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ABSTRACT

The clinical association between obesity-associated type 2 diabetes (T2D) and periodontitis, coupled with the increasing prevalence of these diseases, justifies studies to identify mechanisms responsible for the vicious feed-forward loop between systemic and oral disease. Changes in the immune system are critical for both obesity-associated T2D and periodontitis and therefore may link these diseases. Recent studies at the intersection of immunology and metabolism have greatly advanced our understanding of the role the immune system plays in the transition between obesity and obesity-associated T2D and have shown that immune cells exhibit similar functional changes in obesity/T2D and periodontitis. Furthermore, myeloid and lymphoid cells likely synergize to promote obesity/T2D-associated periodontitis despite complexities introduced by disease interaction. Thus the groundwork is being laid for researchers to exploit existing models to understand immune cell dysfunction and break the devastating relationship between obesity-associated T2D and oral disease.

KEY WORDS: periodontal disease, inflammation, cell biology, cytokine(s).

Immune Cells Link Obesity-associated Type 2 Diabetes and Periodontitis

INTRODUCTION

Obesity prevalence has dramatically increased over the past 2 decades such that over 500 million adults are now categorized as obese [body mass index (BMI) ≥ 30.0 kg/m²] (Swinburn *et al.*, 2011). Obese individuals have a more than ten-fold risk of developing type 2 diabetes (T2D) compared with normal-weight individuals (Kopelman, 2007), although the appreciation of the subset of obese individuals who remain metabolically healthy (the metabolically healthy obese [MHO]; Fig. 1A) is growing (Denis and Obin, 2013). Classification of metabolic status in obese individuals is important for the clinician to interpret accumulating evidence of an association between non-diabetic obesity and periodontitis (Suvan *et al.*, 2011), and the increased prevalence, progression, and severity of periodontitis in obese individuals compared with lean individuals (Gorman *et al.*, 2012; Jimenez *et al.*, 2012). This newly appreciated relationship between obesity and periodontitis is consistent with the well-established paradigm of a bi-directional relationship between obesity-associated T2D and periodontitis, including work showing that T2D increases susceptibility to periodontitis and that periodontal inflammation negatively affects glycemic control (Preshaw *et al.*, 2012). Because obesity and T2D are highly interrelated, and both contribute to the development and progression of periodontal diseases, it is extremely challenging to delineate causal relationships among obesity, T2D, and periodontitis through clinical studies (Fig. 1B). Evidence from the clinic and experiments in model systems (*in vivo* and *in vitro*) show that multiple factors contribute to the pathogenesis of periodontitis in both obesity and obesity-associated T2D. Herein we highlight immune system changes that drive inflammation in obesity, obesity-associated T2D, and periodontitis and may mechanistically explain the clinical associations among these diseases, and may justify sub-classification of obese animals and people into MHO and metabolically unhealthy in terms of both experimental outcomes and treatment options.

IMMUNE RESPONSES IN OBESITY AND OBESITY-ASSOCIATED T2D

In contrast to MHO, which is generally characterized as non-inflammatory (Denis and Obin, 2013), obesity-associated T2D is characterized by chronic low-grade inflammation. Most animal models of obesity have metabolic disease, including insulin resistance. Thus the “MHO” phenotype for animals occurs only under artificial conditions and is a relatively common outcome in mice that lack immunity-related genes. Regardless, the inflammatory nature of obesity-associated T2D raises the possibility that inflammation combines with obesity to promote T2D. Multiple studies have investigated the relationship between immunological and metabolic processes to explore the pathogenesis of obesity-associated T2D. One focus of this work is the dynamic

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nature of immune cell activation and tissue infiltration in obesity, with growing emphasis on pro-inflammatory functions of adipose-tissue-associated and re-circulating cells of the immune system.

