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Effect of Aging on Periodontal Inflammation, Microbial Colonization, and Disease Susceptibility

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Abstract

Periodontitis is a chronic inflammatory disease induced by a biofilm that forms on the tooth surface. Increased periodontal disease is associated with aging. We investigated the effect of aging on challenge by oral pathogens, examining the host response, colonization, and osteoclast numbers in aged versus young mice. We also compared the results with mice with lineage-specific deletion of the transcription factor FOXO1, which reduces dendritic cell (DC) function. Periodontitis was induced by oral inoculation of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in young (4 to 5 mo) and aged (14 to 15 mo) mice. Aged mice as well as mice with reduced DC function had decreased numbers of DCs in lymph nodes, indicative of a diminished host response. In vitro studies suggest that reduced DC numbers in lymph nodes of aged mice may involve the effect of advanced glycation end products on DC migration. Surprisingly, aged mice but not mice with genetically altered DC function had greater production of antibody to *P. gingivalis*, greater IL-12 expression, and more plasma cells in lymph nodes following oral inoculation as compared with young mice. The greater adaptive immune response in aged versus young mice was linked to enhanced levels of *P. gingivalis* and reduced bacterial diversity. Thus, reduced bacterial diversity in aged mice may contribute to increased *P. gingivalis* colonization following inoculation and increased periodontal disease susceptibility, reflected by higher TNF levels and osteoclast numbers in the periodontium of aged versus young mice.

Keywords: bacteria, dendritic cell, DNA-seq, osteoclast, periodontitis, lymphocyte

Introduction

Periodontitis is the most common osteolytic disease in humans and the most common cause of tooth loss in adults, with an estimated prevalence of 25% to 47% (Dye 2012). It is a chronic inflammatory condition initiated and maintained by a dysbiotic dental biofilm. The microbial challenge induces a host response that ultimately results in the destruction of connective tissue attachment and alveolar bone (Graves et al. 2011). Tissue destruction is caused by the immune response of the host. Variations in the host response may increase or decrease the susceptibility of different individuals to destructive periodontal disease (Garlet 2010; Benakanakere et al. 2015).

Dendritic cells (DCs) are professional antigen-presenting cells that initiate primary and secondary T-cell responses (Wilensky et al. 2014). DCs play an essential role in resistance to infection by their ability to present bacterial antigen to lymphocytes, initiating the adaptive immune response (Cutler and Teng 2007; Hovav 2014). The interactions between DCs and other cells of the innate and adaptive immune responses are thought to be important in the pathogenesis of periodontal disease (Arizon et al. 2012). DC function is regulated by transcription factors, including FOXO1. We recently showed that the deletion of FOXO1 reduces DC function and impairs the ability of DCs to activate the adaptive immune response (Dong et al. 2015). Previous results demonstrated that FOXO1 mediates lipopolysaccharide-induced cytokine expression in DCs (Brown et al. 2011). FOXO1 is needed for DC migration and homing to lymph nodes by regulating CCR7 and ICAM-1 expression (Dong et al. 2015). Evidence also shows that aging is associated with a number of factors, including increased periodontal disease, decreased FOXO1, and increased formation of advanced glycation end products (AGEs; Salih and

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Brunet 2008). AGEs accumulate during the physiologic process of aging and have been implicated in numerous age-related pathologic processes (Gkogkolou and Böhm 2012).

Aging is a complex multifactorial process that increases susceptibility to chronic inflammatory diseases and microbial infections, such as periodontitis (Hajishengallis 2010). Older individuals have higher levels of some Gram-negative bacilli, such as *Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Enterobacter*, as compared with young individuals (Bodineau et al. 2009). However, relatively little is known on how aging affects oral bacterial communities.

