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Experimental periodontitis induced by *Porphyromonas gingivalis* does not alter the onset or severity of diabetes in mice

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Background and Objective: Diabetes mellitus is believed to increase the risk and severity of periodontitis. However, less evidence is available on the converse effects of periodontitis on diabetes. The objective of the study was to investigate to what degree experimental periodontitis induced by *Porphyromonas gingivalis* might influence the onset and severity of diabetes in different mouse models.

Material and Methods: Twenty-eight male Tallyho/JngJ mice (type 2 diabetes), 20 male streptozotocin-induced diabetes C57BL/6J mice (type 1 diabetes) and 20 male C57BL/6J mice at 4 wks of age were evenly divided into two groups: periodontal infection and sham infection. Periodontitis was induced by *Porphyromonas gingivalis* W50 (*P. gingivalis*) oral inoculation before the development of diabetes. Sham-infected mice received vehicle as control. *P. gingivalis* in the oral cavity were identified by quantitative polymerase chain reaction. Fasting glucose, body weight and food intake levels were monitored and glucose tolerance tests were performed to assess glucose homeostasis for the onset and progression of diabetes. The level of alveolar bone loss and tumor necrosis factor-alpha were determined in week 20 when mice were killed.

Results: Mice in the infection groups developed more alveolar bone loss than those in sham-infection groups (Tallyho $p = 0.021$; C57-STZ $p = 0.014$; C57 $p = 0.035$). Hyperglycemic mice exhibited significantly more bone loss compared to those normal glucose mice (Tallyho vs. C57 $p = 0.029$; C57-STZ vs. C57 $p = 0.024$). The level of tumor necrosis factor-alpha was consistent with that of periodontal bone loss and hyperglycemia. There was no significant effect of mouse species on the amount of bone loss at the same level of blood glucose. No statistically significant difference or trend in glucose metabolism was found between the infection and sham-infection group.

Conclusion: Diabetes enhanced the risk for periodontal disease induced by *P. gingivalis*. However, no converse impact was found between this periodontal infection and onset and severity of diabetes in both type 1 and 2 diabetes mice.

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Tooth loss because of periodontitis is a major dental health concern (1). It has been believed that this inflammation of periodontal tissue is strongly related with diabetes mellitus, one of the largest public health problems in the world (2). Clinical studies on the complications of diabetes have implied that diabetes may be a substantial risk factor for developing periodontitis (3,4), and periodontal inflammation may negatively affect glycemic control (5). The reasons behind this link have yet to be fully understood. A hyperactive innate immune response may be the antecedent of both diseases. Proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-6 have been found to increase in serums of diabetics with periodontitis (6). However, there is still controversy on the two-way interaction between periodontal clinical parameters and the onset and severity of diabetes (7). A better understanding of the relationship between these two diseases is necessary before therapeutic interventions can be developed.

Rodents provide unique characteristics to evaluate microbial and host responses to complement primate and human studies (8). Studies using rodents have stimulated gingival epithelial disruption and periodontal bone loss via oral infection with selected human pathogens (9). For diabetes, chemical injection and transgenic methods are commonly used to mimic insulin secretion destruction of type 1 diabetes (10) and insulin resistance of type 2 diabetes (11). Chemical-induced diabetic mice were the first murine model to study the effects of hyperglycemia on periodontal tissue (12); however, there is no comprehensive investigation on the animal models with both types of diabetes and periodontal disease. In this study, we attempt to demonstrate in different mouse models for diabetes to what degree periodontal infection may influence both the onset and severity of diabetes, which will substantially improve our knowledge on the mechanism how chronic inflammation relates to the clinical features of diabetes.

Material and methods

Animals

Twenty-eight male Tallyho/JngJ (TH) mice (Jackson Laboratories, Bar Harbor, ME, USA) and 40 male C57BL/6J (C57) mice (Dossy Experimental Animals Co., Chengdu, China) were purchased at 4 wk of age. Mice were fed a finely milled autoclaved normal low fat diet (12.3 kcal% fat; Research Diets, New Brunswick, NJ, USA) and housed at a constant temperature (22°C) with humidity at 45–55% in a 12 h light/dark cycle for the whole experimental process. The study was conducted in accordance with the Loma Linda Animal Care guidelines and Sichuan University Animal Care Regulations.