Adipose tissue cells, or adipocytes, manage lipid availability and secrete hormones (adipokines), including anti-inflammatory adiponectin and pro-inflammatory leptin, to maintain metabolic homeostasis. Adipose tissue macrophages reside adjacent to adipocytes and exhibit an anti-inflammatory phenotype that maintains a non-inflammatory state in lean and perhaps MHO individuals (Lumeng *et al.*, 2007; Chawla *et al.*, 2011; Denis and Obin, 2013). Adipose tissue homeostasis is further reinforced by a unique population of CD4⁺ regulatory T cells (Tregs), which function largely as negative regulators of adipose tissue inflammation and systemic metabolic imbalance (Feuerer *et al.*, 2009).

As obesity progresses and adipose tissue expands, adipocytes secrete less adiponectin and more leptin and chemokines, and adipose-associated immune cells change in parallel. It is not clear whether the original adipose-tissue-resident immune cells become directly activated in response to obesity; however, evidence indicates that circulating immune cells infiltrate the expanding adipose tissue in response to high-fat feeding (Oh *et al.*, 2012). Although the exact order of immune cell infiltration into the expanding adipose tissue remains controversial, increased numbers of both neutrophils and macrophages appear early in disease pathogenesis (Elgazar-Carmon *et al.*, 2008; Oh *et al.*, 2012). B-cell infiltration followed by T cells (Duffaut *et al.*, 2009; Winer *et al.*, 2011) is also essential to obesity-associated inflammation. Although the proposed role of B-cell antibodies in inflammatory obesity remains controversial (Winer *et al.*, 2011; DeFuria *et al.*, 2013), B cells likely function at least in part through an ability to secrete pro-inflammatory cytokines and to regulate T-cell and macrophage-mediated inflammation (Winer *et al.*, 2011; DeFuria *et al.*, 2013). Infiltrating CD8⁺ T cells similarly regulate adipose tissue inflammation, as evidenced by demonstrations that obesity-associated CD8⁺ T cells secrete IFN- γ , an important pro-inflammatory cytokine for macrophage activation and obesity-induced inflammation (Rocha *et al.*, 2008; Nishimura *et al.*, 2009). CD8⁺ T-cell deletion results in a relative lack of inflammatory macrophages in obese adipose tissue and, importantly, systemic insulin sensitivity (Nishimura *et al.*, 2009). Finally, in contrast to the decreased numbers and percentages of Tregs in adipose tissue and in circulation during obesity (Feuerer *et al.*, 2009; DeFuria *et al.*, 2013), CD4⁺ Th1 cells expand and further contribute to overall IFN- γ amounts (Rocha *et al.*, 2008; Winer

Figure 1. The relationships among obesity, type 2 diabetes (T2D), and periodontitis. **(A)** Obesity and T2D are highly interrelated in humans. Under conditions of calorie excess (diet-induced obesity), many people develop metabolically abnormal obesity (MAO), which includes individuals with obesity-associated T2D. However, some with T2D are not obese, and this is more prevalent in some genetic backgrounds. There are also individuals labeled 'metabolically healthy obese' (MHO) who are relatively protected from obese-associated co-morbidities including inflammation and insulin resistance. These distinct populations are not seen in wild-type mouse models, since mice usually develop obesity and metabolic abnormalities simultaneously. **(B)** The recognition of obesity as a cause of T2D and the bi-directional relationship between T2D and periodontitis are well-appreciated. It has been recently established that there is an association between obesity and periodontitis. Analysis of accumulating data, taken together with evidence that T2D potentiates periodontitis, suggests a three-way relationship among obesity, T2D, and periodontitis, with each condition influencing the other 2. The altered microbiota and dysregulated host response (including immune cell dysfunction) could be dynamic drivers (arrows) of these disease gears. Studies often fail to identify obese individuals adequately as MHO or as metabolically unhealthy; thus the location of MHO in this panel is unknown.

et al., 2009; Nikolajczyk *et al.*, 2012). Pro-inflammatory CD4⁺ Th17s also accumulate in obese adipose tissue (Winer *et al.*, 2009; DeFuria *et al.*, 2013). Overall, the shift from dominance of anti-inflammatory Tregs to pro-inflammatory Th1/Th17s (Feuerer *et al.*, 2009; Winer *et al.*, 2009; Nikolajczyk *et al.*, 2012) reinforces macrophage and B-cell pro-inflammatory cytokine profiles in obesity-associated T2D.