We investigated factors that may contribute to aged-associated periodontal disease susceptibility. Aged mice and mice with DC FOXO1 deletion had reduced numbers of DCs in lymph nodes, consistent with reduced DC function. However, aged mice compared with young mice had greater activation of the adaptive immune response challenged by oral infection, which was not observed in mice with DC FOXO1 deletion. The greater adaptive immune response in aged mice was linked to greater colonization of the tooth surface by *Porphyromonas gingivalis*. Reduced bacterial diversity in aged versus young mice may be responsible for the increased *P. gingivalis* colonization following inoculation and may increase periodontal disease susceptibility, reflected by increased tumor necrosis factor (TNF) levels in the periodontium and greater osteoclast numbers.

Materials and Methods

Inoculation of Mice

FOXO1$^{L/L}$ mice were bred with CD11c.Cre recombinase mice to generate mice with lineage-specific FOXO1 deletion in experimental (CD11c.Cre^{+}.FOXO1$^{L/L}$) versus wild-type control mice littermates (CD11c.Cre^{-}.FOXO1$^{L/L}$). All mice were housed in the same colonies under specific pathogen–free conditions. Both *P. gingivalis* and *Fusobacterium nucleatum* were grown in broth, in an anaerobic chamber with 85% N_2, 5% H_2, and 10% CO_2. All animals were given 10^9 colony-forming units of *P. gingivalis* and 10^9 colony-forming units of *F. nucleatum* suspended in 100 µL of 2% carboxymethyl cellulose in sterile phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA) into the oral cavity as described (Xiao et al. 2015). Bacteria were administered orally 3 times per week for 2 wk. Mice were euthanized by an overdose of intraperitoneal injection of ketamine and xylazine, followed by decapitation 6 wk after the last oral inoculation. Young (~4 to 5 mo) and aged mice (~14 to 15 mo) were used. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Microscopic Computerized Tomography Analysis and TRAP Analysis

Maxillae were dissected after euthanasia and scanned with the µCT-40 (Scano Medical AG, Bassersdorf, Switzerland). Histomorphometric analysis was performed with Image ProPlus software (version 7.0; Media Cybernetics, Silver Spring, MD, USA). Bone loss was measured by the residual bone area between the first and second molars. Histologic sections were prepared after decalcification in 10% ethylenediaminetetraacetic acid at pH 7.4 for 6 wk. Paraffin-embedded sections were examined for the presence of osteoclasts, which were identified as multinucleated tartrate-resistant acid phosphatase (TRAP)–positive cells in the vicinity of bone tissue. The region of interest was the coronal 0.35 mm of alveolar bone following the approach that we described previously (Liu et al. 2006).

Immunofluorescence and Immunohistochemistry

TNF expression was measured by immunofluorescence. Primary antibody (Abcam, Cambridge, MA, USA) was detected by a specific biotinylated secondary antibody, followed by fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA, USA). Coverslips were mounted with Fluoroshield with DAPI (Sigma-Aldrich) to allow visualization of the cell nuclei. To enhance antigen detection, antigen retrieval was performed with sodium citrate buffer at pH 6.0 (Sigma-Aldrich), followed by the use of a tyramide signal amplification system (PerkinElmer, Waltham, MA, USA).

Cervical lymph nodes were collected and fixed immediately after euthanasia. DCs in lymph nodes were identified by immunohistochemistry via a specific antibody to CD205 (NLDC145; Serotec, Oxford, UK). DCs in lymphoid follicles and paracortical area were assessed with the 20× objective of microscope. The number of plasma cells and IL-12 expression in lymph nodes were measured as described before (Xiao et al. 2015). Briefly, plasma cells were detected by a specific antibody to CD138 (BD Biosciences, San Jose, CA, USA). IL-12 production in the germinal centers of lymph nodes was quantified by immunofluorescence measuring mean fluorescence intensity (R&D Systems, Minneapolis, MN, USA). Primary antibodies were followed by a species-specific biotinylated secondary antibody and then by fluorescein-conjugated avidin (Vectastain ABC Kit; Vector Laboratories). Coverslips were mounted with Fluoroshield (Sigma-Aldrich). Images were captured with a fluorescence microscope and Nikon NIS-Elements software (version 3.2; Nikon, Melville, NY, USA) and analyzed by trained examiners blinded to the experimental groups.