Study design

All mice were placed on Sulfatrim (Goldline Laboratories, Ft. Lauderdale, FL, USA) 5/500 mL of drinking water for 5 d. After 5 d, the Sulfatrim drinking water was replaced with autoclaved tap water for the remainder of the experiment. At 6 wk of age, 20 C57 mice were intraperitoneally given streptozotocin (STZ, 55 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) for 5 d successively to induced diabetes (C57-STZ group). All mice (TH, C57-STZ and C57) were evenly divided into infection and sham-infection group. Periodontal disease was induced by oral inoculation as described in the literature (13) with some improvement. Briefly, *Porphyromonas gingivalis* W50 (*P. gingivalis*, ATCC 53978, Manassas, VA, USA) were grown in brain heart infusion (Oxoid, Hampshire, UK) in an anaerobic chamber with 85% N_2, 5% H_2 and 10% CO_2. On the day of gavage, live *P. gingivalis* were suspended in phosphate-buffered saline (PBS) plus 2% w/v carboxy methyl cellulose (Sigma-Aldrich) to a concentration of 10^9 CFU/mL. Each mouse in the infection groups was orally inoculated with 100 μL bacteria three times at 2 d intervals. Sham-infected mice received 100 μL PBS with 2% carboxy methyl cellulose. During the 16 wk observation time, the mice received three batches of the oral infection procedure at weeks 6, 10 and 14 respectively. Animals were killed 6 wk after the last *P. gingivalis* inoculation.

Recovery of *P. gingivalis* from the murine oral cavity

At termination of the experiments, a sterile calcium alginate swab (Fisherbrand; Fisher Scientific, Pittsburgh, PA, USA) was held against the gumline of the upper and lower molars for 20 s. The swab was then placed in PBS and mixed for 30 s. Gingival tissues were collected from both sides of the molar area of mandibles. Total DNA was isolated from the homogenized bacterial suspensions and gingival tissue samples using the DNeasy Mini kit (Qiagen, Hilden Hamburg, Germany) and quantitated by Nanovue Plus Spectrophotometer (GE Life Science, Piscataway, NJ, USA). *P. gingivalis* was identified by polymerase chain reaction (PCR) using special primers (Integrated DNA Technologies, Coralville, IA, USA) for 16s ribosome of *P. gingivalis*: forward 5′-TGTAGATGACTGATGGTGAAAACC-3′, reverse 5′-ACGTCATCCACACCTTCCTC3′. Quantitative PCR was performed using the Light Cycler 2.0 PCR Detection System (Roche, Indianapolis, IN, USA). Pure *P. gingivalis* was used as control. Data were collected and expressed as a function of crossing point (*Cp*). The sample mRNA abundance was calculated by the formula $2^{-\Delta\Delta Cp}$. Results were expressed as fold changes from control.

Determination of fasting glucose and glucose metabolism

Fasting blood glucose levels following an 8 h fast were determined in blood collected from tails every 2 wk using a glucometer (OneTouch; LifeScan, Milpitas, CA, USA) under general anesthesia with isoflurane. During the observation period, body weight and food intake was monitored every other week.

Intraperitoneal glucose tolerance test

Intraperitoneal glucose tolerance test (ipGTT) was performed on samples collected from randomly selected mice (six per group) at weeks 5, 12 and 20. Briefly, following an 8 h fast, dextrose (1 g/kg body weight, Sigma-Aldrich) was administered intraperitoneally, and the plasma glucose levels in tail blood were determined after 0 (baseline), 15, 30, 60 and 120 min using a glucometer (OneTouch).

Alveolar bone loss

Level of alveolar bone loss area was measured by a scanning electronic microscope (Zeiss EVO, CRAIC Technologies Inc., Kirchdorf, Germany) and images were analyzed (NIH ImageJ, National Institutes of Health, Bethesda, MD, USA) as described by Tatakis and Guglielmoni (14) at the conclusion of the study. Briefly, bone loss was calculated as the average area (mm^2) bordered by the cemento-enamel junction, the crest of alveolar bone, and the mesial and distal line angles of the first and second molars from both the lingual sides of the mandibles. All bone measurements were done three times in a random and blinded protocol by two evaluators.

Serum tumor necrosis factor-alpha levels

Concentrations of serum TNF-α collected from all groups at the time mice were killed (week 20) were determined using ELISA kits (CUSABIO; Sino-American Biotechnology, Wuhan, China) according to the manufacturer's instructions.

Histological examination of pancreas tissue

At the time mice were killed, pancreas tissue samples were harvested, rinsed in PBS and fixed in 4% paraformaldehyde (Sigma-Aldrich) for 24 h. Tissues were then dehydrated, cleared and infiltrated with a histoprocessor (2035 Leica, Leica Microsystems Inc., Wetzlar, Germany) for 16 h. These samples were further made into a series of 4 μm section slides by tissue cutting, and stained with hematoxylin and eosin (Sigma-Aldrich) for histopathological analysis. The sections were stained with hematoxylin solution for 5 min followed by eosin for 5 min. Images were captured with an optical microscope (Nikon 80i, Nikon Corp., Tokyo, Japan). Pancreatic section images of all mice were analyzed using ImageJ software to determine islet size. All islets were photographed at 100 × magnification, and each islet in the images was outlined manually. Individual islet areas were recorded in square pixels. The number of islets was counted on the slide at 10 × magnification.