B cells and T cells play critical roles in obesity-associated inflammation and insulin resistance, but myeloid cells eventually dominate obese adipose tissue inflammation through relatively well-understood mechanisms. Saturated free fatty acids and/or hyperglycemia activates macrophages, thus triggering a cytokine-driven inflammatory cascade and insulin resistance, primarily via TNF- α , IL-6, and IL-1 β , among others (Saber *et al.*, 2009; Chawla *et al.*, 2011). Related work on obesity-associated dendritic cells (DCs), a macrophage cousin, shows that DCs contribute to inflammatory obesity by regulating a pro-inflammatory CD4⁺ T-cell response (Bertola *et al.*, 2012). DCs also initiate and regulate macrophage adipose tissue recruitment in obesity to fuel the feed-forward inflammatory loop (Stefanovic-Racic *et al.*, 2012). Overall, over-nutrition and the resultant obesity (often confounded by metabolic imbalance) change both immune cell trafficking and function, and the vast majority of animal studies show that loss of pro-inflammatory immune cell function protects against obesity and/or obesity-associated insulin resistance.

IMMUNE RESPONSES IN PERIODONTITIS

If obesity and/or obesity-associated T2D (obesity/T2D) potentiates periodontitis by priming the immune system, one might expect similar alterations in innate and adaptive immune cell function in the 2 diseases. Although the temporal order of immune cell infiltration and inflammation in chronically infected gingiva may differ from the order described above for the expanding adipose tissue in inflammatory obesity/T2D, many studies highlight the parallels between pro-inflammatory changes in individuals with periodontitis and obese individuals with T2D. Although many periodontitis studies have not reported the BMI or metabolic health status of human participants, most murine periodontitis studies have been completed on chow-fed mice, which (excepting select genotypes) are generally lean. For clarity, we have mentioned the obesity status of the individuals (when known) in the periodontitis studies highlighted below. However, very few studies have directly compared mechanisms of periodontitis in lean/non-diabetic and obese/non-diabetic individuals.

Just as adipocytes are the dominant cell type in lean and obese adipose tissue, gingival epithelial cells and fibroblasts are major cell types of gingival tissue that help maintain homeostasis. In healthy gingiva, epithelial cells and fibroblasts mediate a “simmering” innate immune response and direct gingival trafficking of more traditional immune system cells (Bosshardt and Lang, 2005). Gingival trafficking coordinates movement of neutrophils, among other cell types, which migrate through the junctional epithelium and into the gingival crevice to form a barrier against plaque biofilm in healthy gingiva. Although neutrophils also phagocytose and kill potentially pathogenic microorganisms, timely clearance of apoptotic neutrophils by macrophages (Hajishengallis and Hajishengallis, 2014) also links innate immune mechanisms to oral health.

Periodontitis in lean individuals is characterized by multiple changes in gingival innate immunity. Gingival epithelial cells and fibroblasts respond to pathogen LPS by the transient expression of cytokines such as TNF- α and IL-1 β ; thus these cells likely play an active role in the initiation and maintenance of gingival inflammation (Miyauchi *et al.*, 2001). In addition, neutrophils exhibit pro-survival and hyper-responsive phenotypes that extend neutrophil life and promote excessive production of reactive oxygen species (Dias *et al.*, 2011; Lakschevitz *et al.*, 2013), which further contribute to periodontitis pathogenesis. Interestingly, early neutrophil function in periodontitis is similar to the early contribution of neutrophils to obesity-associated adipose tissue inflammation, despite the sterile nature of the latter process. Periodontitis is also characterized by monocyte infiltration. The infiltrating monocytes differentiate into macrophages or, in some cases, into dendritic cells (DCs). Like neutrophils, macrophages phagocytose periodontal pathogens, and furthermore orchestrate wound repair by functionally coordinating innate and adaptive immune responses. Macrophages recognize oral microbes through surface Toll-like receptors (TLRs) (Muthukuru *et al.*, 2005), which activate pro-inflammatory cytokines, chemokines, and microbicidal molecules. Thus, classically defined innate responses mediate multiple outcomes in chronically infected oral tissue.

