized by Amar *et al.* (2007), in showing a reduced proinflammatory cytokine response, specifically using *P. gingivalis* infection to measure the cytokine response elicited in obese vs. lean mice. Taken together, both obesity and chronic exposure to periodontal disease-associated bacteria alone appear to mute the local immune response, and in combination may synergize in suppressing the innate immune system, so exacerbating periodontal disease.

The picture emerging shows that tolerance in the innate immune system occurring as a result of persistent low-level exposure to *P. gingivalis* infection or obesity paralyzes the innate immune response and further aggravates periodontal disease. As a consequence of enhanced periodontal disease, an individual is subjected to repeated bacteremia with *P. gingivalis*, which can lead to repeated bouts of inflammation.

NORMAL IMMUNE RESPONSE TO MICROBIAL PATHOGENS VS. COMMENSAL

The innate immune system plays a prominent role as a first line of defense against pathogens. The response of a mammalian host to microbial pathogens involves the activation of both innate and adaptive components of the immune system. It is based on the recognition of conserved pathogen-associated molecular patterns (PAMPs) that are present on pathogens, but are not found on the host cells. The PAMPs are recognized by PRRs, such as the TLRs, nucleotide-binding oligomerization domains (NODs), cluster of differentiation 14, complement receptor-3, lectins, and scavenger receptors (Areschoug & Gordon, 2008). The TLRs sense the presence of microbial infection and are crucial for the initiation of inflammatory and immune defense responses. An important cellular component of the innate immune response is the macrophage axis, because macrophages are among the first to respond to invasion by a microbial pathogen, along with polymorphonuclear leukocytes. Macrophages are also involved in activating the adaptive arm of the immune response via antigen presentation, so linking the innate response to adaptive immunity. Therefore, macrophages play a critical role in proper immune function and it is important to understand how macrophages detect pathogens and their by-products, and the effect that the detection of a pathogen has on macrophages (Zelkha *et al.*, 2010).

Macrophages are able to detect the presence of a pathogen by recognizing a variety of repetitive motifs commonly found on pathogens referred to as PAMPs. Macrophages express a limited number of PRRs on their surface that can bind to PAMPs and, through subsequent intracellular signaling, trigger the inflammatory response (Medzhitov & Janeway, 2000; Aderem, 2003; Akira *et al.*, 2006; Beutler *et al.*, 2006).

One type of PRR that is capable of recognition and signaling associated with *P. gingivalis* exposure is the TLR family (Dobrovolskaia *et al.*, 2003; Michelsen *et al.*, 2004). The TLRs monitor the extracellular environment and phagolysosomal compartments, and recognize PAMPs such as lipopolysaccharide (LPS), flagellar proteins, CpG DNA, oxidized low-density lipopeptide, and endogenous proteins like heat-shock protein 60 (Zuany-Amorim *et al.*, 2002), and bacterial lipoprotein (Medzhitov, 2001). More specifically, activation of TLR2, known to recognize gram-negative bacterial cell wall components, has been shown to lead to an increase in the inflammatory response (Burns *et al.*, 2006). Hence, the normal role of TLR2 is critical to the detection and clearance of gram-negative microbial pathogens such as *P. gingivalis*.

Another major class of PRRs includes soluble cytosolic nucleotide-binding oligomerization domain-like receptors, which complement host defenses by providing an intracellular layer of surveillance. Nucleotide-binding oligomerization domain (NOD) proteins recognize cell wall fragments from both gram-negative and gram-positive bacteria (Chamaillard *et al.*, 2003; Girardin *et al.*, 2003; Inohara *et al.*, 2005). Nucleotide-binding oligomerization domain-1 (NOD1) recognizes a specific peptidoglycan fragment containing diaminopimelic acid, while NOD2 recognizes a muramyl dipeptide fragment of peptidoglycan. Both TLR and NOD proteins are found on macrophages, although how these cells initiate a threat-specific transcriptional

response is poorly understood. Combined, the TLRs and NODs represent two critical layers of defense to detect pathogens and differentially modulate the immune response (Takeda & Akira, 2003; Girardin & Philpott, 2004).