Statistical analysis

Statistical analysis was performed on alveolar bone loss, body weight, food intake, fasting blood glucose, ipGTT, pancreas histopathological analysis and serum TNF-α level data. Data are presented as mean ± SEM. Two groups were being compared by a one- or two-tailed Student *t* test depending on the hypothesis in question. When comparing three or more groups, ANOVA followed by Bonferroni's method to control for multiple comparisons was used. A p value of ≤ 0.05 was considered statistically significant. These methods were performed using SPSS for Windows (Graph Pad Software).

Results

Alveolar bone loss due to periodontitis

Significant amounts of *P. gingivalis* were detected in both swabs (Fig. 1, TH $p = 0.023$) and gingival tissue (Fig. 1, TH $p = 0.031$) samples compared with the control groups. Mice in the infection groups exhibited significantly more bone loss compared to those in sham-infection groups (TH $p = 0.021$; C57-STZ $p = 0.016$; C57 $p = 0.035$; Fig. 2). The levels of bone loss were not affected by the mouse species at the same level of blood glucose (TH vs. C57-STZ $p > 0.05$; Fig. 2). These data confirmed that oral inoculation of *P. gingivalis* induced alveolar bone loss in mice relative to controls.

Alveolar bone loss due to high glucose

Generally, hyperglycemic mice exhibited significantly more bone loss compared to those normal glucose mice. Within the infection groups, higher average bone loss level was found in mice with high blood glucose than those with normal glucose (TH vs. C57 $p = 0.029$, C57-STZ vs. C57 $p = 0.024$, Fig. 2). Similarly, we found the statistically significant difference between these groups without *P. gingivalis* infection (TH vs. C57 $p = 0.031$, C57-STZ vs. C57 $p = 0.021$, Fig. 2), which may be due to hyperglycemia. TH and C57-STZ mice without bacteria infection showed the same amount of bone loss at this time point compared to C57 mice with *P. gingivalis* infection ($p > 0.05$).

Effects of periodontitis on pancreas morphology

Islet sizes and numbers in the pancreas of mice with diabetes decreased dramatically on hematoxylin and eosin-stained slides compared with that of normal glycemia mice; no significant differences were observed between the islets of mice with *P. gingivalis* infection and their controls (Table S1, S2). The average islet size in the pancreas was calculated in 10^3 square pixels (infected TH vs. sham-infected C57 $p = 0.021$; sham-infected TH vs. sham-infected C57 $p = 0.036$; infected C57-STZ vs. sham-infected C57 $p = 0.005$; sham-infected C57-STZ vs. sham-infected C57 $p = 0.011$; Table S1). The average number of islets was counted at 10 × magnification (infected TH vs. sham-infected C57 $p = 0.028$; sham-infected TH vs. sham-infected C57 $p = 0.039$; infected C57-STZ vs. sham-infected C57 $p = 0.014$; sham-infected C57-STZ vs. sham-infected C57 $p = 0.017$; Table S2).

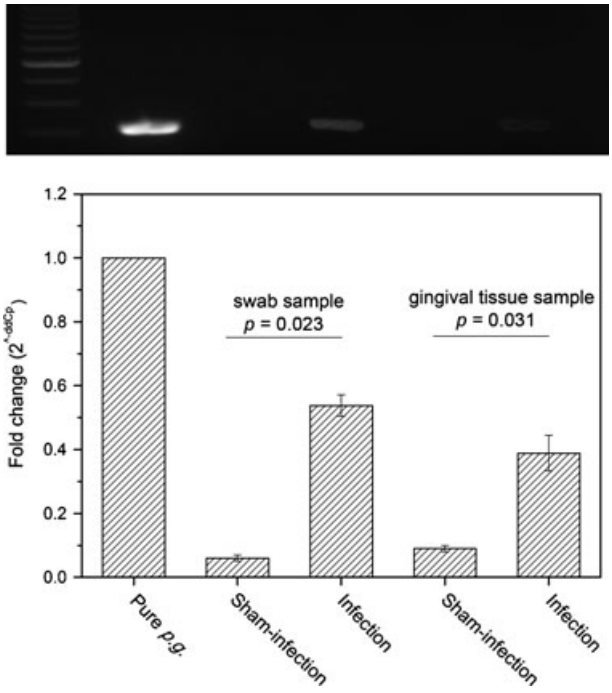

Fig. 1. Quantitative PCR results of bacteria recovery from the oral cavity of both infection and control groups. Graphs were shown based on data from Tallyho mice. p.g., *Porphyromonas gingivalis*.