In addition to the role cellular mechanisms play in periodontitis in lean individuals, molecular mechanisms also determine the outcome of oral infection. TLRs regulate oral inflammation in part through a chronic infection-associated mechanism termed ‘endotoxin tolerance’. Endotoxin tolerance is defined as a severely blunted cytokine response to a secondary dose of endotoxin that follows closely on the heels of initial endotoxin challenge. Interestingly, *P. gingivalis* LPS, a TLR2-agonist, induces macrophage tolerance (Muthukuru *et al.*, 2005), perhaps to facilitate *P. gingivalis* evasion from the immune system. However, new work surprisingly concludes that *P. gingivalis* exploits TLR2 in *Escherichia coli* LPS-tolerized macrophages to induce excessive production of TNF α , thus driving oral bone loss (Papadopoulos *et al.*, 2013). Although a parallel role for endotoxin tolerance or the ability of *P. gingivalis* LPS to ignore *E. coli* LPS-induced tolerance has not been described in obesity, the demonstration that tolerance requires adiponectin (Zacharioudaki *et al.*, 2009), an anti-inflammatory adipokine that is severely decreased in obesity, allows us to speculate that macrophage tolerance may not be critical in periodontitis in obese individuals.

Besides the innate immune responses of cells like fibroblasts and myeloid cells, multiple lines of evidence implicate lymphocytes in unresolved oral infection. B cells and their terminally differentiated offspring, plasma cells, dominate chronic periodontitis lesions (Berglundh and Donati, 2005). B cells spearhead multiple host defense mechanisms, including the production of antibodies that identify and bind to antigen in a highly specific “lock and key” mechanism. B-cell IgG has been proposed to protect the host from periodontopathogen infection, consistent with the finding of an inverse correlation between anti-pathogen antibodies and disease severity (Hall *et al.*, 2012). However, polyclonal antibodies to periodontopathic bacteria are often non-specific and/or of low avidity, and thus may be ineffective in clearing the infection (Tew *et al.*, 1989). Moreover, the existence of a destructive autoantibody has been proposed to contribute to periodontitis (De-Gennaro *et al.*, 2006), which directly contradicts the protective antibody hypothesis outlined above. Related studies also showed that B cell-expressed IgD contributes to alveolar bone loss following *P. gingivalis* infection (Baker *et al.*, 2009). However, the exact role of IgD and the specific mechanism underlying IgD function in bone loss are not known. Given the context of contradictory outcomes outlined above, the overall role of antibodies in periodontitis remains unclear. Because the role of antibodies in T2D is similarly controversial (Winer *et al.*, 2011; Nikolajczyk *et al.*, 2012), a definitive understanding of the role antibodies play in both diseases will require more focused approaches, such as antibody-specific knock-out mice. In addition to antibodies, B cells from periodontitis patients also secrete a pro-inflammatory cytokine profile that is similar to the profile expressed by B cells from individuals with T2D (Nikolajczyk *et al.*, 2012). The role for B-cell cytokines in oral pathogenesis remains to be rigorously established, in part because other cell types also secrete B-cell cytokines. A final function of B cells in periodontitis is based on demonstrations that B cells are major, and in some cases functionally unique, sources of pro-osteoclastogenic RANKL (Onal *et al.*, 2012). RANKL expression increases in animals with

obesity-associated T2D (Cao *et al.*, 2010), raising the possibility that B-cell RANKL may potentiate T2D-associated periodontitis.

The second major lymphocyte subset, T cells, constitutes the second most numerous immune cell in chronic periodontal lesions, outnumbered only by B cells (Berglundh and Donati, 2005). The CD4⁺ T-cell subset plays a significant role in periodontitis, as evidenced by the demonstration that CD4⁺ (but not CD8⁺) T-cell deficiency decreases *P. gingivalis*-induced oral bone loss (Baker *et al.*, 2002). Recent studies also showed that *P. gingivalis* induces RANKL expression on CD4⁺ T cells (Belibasakis *et al.*, 2011), and that RANKL neutralization can block the bone destruction presumably induced by these cells (Lin *et al.*, 2011). Although analysis of these data suggests that CD4⁺ T-cell RANKL is critical for periodontitis, definitive experiments need to be completed to determine whether T-cell RANKL alone or in combination with B-cell RANKL plays major roles in pathogen-induced oral bone loss, and whether obesity similarly increases RANKL from multiple cellular sources.