Activation of TLR2, NOD1, or NOD2, which is common after *P. gingivalis* exposure, results in the activation of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) protein complex and its intracellular signaling pathway (Asai *et al*., 2001; Wang & Ohura, 2002). Once NF-κB is activated, expression of many proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-1β and IL-6 (Geivelis *et al*., 1993; Yavuzyilmaz *et al*., 1995; Salvi *et al*., 1998), chemokines including IL-8, monocyte chemotactic protein-1 and chemokine (C-C motif) ligand 5 (RANTES) (Tsai *et al*., 1995; Emingil *et al*., 2004), C-reactive protein (Salzberg *et al*., 2006; Dasanayake, 2009), and mRNA for TLRs (Ren *et al*., 2005) is initiated. In periodontal disease, such signaling is most likely to occur in gingival epithelial cells, gingival fibroblasts, and macrophages (Asai *et al*., 2001; Wang & Ohura, 2002).

It is important to notice that bacterial ligands recognized by TLRs are not unique to pathogens, but rather are shared by entire classes of bacteria, and are also produced, therefore, by commensal bacteria. One important question that remains the focus of intense investigation is how the host distinguishes between pathogenic and commensal bacteria. Several mechanisms have been proposed to explain how the host discriminates between pathogens and commensals so as to trigger TLR signaling when appropriate. These include (i) tolerance of commensal PAMPs, (ii) compartmentalized TLR expression, and (iii) commensal manipulation of TLR signaling inducing tolerant responses (Lewis, 2008). As similar PAMPs are present on both commensals and pathogens, it is probable that the latter differ from the former mainly in terms of the differential expression of cell surface molecules, such as adhesion proteins and other virulence factors that may affect the host (Lewis, 2008). Recently, the select usage of CXCR2 ligands was found to be crucial in allowing the host to homeostatically regulate neutrophil migration to the periodontal tissue in response to – and for immune surveillance of – commensal bacterial colonization (Zenobia *et al*., 2013). This is of great importance given that the main mechanisms by which periodontal tissue regulates the numbers of oral commensal bacteria that reside on the tooth and its root surface depends on the constant transit of neutrophils through gingival tissue and into the gingival crevice (Zenobia *et al*., 2013).

DYSREGULATION OF IMMUNE RESPONSE TO MICROBIAL PATHOGENS AND HOMOTOLERANCE

Lipopolysaccharide is an endotoxin found on the outer membrane of gram-negative bacteria, such as *P. gingivalis*, that has been shown to be a potent activator of the innate immune response, especially in macrophages. It can also be released from dying gram-negative bacteria, which can then bind to lipopolysaccharide-binding protein (LBP). The LPS–LBP complex transfers the LPS first to cluster of differentiation 14, and then to lymphocyte antigen 96 protein (MD-2), which ultimately activates TLR4 pro-inflammatory signaling (Mogensen, 2009) on macrophages and dendritic cells (Johnson & Sayles, 2002; Johnson *et al*., 2002; Jerala, 2007).

In seeming contradiction to this signaling cascade, Tanabe & Grenier (2008) have recently shown that low-level pre-treatment stimulation of macrophages with *Aggregatibacter actinomycetemcomitans* LPS (0.01–0.1 μg ml^{-1}), has been shown to reduce TNF-α levels in response to subsequent stronger (1 μg ml^{-1}) LPS stimulation within 24 h. Dobrovolskaia *et al.* (2003) further characterized this reduced inflammatory response as homotolerance. Homotolerant cells were observed to secrete reduced levels of TNF-α when pre-treated with a specific ligand to a PRR, such as *P. gingivalis* LPS, and then subsequently challenged with a more potent dose of the same agonist.