Effects of periodontitis on glucose intolerance and onset of diabetes

The average fasting glucose level of TH mice increased spontaneously to approximately 400 mg/dL at age 7 wk. Similar hyperglycemia could be observed in STZ-C57 mice 2 wk after STZ injection, while the blood glucose of normal C57 mice was maintained at the same level for the entire study process. During the experimental period, the body weight of TH mice increased continuously to a final value significantly higher than STZ-C57 and C57 mice (Fig. 3, $p = 0.015$). Conversely, reduced body weight was found in STZ-induced diabetic mice. No significant difference was found in the body weight, fasting glucose and food intake levels between infection and sham-infection groups in the three types of mice (Fig. 3, Table 1). To characterize further the effect of periodontitis on the development of impaired glucose homeostasis in different mouse species, we performed ipGTT on six mice from each group at weeks 5, 12 and 20. Both TH and C57-STZ mice exhibited impaired glucose tolerance at 10 and 20 wk as evidenced by glucose levels higher than 300 mg/dL at 120 min (Fig. 4). No statistically significant difference or trend was found between the bacterial and sham-infection group, which was consistent with the level of mean fasting glucose.

Effects of periodontitis and diabetes on serum tumor necrosis factor-alpha levels

As shown in Fig 5, TNF-α serum levels in samples collected at the time mice were killed were higher in *P. gingivalis*-infected mice compared to the sham-infection groups (C57-STZ $p = 0.034$; C57 $p = 0.017$; TH $p = 0.025$). Within the infection groups, the TNF-α in the serum of normoglycemic control mice was less than that of diabetic mice (C57-STZ vs. C57 $p = 0.011$; TH vs. C57 $p = 0.012$). Similar results were observed among the sham-infected groups (C57-STZ vs. C57 $p = 0.002$; TH vs. C57 $p = 0.009$). The average TNF-α level was lower in *P. gingivalis*-infected mice with normal blood glucose compared to diabetic mice without infection, but the difference between these groups was not statistically significant (*P. gingivalis*-infected C57 vs. C57-STZ, *P. gingivalis*-infected C57 vs. TH $p > 0.05$). These results indicated that both periodontitis and diabetes contributed significantly to increased serum TNF-α levels and that periodontitis was a promoter to elevated serum TNF-α in diabetic mice.

Discussion

The two major forms of diabetes are types 1 and 2. Both forms of the disease are believed to be related with a range of complications (15), including periodontitis, which is the major causes of tooth loss in adults (16). Clinical investigations have shown that types 1 and 2 diabetes increase the severity of periodontitis (17), and reversely, periodontal inflammation may negatively affect glycemic control and insulin resistance of people with diabetes in the general population (18). However, the reverse relationship between periodontitis and incidence of diabetes is not completely understood.

Clinical trials of the relationship between diabetes and periodontitis have limitations such as metabolic control, genetic background, onset and duration of the diseases, as well as ethical problems and logistics reasons (19). Therefore, appropriate use of animal models can help us answer these unsolved questions. Mouse models of experimental diabetic periodontitis have the advantage of short generation time, easy handling and relatively lower cost than other species (8,20). In this study, we investigated the impact of periodontal infection on the onset and severity of diabetes in mouse models representing both types 1 and type 2.

There are two main types of diabetic mice, chemical induced and transgenic mice. The advantage of chemically induced diabetes models is the low cost because the disease can be induced in regular, easily accessible laboratory mice strains. STZ is the most commonly used chemical to