Of the multiple types of CD4⁺ T cells (Th1, Th2, Th17, Tregs), Th2 cells are thought to dominate over an initial Th1 response in progressive periodontitis (Yamamoto *et al.*, 1997), perhaps reminiscent of Th2-dominated immune hyper-reactivity in asthma. A more recently appreciated CD4⁺ T-cell subset, the anti-inflammatory Tregs, is also present in the periodontal tissue (Cardoso *et al.*, 2008) and could theoretically attenuate the severity of experimental periodontitis, regardless of Th1 vs. Th2 origins. Diseased periodontal tissues are also populated by abundant Th17 cells (Moutsopoulos *et al.*, 2012), which express the osteoclastogenic cytokine IL-17. Interestingly, *P. gingivalis* favors Th17 differentiation not only by triggering antigen-presenting cells to produce Th17-supporting cytokines, but also by differentially degrading cytokines that promote Th1 (over Th17) differentiation (Moutsopoulos *et al.*, 2012). The speculation that *P. gingivalis* “engineers” a Th17 environment over a developmentally related Th1 milieu is inconsistent with the idea that Th1s characterize non-progressive lesions (Yamamoto *et al.*, 1997), and suggests instead that Th17s represent the lesser of 2 evils for the bacteria. Overall, analysis of the data provides controversy yet little concrete evidence regarding the role of specific CD4⁺ T-cell subsets in periodontitis.

IMMUNE RESPONSES IN T2D-ASSOCIATED PERIODONTITIS

Examinations of the immune responses that characterize T2D-associated periodontitis have begun to query the possibility that dysregulated immune responses potentiate periodontitis in T2D. T2D-associated changes in innate responses of gingival epithelial cells, fibroblasts, and endothelial cells include increased expression of receptors of advanced glycation end-products, or RAGEs (Katz *et al.*, 2005), which broadly activate inflammation and thus may predispose toward periodontitis (Amir *et al.*, 2011). The importance of RAGE/AGE interaction in T2D-associated periodontitis is experimentally supported by studies in which systemic administration of soluble RAGE prevented periodontal disease progression in a lean type 1 diabetes mouse model that recapitulates the hyperglycemia of obese/T2D mice (Lalla *et al.*, 2000). Clinical and animal studies focused on

cellular mechanisms also provide evidence for aberrant immune cell function – for example, enhanced neutrophil superoxide production – in T2D-associated periodontitis (Karima *et al.*, 2005). Like altered RAGE/AGE outcomes, altered neutrophil function may stem, at least in part, from T2D-associated hyperglycemia, which primes mouse gingival neutrophils for marginalization and superoxide production (Gyurko *et al.*, 2006).

In contrast to the rich literature implicating macrophages in obesity-associated inflammation and T2D, outlined above, there is, perhaps surprisingly, no direct evidence of a role for monocytes/macrophages in T2D-associated periodontitis in humans. However, one animal study showed that macrophages in obese mice respond to *P. gingivalis* with lower inflammatory cytokine production compared with macrophages from lean hosts (Amar *et al.*, 2007). Importantly, these mice differed from standard diet-induced obese mice, as evidenced by the absence of hyperglycemia. This unexpected decrease in macrophage response is consistent with the idea that some immune system functions may be blunted by obesity/T2D and is consistent with the dysregulated TLR expression and significantly reduced signal transduction in peritoneal macrophages responding to *P. gingivalis* in work from the same group (Zelkha *et al.*, 2010). Because both saturated fatty acid and LPS could require TLRs to activate cells, it is possible that chronic exposure to saturated fatty acids in obesity with confounding metabolic disease induces macrophage tolerance *in vitro* (Zelkha *et al.*, 2010). However, recent studies showed that saturated fatty acids instead amplified inflammatory signaling triggered by LPS in macrophages *in vitro* (Jin *et al.*, 2013), directly contradicting the tolerance hypothesis in obesity/T2D-associated periodontitis. Analysis of macrophages in a more standard *in vivo* mouse model (discussed below) may clarify these seemingly disparate results. Finally, one underappreciated possibility to explain how altered immune responses support both obesity-associated inflammation and oral pathogen infection is work showing that, surprisingly, abnormal cytokine persistence alone can prevent pathogen clearance (Wilson *et al.*, 2013).