Further work by Muthukuru *et al.* (2005) has shown that similar pre-treatment and subsequent strong challenge with *P. gingivalis* LPS specifically demonstrated homotolerance by reduction of TLR2 and TLR4 mRNA levels, suggesting alterations in the transcription factors present at TLR promoters or the TLR genes themselves are responsible for the reduced inflammatory response. The decreased secretion of a critical inflammatory mediator TNF-α was shown by Tanabe & Grenier (2008) to be one effect of *Aggregatibacter actinomycetemcomitans* LPS induction of a homotolerance response in

macrophages. However, homotolerance was not observed across the entire spectrum of pro-inflammatory cytokines. Both IL-1β and matrix metalloproteinase 9 showed increased secretion upon low-level pre-treatment stimulation and subsequent strong LPS stimulation (Tanabe & Grenier, 2008).

Additionally, the reduced immune response was not observed when cells were pre-treated with a ligand for one PRR and subsequently challenged with a ligand for a different PRR [for example, pre-treatment with *P. gingivalis* LPS and challenge with *N*-palmitoyl-*S*-[2,3-bis(palmitoyl)-(2RS)-propyl]-(R)cysteinyl-alanyl-glycine (Pam3Cys), a synthetic lipopeptide that activates different PRRs]. Dobrovolskaia *et al.* (2003) called this response heterotolerance.

Findings concerning macrophage stimulus tolerance demonstrate the role of *P. gingivalis* in fine-tuning the host inflammatory response and progression of periodontitis, resulting in a muting of the innate immune response, and enabling *P. gingivalis* to evade the host immune defense mechanisms. This appears to be a critical evolutionary feature of *P. gingivalis* that provides the bacterium with a means to survive and thrive in periodontal sites. Alternatively, homotolerance could also represent a host protective measure aimed at protection against endotoxin-induced septic shock to prevent a potentially fatal systemic inflammatory response/cytokine release (Biswas & Lopez-Collazo, 2009).

Macrophages of obese animals are functionally impaired, which leads to reduced phagocytic capacity and defective oxidative burst (Lee *et al.*, 1999; Mancuso *et al.*, 2002; Ryan *et al.*, 2008), making the body of the host more susceptible to infections. The lack of cytokine expression in response to infections has been linked to dysfunction in macrophages and/or a defect in the maturation of monocytes (Mito *et al.*, 2000; Amar *et al.*, 2007; Smith *et al.*, 2007). It is increasingly recognized that obesity is characterized by a dysregulation of inflammatory pathways such as those that are activated by TLR2. However, the fundamental mechanisms responsible for this dysregulation are poorly understood.

Recent data have demonstrated that diet-induced obesity dysregulates TLR2 and TLR4 expression, significantly reduces protein kinase B (Akt/PKB) phosphorylation in peritoneal macrophages, and reduces the immune response to *P. gingivalis* (Zhou *et al.*, 2009). TLR2 may be a candidate for participation in the cross-talk between inflammation and metabolic signals because both *P. gingivalis* and free fatty acids (FFAs) can activate it (Shi *et al.*, 2006; Nguyen *et al.*, 2007), due to the fact that TLR2 recognizes lipids (Liang *et al.*, 2011). Recent studies have shown that *P. gingivalis* LPS activates phosphoinositide 3-kinases and Akt/PKB through TLR2, leading to extracellular signal-regulated kinases (ERKs), or classical mitogen-activated protein kinases (ERK1/2) activation, and TNF-α expression (Shi *et al.*, 2002). Pre-treatment of macrophages with *P. gingivalis* LPS induces TLR2 homotolerance, as previously discussed, and both *P. gingivalis* and FFAs activate the innate immune response through the TLR2 pathway.

Zhou *et al.* (2009) have proposed a 'homotolerance' hypothesis to explain the immune dysregulation observed in a DIO model upon exposure to *P. gingivalis*. Previous work from Muthukuru *et al.* (2005) showed that pre-treatment with *P. gingivalis* LPS (1000 ng ml^{-1}) induced similar tolerance in innate immune response independently of obesity. There may be an additive effect when obesity is combined with *P. gingivalis* LPS exposure, further enhancing tolerance and immune dysregulation in obese patients with periodontal disease. In addition, the role of TLRs has recently been advocated in periodontal disease, as evidenced by reduced bone loss in periodontal disease for TLR2- and TLR4-deficient mice (Hou *et al.*, 2000; Costalonga *et al.*, 2009). Indeed we recently looked at the effect of *P. gingivalis* LPS on the recruitment of NF-κB to the TNF-α and IL-10 promoters in DIO vs. lean mouse macrophages. Recruitment of NF-κB to both TNF-α and IL-10 promoters in macrophages from lean mice at 30 min was readily detected, whereas this recruitment was substantially reduced in macrophages from mice with DIO, probably resulting from a homotolerance (Amar *et al.*, 2007).