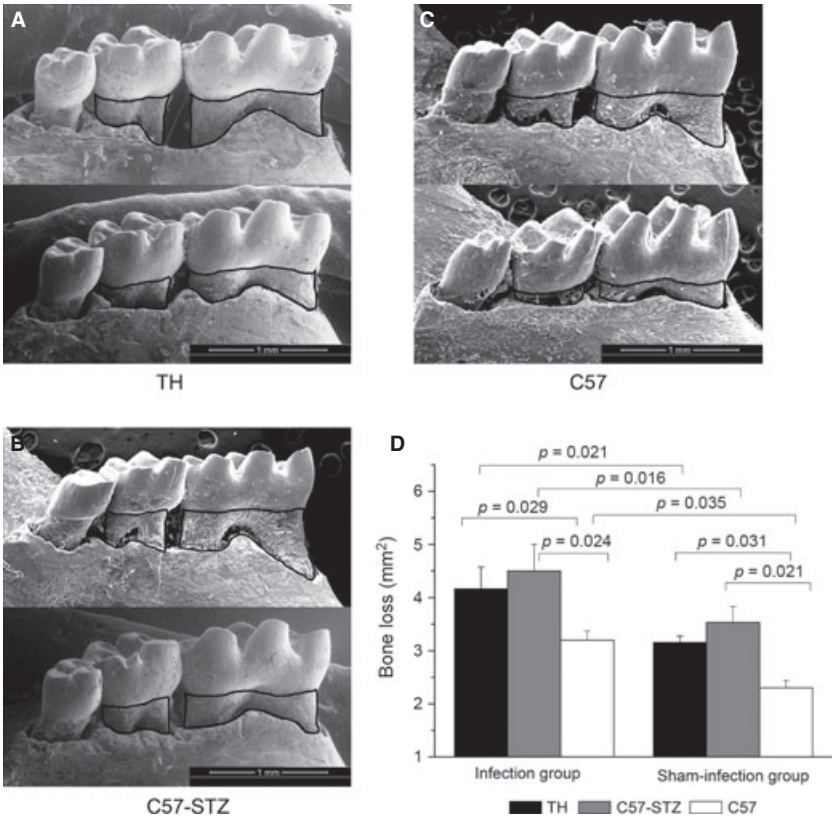

Fig. 2. Scanning electronic microscope images of defleshed right mandible from mice in all groups: (A) TH mice; (B) STZ-C57 mice; (C) C57 mice. Area within the line was calculated as bone loss on the lingual sides of mandibular first and second molars. (D) Comparison of alveolar bone loss level in different diabetic mouse models. C57, C57BL/6J; STZ-C57, streptozotocin-induced diabetes C57BL/6J; TH, Tallyho/JngJ.

induce the mouse model of type 1 diabetes (21). The present study confirmed that male C57 mice develop hyperglycemia 2 wk after STZ administration (22). Histological images of the pancreas demonstrated lack of Langerhans islands leading to reduced insulin secretion level. Genetic diabetes models possess the advantage of a disease course that is more predictable. Male TH mice mimic many characteristics of human type 2 diabetes mellitus such as hyperglycemia, hyperlipidemia, obesity and loss of pancreatic β cells (23,24). Although the TH mouse harbors a complex inheritance pattern, its homogeneous genetic background is a powerful adjunct to human studies. When compared to patients with type 2 diabetes, it exhibits similar genetic factors that contribute to type 2 diabetes-related characteristics in genomic analysis (25,26). TH mice have provided samples required for the study of different complications of diabetes, including vascular dysfunction and bone loss (27,28). These previous reports suggested that patients with type 2 diabetes and TH mice shared significant similarities in pathogenesis and physiological states of diabetes and complications. Additionally, we learned from this study that the onset of diabetes of TH mice was between 7 and 8 wk of age. Other type 2 diabetes species such as *db*/*db* and *ob*/*ob* would develop hyperglycemia as early as 4–5 wk old (29,30). The relatively late onset of hyperglycemia of TH mice gives us enough time to induce periodontal disease and observe the two-way relationship.

Mice have only one incisor and three molars of each quadrant in the oral cavity. Because of limited space, oral inoculation of periodontal pathogens has often been used to elicit periodontal disease in mouse models (13). Inoculation models may have the advantage of inducing specific host responses to certain human periodontopathogens (31). Live *P. gingivalis* is widely used because it is the main pathogen of adult periodontitis. The selection of strains that are different in virulence is important in determining whether alveolar bone loss could ensue. *P. gingivalis* W50 was originally isolated from an adult patient with periodontitis (32). Its high virulence makes it more invasive in mouse models for hard tissue destruction, which accompanies periodontal disease compared to other strains such as *P. gingivalis* 381 and W83 (33,34). In the preliminary experiment, we tried to use the method proposed by Baker *et al.* (13) to induce the periodontitis model. The results showed that mice with only one batch of oral induction did not exhibit typical alveolar bone loss. Alternatively, significant bone loss was efficiently induced after three batches of the oral infection procedure. Thus, we chose to use this improved method to achieve periodontal infection in the present study. This study showed that *P. gingivalis* W50 could effectively induce significant periodontal bone loss in both diabetic and normal mice, indicating that oral infection of W50 is an effective way to create periodontitis in mice with both types of diabetes.