Evidence for a role of altered lymphocyte function in diabetic patients with periodontitis is similarly limited. Preliminary evidence for an association between glycemic control and IFN- γ /IL-17 concentrations in gingival crevicular fluid from periodontitis patients with diabetes (Santos *et al.*, 2010; Ribeiro *et al.*, 2011) suggests that Th1/Th17 might link periodontitis and T2D. A recent immunohistological study (Duarte *et al.*, 2011) also provides evidence for elevated percentages of Th17 and Treg cells in patients with T2D plus periodontitis as compared with patients with periodontitis alone. Similarly, few studies have tested roles for B cells in T2D-associated periodontitis. Preliminary work showed no significant difference in B-cell infiltrate in gingival tissue in T2D- vs. non-diabetes-associated periodontitis (Duarte *et al.*, 2011). However, given the predominance of B cells in periodontitis lesions, our demonstration that B cells from obese individuals with T2D express a pro-inflammatory cytokine profile similar to that of B cells from lean individuals with periodontitis (Nikolajczyk *et al.*, 2012; DeFuria *et al.*, 2013) leads us to speculate that the phenotypic changes in B cells in obesity may have the unexpected consequence of promoting periodontal disease. Overall, the role of

Figure 2. Working hypothesis of the link between obesity-associated type 2 diabetes (T2D) and periodontitis. Obesity-associated T2D results in increased pro-inflammatory metabolites such as free fatty acids and glucose as well as endotoxin from increased gut permeability. Obesity also results in higher concentrations of adipocyte cytokines, as well as the altered intestinal microbiota, all of which trigger surface signaling molecules including Toll-like receptors (TLRs) and cytokine receptors. These receptors activate a wide array of immunological cells types, including most immune system cells as shown, and structural cells with innate immune responses such as adipocytes, gingival epithelial cells, and fibroblasts (not shown). Thus, obesity/T2D primes immune mechanisms for an inappropriately robust response to oral pathogens that results in periodontal inflammation and exaggerated tissue destruction.

lymphocytes in periodontal pathogenesis in T2D is worthy of more comprehensive investigation in both animal models and humans, the latter of which will require logistically challenging collaborations between endocrinology and periodontology clinics to ensure thorough characterization of both metabolic and periodontal status in study participants.

ANIMAL MODELS AS TOOLS FOR MECHANISTIC STUDIES OF OBESITY/T2D-ASSOCIATED PERIODONTITIS

Multiple models of periodontitis in animals with a combination of obesity and metabolic disease suggest that immune responses predispose obese/T2D individuals to periodontitis, but these studies have limitations. For example, the monogenic nature of some animal models may not accurately recapitulate human pathogenesis, since only a few cases of human obesity/T2D are due to gene mutations (Nilsson *et al.*, 2012). The use of genetically obese ob/ob or db/db mice, which contain a natural mutation that inactivates leptin or the leptin receptor, respectively, must be

interpreted with special caution because of the appreciation that T cells require leptin for normal development and function (Lord *et al.*, 1998; Palmer *et al.*, 2006). Furthermore, current animal models of obesity are generally characterized by insulin resistance; therefore, these models cannot address periodontal diseases in MHO or in obese subjects who are creeping toward T2D yet maintain some insulin sensitivity and beta cell function. Despite the limitations of the available animal models, the diet-induced obese (DIO) model, in which animals become both obese and insulin-resistant in response to a high-fat diet (Nilsson *et al.*, 2012), may be an important tool for understanding periodontitis potentiated by obesity-associated T2D. Genetic manipulations have been used in conjunction with DIO to establish the role of specific immune cell types in obesity/T2D, including but not limited to B cell-deficient mice (Winer *et al.*, 2011; DeFuria *et al.*, 2013) and CD8⁺ T cell-deficient mice (Nishimura *et al.*, 2009). In theory, DIO studies in such mice could be combined with properly timed oral pathogen inoculation to identify mechanisms that promote periodontitis in obesity/T2D (proof of principle) and/or produce outcomes that can be translated to humans (*e.g.*, phenotype of B cell-null mice can be mimicked by generally safe B-cell depletion drugs). However, caution is important in identifying genetically altered mice that are appropriate for DIO studies. Genetic