It is, however, still possible that this tolerance might be TLR-independent as reported for foam cell formation in the atherosclerosis process. A study reported that that 25-hydroxycholesterol inhibits an LPS-induced inflammatory process by inducing an LPS tolerance in macrophages. 25-Hydroxycholesterol that induces LPS tolerance in macrophages could therefore contribute to a decreased inflammatory activity in foam cells. This could be seen in lesions as a low chronic inflammation in the presence of macrophages and foam cells. The mechanisms for LPS tolerance seem to be multiple (Englund *et al.*, 2001).

OBESITY AND PERIODONTAL DISEASE

It is known that the innate and adaptive immune responses are affected by obesity (Tanaka *et al.*, 1993; Stallone, 1994; Marti *et al.*, 2001). Obesity has been characterized as an altered systemic inflammatory state resulting from an imbalance in the cytokine network, and increased levels of acute-phase proteins and pro-inflammatory cytokines such as TNF-α and leukocytes (Aronson *et al.*, 2004; Genco *et al.*, 2005) in the plasma of obese subjects (Kanbay *et al.*, 2008; Perez-Echarri *et al.*, 2008). Furthermore, it has been observed that macrophage infiltration of the white adipose tissue of obese mice in numbers occurs in direct proportion to adipocyte size and number (Weisberg *et al.*, 2003; Faintuch *et al.*, 2007). However, macrophage effector functions are impaired in obese animals, with a reduced phagocytic capacity and a defective oxidative burst (Lee *et al.*, 1999; Mancuso *et al.*, 2002; Ryan *et al.*, 2008). As in humans, other species of obese animals also display delayed wound healing that is associated with dysregulated polymorphonuclear leukocytes and macrophage infiltration (Goren *et al.*, 2003). Acquired immunity is also affected by obesity, as evidenced by impaired T-cell- and B-cell-mediated immune responses in obese *ob*/*ob* and diabetic *db*/*db* mice (Mandel & Mahmoud, 1978; Chandra & Au, 1980). In addition, the levels of classical immune cytokines (TNF-α, IL-6, IL-1 receptor antagonist, and transforming growth factor-β) secreted by adipocytes are significantly increased in obesity (Fantuzzi, 2005).

Consumption of a high-fat diet leads to elevated plasma FFA levels. The FFAs are able to activate TLR-mediated signaling pathways and thereby induce TNF-α expression. TLR2 can be activated by palmitate, a nutritional FFA. Although TLR2 normally downregulates the TNF-α expression induced by FFAs, it is dysfunctional in the macrophages of individuals with diet-induced obesity, and TNF-α expression remains high. As a result, TNF-α is often chronically elevated in individuals with obesity. Therefore, the decreased immune response observed in DIO individuals may be related to the disruption of TLR2 signaling pathways by elevated plasma FFAs or by a DIO-related state of chronic inflammation.

High-fat diet also raises the intracellular pool of C-terminal modulator protein (CTMP), which inhibits Akt phosphorylation and attenuates innate immune responses in the macrophages of individuals with obesity. At first, FFAs induce the upregulation of CTMP by activating TLR2. Later, CTMP remains high when the aforementioned defective TLR2 is unable to inhibit TNF-α-induced CTMP. Elevation of CTMP by FFAs and TNF-α, in addition to the disruption of TLR2 signaling in macrophages, is a factor in the impairment of innate immune function in DIO individuals. In this way, obesity directly causes innate immune dysfunction (Zhou *et al.*, 2009).