Determination of mouse strains is another important factor to ensure successful induction of periodontitis. Male C57BL/6J and female Balb/cByJ mice have been shown to have high susceptibility to *P. gingivalis* so they are usually the previous selections (9) of periodontal mouse models. The TH mouse closely represents the pathogenesis and physiological state preceding type 2 diabetes and its complications (25–28), and it is suitable for mimicking this type of diabetes with periodontitis. Here, we chose sex-matched male C57 mice as normal glucose control to both male TH and STZ-induced hyperglycemia groups. Our results demonstrated that C57 mice developed moderate periodontal bone loss after *P. gingivalis*

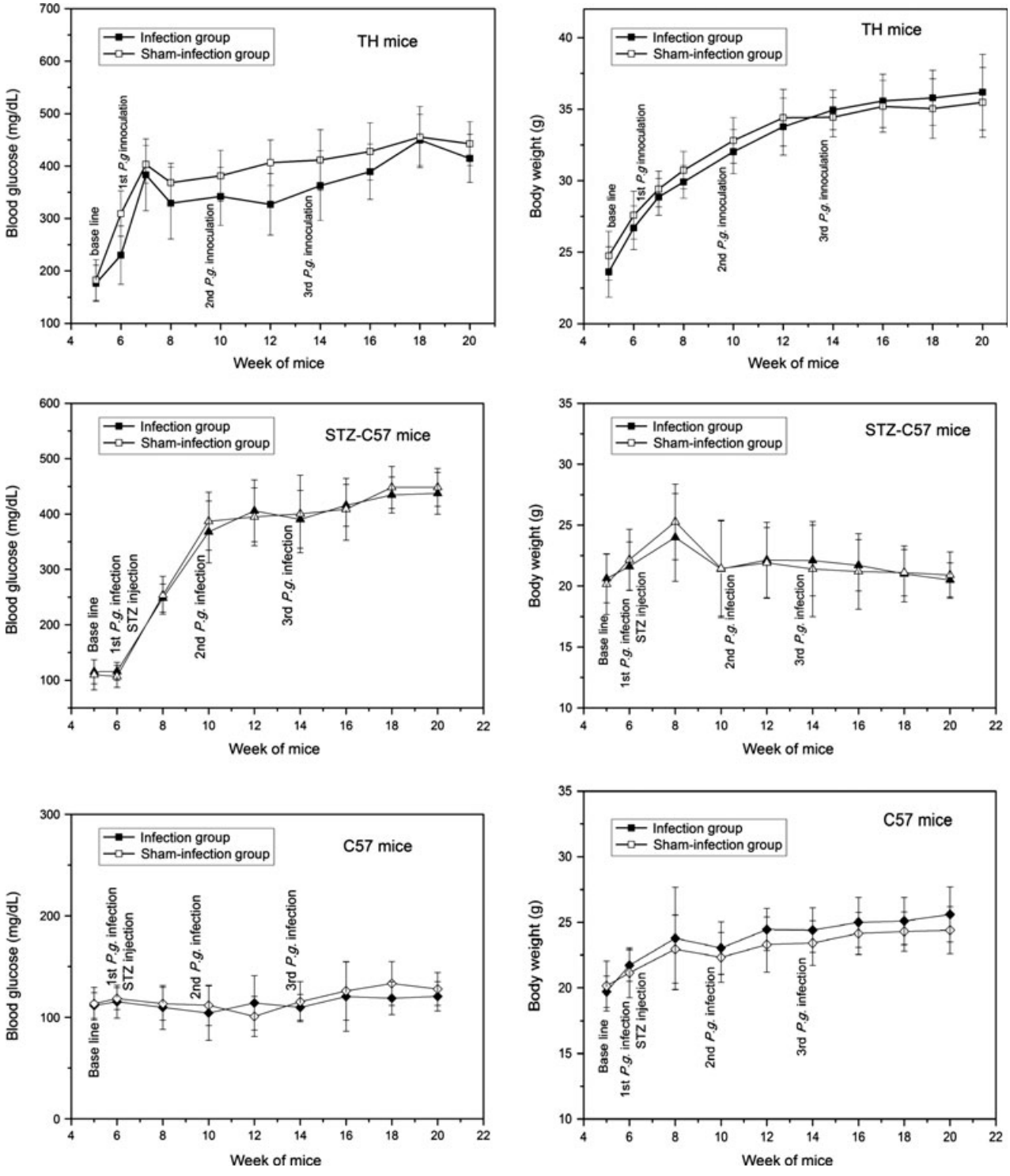

Fig. 3. Comparison of fasting blood glucose and body weight levels in different mouse groups (TH mice: $n = 28$, STZ-C57 mice: $n = 20$, C57: $n = 20$). No statistical differences detected for body weight and blood glucose level between the control and infection groups within the same type of mouse model. Values for each time point are expressed as mean ± SEM. STZ-C57, streptozotocin-induced diabetes C57BL/6J; TH, Tallyho/JngJ.