manipulation may lead to divergence of body weight, an important determinant of inflammation and insulin resistance. In the vast majority of cases, a heavier mouse is more inflamed and insulin-resistant, thus likely more susceptible to periodontitis. In such cases, genetic manipulation probably plays a dominant role in feeding behavior or basal metabolism, which could make results from adding periodontitis to such models tricky to interpret.

An additional consideration for the use of animal models to interpret human disease pathogenesis in obesity-associated periodontitis is the method used to induce periodontitis. Limited numbers of studies have used obese/T2D animals to understand periodontitis in the context of obesity/T2D, with periodontitis initiated by either mechanical methods (*i.e.*, ligature) (Pacios *et al.*, 2012) or by systemic *P. gingivalis* infection (Amar *et al.*, 2007). Despite the insights into obesity/T2D-associated periodontitis reported from these studies, the acute traumatic nature of ligature-induced periodontitis (which mechanistically contrasts with chronic human periodontitis) or the non-physiological route of *P. gingivalis* entry into the infection site (*i.e.*, by venous injection) calls for additional work with more physiological approaches to the understanding of combinatorial disease.

SUMMARY AND FUTURE DIRECTIONS

The establishment of an association between obesity and periodontitis is beginning to shed light on concrete targets for breaking the feed-forward loops among obesity, T2D, and periodontitis and to perhaps stop disease before the dangerous transition to T2D. Currently available work on immune cell function in T2D-associated periodontitis supports an overall hyper-responsive state that reinforces chronic oral inflammation (Fig. 2). Immune cells, especially the B and T cells that dominate periodontitis lesions, are strongly implicated in obesity-associated periodontitis, but require further examination. These and other cell types, including macrophages and neutrophils, will be fruitful areas for detailed investigation.

Analysis of current data suggests several lines of inquiry that will identify targets for interrupting the interaction between obesity and periodontitis, regardless of metabolic status. Foremost, information on the histopathology of periodontal lesions in well-characterized obese individuals, including MHO persons, is incomplete, since many clinical studies have not taken into account and/or described the metabolic status of oral sample donors. Baseline studies of cellular infiltrates are critical for interpretation of, for example, published transcriptional profiling data from gingiva. Second, researchers must take into account differences between humans and experimental animals (mainly rodents) with regard to immune system function and metabolism. In contrast to the uncoupling of metabolic status from obesity in MHO individuals, animal models almost always suffer from simultaneous obesity and metabolic disease. Therefore, studies of db/db mice in the calvarial model of periodontitis, which revealed that T2D causes impaired healing and suppression of bone formation in response to *P. gingivalis* (He *et al.*, 2004), may be irrelevant to MHO people. Similarly, ligature-induced periodontitis in Zucker diabetic fatty rats showed that a T2D-like condition increases the intensity and duration of inflammatory infiltrate as well as bone loss (Liu *et al.*, 2006), but the outcomes under MHO conditions were not tested.

Epidemiological and experimental studies link periodontitis with obesity-associated T2D, but the precise mechanisms underlying the interactions among obesity, metabolic health, and periodontitis remain elusive. A more thorough characterization of obesity-associated periodontitis in rodent models by a combination of oral assessment and whole-body metabolic measures will be required to complement tissue analysis of well-characterized individuals, and to identify mechanisms by which periodontitis may predispose to metabolic imbalance, a “holy grail” of periodontal research. Overall, studies of immune effectors using the most relevant animal model are urged to identify biologically active drugs that effectively break the cycle between obesity-associated T2D and periodontitis, to simultaneously improve multiple measures of health.

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