This immune paralysis is supported in epidemiological studies of obese individuals, which found evidence of increased susceptibility to infections (Espejo *et al.*, 2003), including postoperative infectious complications, and a positive correlation between BMI and the incidence of both community-acquired and nosocomial infections (Canturk *et al.*, 2003). Recent evidence points to a high-fat diet, which interferes with the ability of the immune system to appropriately respond to *P. gingivalis* infection (Amar *et al.*, 2007), as the cause. This has also been observed in mice as greater susceptibility to infection by influenza virus, as well as greater periodontal bone loss following *P. gingivalis* infection (Smith *et al.*, 2007). Dissecting the molecular mechanisms behind this dysregulation will certainly shed light on the most appropriate targets for interventional studies (Schoneveld *et al.*, 2008), which will be discussed at greater length at the end of this chapter.

OBESITY'S EXACERBATION OF PERIODONTAL DISEASE

Figure 1 illustrates the effect of obesity on peripheral innate immune response to *P. gingivalis* infection. In normal mice, a homeostatic cytokine network maintains a regulated response to bacterial challenge through a cycle of transient inflammation, followed by downmodulation with anti-inflammatory cytokines. As obesity develops, monocyte dysregulation develops as a result of the FFA-induced homotolerance along the TLR2 signaling pathway. Together, these perturbations mute the homeostatic network that normally counters the inflammation associated with infections. Obesity becomes associated with an altered proinflammatory and anti-inflammatory network, an altered gene expression profile in monocytes and macrophages, an altered capacity for signaling through TLRs and other microbially induced pathways, and an

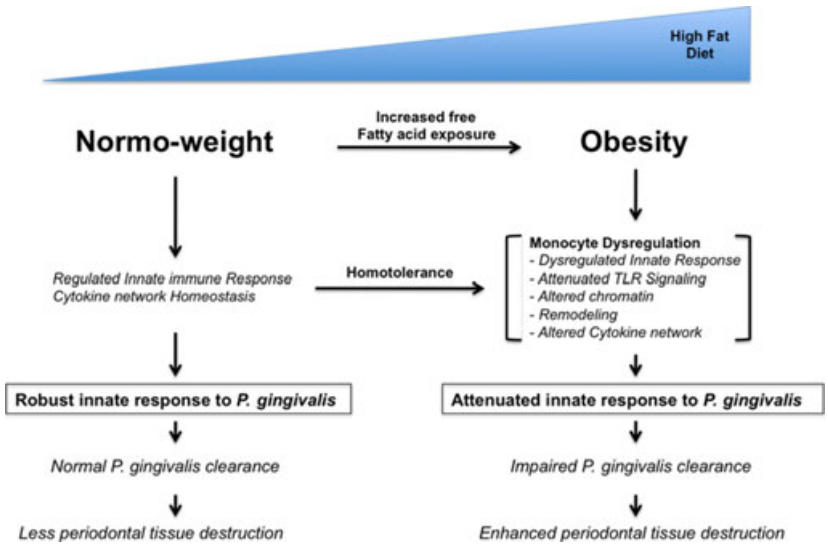

Figure 1 Model for proposed effect of obesity on innate response to *Porphyromonas gingivalis*.

altered chromatin status definable at specific gene loci (Zelkha *et al*., 2010). Ultimately, obesity fosters a state of attenuated innate response to *P. gingivalis* with an impaired *P. gingivalis* clearance that has been established to result in enhanced tissue damage and bone loss (Amar *et al*., 2007).

Obesity coupled with periodontal disease appears to lead to a significantly higher level of dysregulated innate immune responses to *P. gingivalis* infection and increased periodontal morbidity and exacerbation of periodontal disease-linked disorders, such as cardiovascular disease. Furthermore, this dysregulation may be a systemic phenomenon characterized by a muted innate immune response to many pathogens leaving a host potentially susceptible to the deleterious effects of these pathogens (Zelkha *et al*., 2010).

Therapeutically we reported recently that moderate daily exercise with dietary control results in the improvement of host immune responses including periodontal bone loss through multiple molecular mechanisms. Although this restoration was observed with imposed diet control and exercise, human obesity involves important complex behavioral factors that affect diet choices and exercise. Given that human obesity remains a notable epidemic with public health implications, the present results provide further impetus for obese individuals to scale up efforts for a self-imposed regimen of exercise and control of diet (Zhou *et al*., 2011).