inoculation; while STZ-C57 mice, representing type 1 diabetes, had induced periodontitis that was more severe than normal glucose controls. The results were consistent with the finding that chemical-induced diabetes enhanced periodontal bone loss (12). It has been investigated, to a lesser extent, how periodontal disease induced by *P. gingivalis* impacts the genetic rodent models for type 2 diabetes. Most results of the type 2

Table 1. Food intake of different mice species during observation period

Mice species	TH (g)	C57-STZ (g)	C57 (g)
Infection (5 wk)	3.6 ± 0.7	3.5 ± 0.6	3.2 ± 0.5
Control (5 wk)	3.4 ± 0.4	3.8 ± 0.9	3.3 ± 0.8
Infection (20 wk)	6.8 ± 1.6	7.4 ± 1.3	4.5 ± 0.3
Control (20 wk)	7.8 ± 0.9	6.9 ± 0.8	4.7 ± 0.7

Note: No statistical differences detected for food intake levels.
C57, C57BL/6J; STZ-C57, streptozotocin-induced diabetes C57BL/6J; TH, Tallyho/JngJ.

explored this. In a lean model of type 1 diabetes and in a model of prediabetes, periodontitis was not associated with the onset and severity of diabetes (37). The results from a rat study found that ligature-induced periodontitis seemed to accelerate the onset of insulin resistance and type 2 diabetes in animals fed a high-fat but not low-

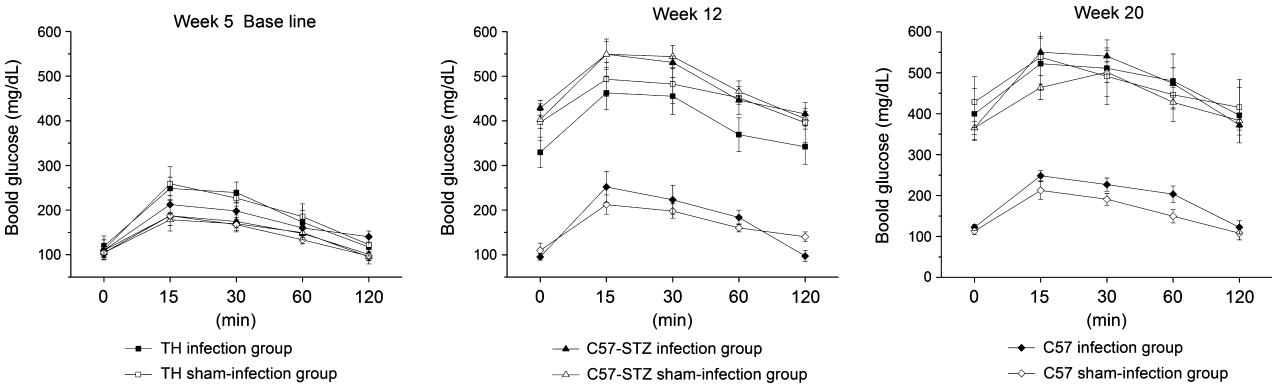

Fig. 4. Glucose concentration levels from the intraperitoneal glucose tolerance test performed at weeks 5, 12 and 20 ($n = 6$). Values for each time point are expressed as mean ± SEM. C57, C57BL/6J; STZ-C57, streptozotocin-induced diabetes C57BL/6J; TH, Tallyho/JngJ.

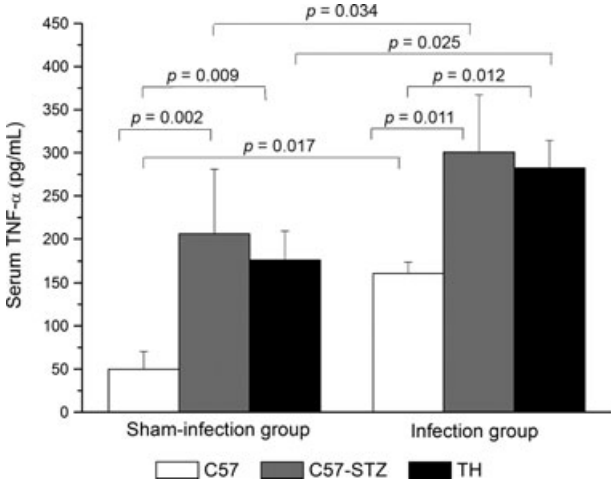

Fig. 5. Serum TNF-α level at time of termination determined by enzyme-linked immunosorbent assays. C57, C57BL/6J; STZ-C57, streptozotocin-induced diabetes C57BL/6J; TH, Tallyho/JngJ; TNF, tumor necrosis factor.

diabetes models used in studies on periodontitis are from rat models (35,36). Research on TH mice might assist in choosing appropriate methods and explain the contribution of periodontal bacteria. Similar to C57-STZ mice, TH mice had periodontal manifestation that was more severe than C57 controls. The results further supported the relationship between the degree of hyperglycemia and severity of periodontitis.

Although intriguing interests have been aroused on whether periodontitis plays a role in the incidence of diabetes, few animal investigations have fat diet (38). We next looked further at the impact of periodontitis on the incidence of diabetics from the literature. In a 7 year prospective study of 5848 individuals without diabetes, the effect of periodontitis on diabetes incidence was assessed (39). Moderate and severe periodontitis were significantly associated with an increased risk of diabetes incidence, but significance was lost after adjusting for sex, smoking, body mass index, triacylglycerol, hypertension and high-density lipoprotein cholesterol. Another prospective cohort study revealed that teeth loss was a risk factor of death in people with type 2 diabetes, but periodontal diseases was not associated with mortality due to severe diabetes (40). An explanation for the uncertain effect of periodontal infection on diabetes may be relevant to the etiology of diabetes. Many factors affect the onset and development of this metabolic disease. Although environmental factors such as periodontal infection may affect this process, the genetic propensity of diabetes is considered an important contributor.

Therefore, the effect of *P. gingivalis* infection might have been masked by the strong propensity of type 2 diabetes development in TH mice in this study. It may be the reason for the result that periodontal infection showed no effect on the onset and severity of type 2 diabetes in TH mice. Thus, further investigations are needed to study the association between diabetes and periodontal infection, for example, using different diabetic rodent models.

Hyperglycemia in diabetes has been proved to promote a proinflammatory environment. Previous studies have showed that high blood glucose level is associated with enhanced serum TNF-α, an adverse inflammatory cytokine on periodontal tissues (41). Expression of TNF-α was prolonged in diabetes compared to normal animals during wound healing (42). In the present study, we compared serum TNF-α levels of diabetic mice and their healthy controls, and the results showed that mice with hyperglycemia have higher TNF-α levels. Moreover, we found that diabetic mice (both TH and STZ-C57) with periodontitis exhibited higher serum TNF-α and alveolar bone loss than the infection group in C57 mice. This indicates that hyperglycemia can aggravate TNF-α production caused by *P. gingivalis* oral infection, and further leads to exacerbated periodontal damage. Therefore, increased TNF-α in diabetes may be an independent risk factor for the development of periodontal disease.

Furthermore, our results showed that during active *P. gingivalis* infection, expression of TNF-α in both types of diabetic mice were stimulated more than in normal C57 controls. However, increased TNF-α does not impact on the severity of diabetes. The results implied that periodontal infection alters the production of TNF-α, but diabetes development may correlate with other factors besides TNF-α. The effect of TNF-α on type 1 diabetes is indeterminate. It is believed that progressive pancreatic β cell loss is caused by combinations of proinflammatory cytokines such as TNF-α, interleukin-6 and interferon-γ (43). Thus, previous experiments on blocking TNF-α alone showed varied effects in rodent diabetes models, including protective, neutral or deleterious (44). In type 2 diabetes, TNF-α plays a specific role in promoting insulin resistance, which is an important risk for this metabolic disease (38,45). However, besides insulin resistance, defective insulin secretion is a critical prerequisite for the development of overt type 2 diabetes (46). The inability of the pancreatic β cells to maintain normal insulin secretion has recently believed to be associated with chronic exposure to high glucose and saturated free fatty acids (47), termed glucotoxicity and lipotoxicity (48). However, few studies in the literature reported this inability linked to TNF-α expression. Taken together, the role of TNF-α in the onset and development of type 2 diabetes is indefinite. Therefore, more experiments are needed to establish whether periodontal infection affects diabetes, and the mechanism by which the infection interacts with diabetes remains to be studied.

Conclusions

Animal models have been very useful, and they hold great promise for future studies given a variety of possibilities for testing biologic hypotheses. *P. gingivalis* infection aggravates alveolar bone loss and induces a stronger inflammatory response compared with that without the infection. The results suggest that oral infection of TH and STZ-induced mice with *P. gingivalis* is a potential model of experimental periodontitis with different diabetes types.

Within the limitation of this study, it can be concluded that diabetes increases the risk for periodontal disease induced by *P. gingivalis*. However, conversely, no significant difference was found between periodontal infection and the control group in any mouse species selected in this work. Although there is evidence that periodontal infection may accelerate the onset of severe insulin resistance and impaired glucose homeostasis in a high-fat food-induced diabetes rat model (38), the association between periodontal infection and the onset and progression of diabetes will become clearer as more clinical research is conducted.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Average islet sizes in the pancreas of different mice (103 square pixels/islet).

Table S2 Average numbers of islets in the pancreas of different mice.

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