CONCLUSIONS

In this review we have strived to link exposure of *P. gingivalis* to exacerbations in periodontal disease through induction of homotolerance by obesity.

Obesity is a condition that is known to affect the periodontal complex. Although obesity is a state of hyper-inflammation characterized by expanded numbers of macrophages, leukocyte and lymphocyte infiltration into adipose tissue, and an activated cytokine network, sustainability of the immune system seems paralyzed in response to various infections. Recent findings by Zhou *et al*., 2009, 2011. have demonstrated a form of tolerance in reduced TLR expression levels when obese mice are repeatedly infected with *P. gingivalis* compared with lean infected mice. This has the effect of reducing the TNF-α level similar to the homotolerance seen when low levels of LPS pre-treatments with macrophages (Kim & Amar, 2006; Zhou *et al*., 2009). Furthermore, the contribution of homotolerance induced by obesity may be additive to the homotolerance induced by *P. gingivalis* exposure, such that a higher degree of homotolerance exists in the combined obese plus *P. gingivalis*-infected individual than in individuals with either condition alone. Taken together, obesity mutes the immune response to *P. gingivalis*, which may be critical in providing an ideal environment for *P. gingivalis* to thrive and so exacerbate periodontal disease.

As exposure to dietary FFAs increases, homotolerance is induced along the TLR2 pathway in the innate immune system. Homotolerance alters TLR signaling pathways by altering the expression levels of TLR2 and possibly chromatin remodeling at the TLR2 gene or other gene loci involved in the signaling pathway or cytokine release. The effect of homotolerance leads to a dysregulated innate immune response and altered cytokine network upon exposure to *P. gingivalis*. As a result, the innate immune system has impaired clearance of *P. gingivalis*, which leads to enhancement of periodontal tissue destruction.

The concept of homotolerance is emerging as a critical driver in periodontal disease progression and effects of obesity on the immune system. Endotoxin tolerance has been a known phenomenon since 1950 when Neva & Morgan (1950) demonstrated tolerance in the exposure of enteric bacteria in patients with typhoid fever. However, only in the past decade have the molecular mechanisms of how tolerance is achieved in the innate immune system been elucidated, and only very recently have we had an understanding of its implications on various disease states, like periodontal disease.

Homotolerance appears to be a mechanism designed to fine-tune the immune system to ignore a low-level stimulation of PRRs, thereby preventing repeated bouts of inflammation or even sepsis. This would certainly be ideal in areas of the body with repeated exposure to such pathogens, such as the lung or colon. However, in the oral cavity it appears *P. gingivalis* is able to take advantage of this mechanism by using it to tolerate resident and infiltrating leukocytes and effectively mute any immune response against it, so exacerbating periodontal disease.

Further understanding of the mechanisms that generate this homotolerant state are required to fully understand how this state is induced, what its effects are on the immune system, and what pathological conditions are affected by homotolerance. Current findings by Muthukuru *et al.* (2005) showed that the PRR expression levels drop in response to low-level pre-treatments with TLR2 and TLR4 agonists, pointing to a reduction in mRNA production. The reduced expression could possibly be caused by alterations in transcription factors at the promoter, the context of the gene at the promoter region itself, like epigenetic regulation, or even by the induction of small interference RNAs to silence TLR-generated mRNAs, to name a few.

In addition, research on how low-level stimulation, but not a high-level stimulation, induces tolerance would be critical in furthering the understanding of homotolerance. Specifically, further research is needed to determine the induction threshold, where too little stimulation induces tolerance vs. too much stimulation induces inflammation. Answers to questions such as these would also be critical in our understanding of homotolerance. These understandings could then be translated into better identification of rational therapeutic designs to better focus targets on key processes of disease progression. The implications are staggering when thinking of the obesity epidemic and the prevalence of periodontitis. Furthermore, treatments targeted at homotolerance would be more of a preventive measure instituted pre-clinically, before the overt debilitating and potentially fatal secondary effects of obesity manifest in the patient.

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