DC Migration

DC migration was carried out in primary bone marrow–derived DCs or the DC2.4 cell line as described (Dong et al. 2015). Briefly, cells were harvested and incubated with 1 µg/mL of lipopolysaccharide for 24 h following incubation with bovine serum albumin or carboxymethyl-lysine-albumin, an AGE that binds to the receptor for AGE or unmodified albumin (200 µg/mL) for 48 h as described in (Zhang et al. 2015). Chemotaxis was measured in polycarbonate-filter Transwell chambers (5-µm pore size; Corning) with or without CCL21 or CCL19 (Peprotech) for 3 h at 37 °C. DCs that migrated to the bottom chamber were counted by fluorescence microscopy after staining of cell nuclei with DAPI.

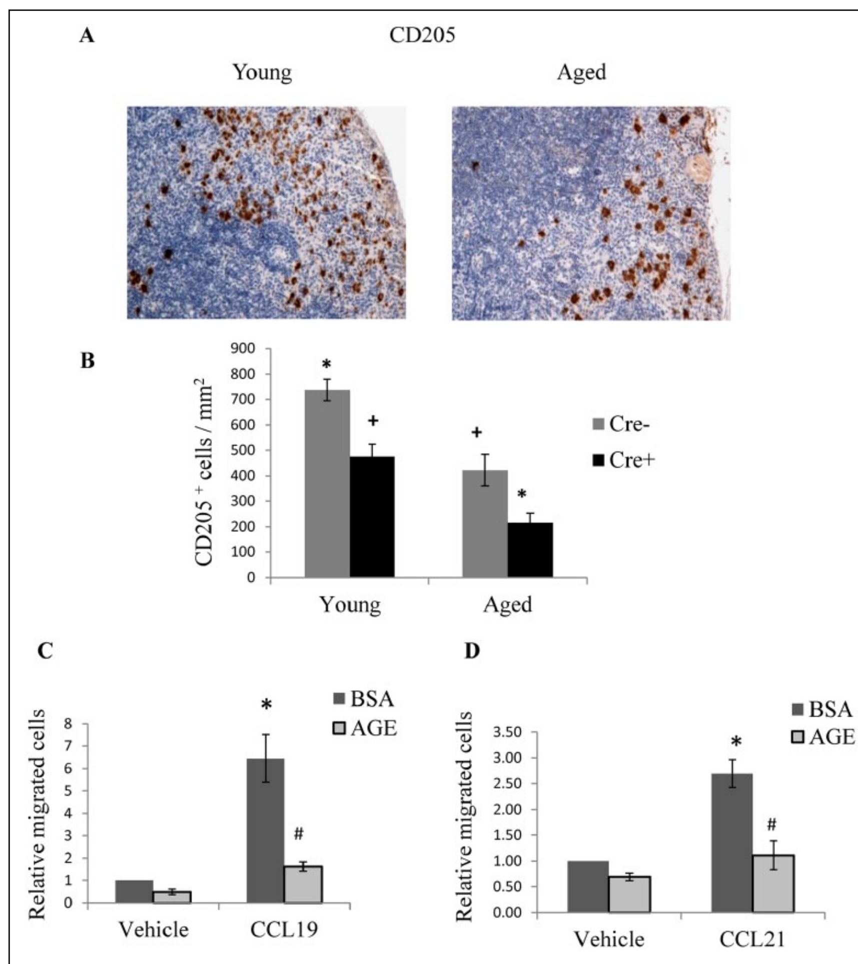

Figure 1. Aging and dendritic cell (DC) FOXO1 deletion reduce the number of DCs in lymph nodes and reduce DC migration. DCs in the lymph nodes were identified by immunohistochemistry with a specific antibody to CD205. DC migration was examined in DC2.4 cells or bone marrow–derived DCs after treatment with bovine serum albumin (BSA) or advanced glycation end products (AGEs) and stimulated with CCL19 or CCL21 added to the bottom chamber in a Transwell assay. (**A**) Images of CD205-positive immunostaining in cervical lymph nodes. (**B**) Quantification of CD205-positive immunostaining in lymph nodes. (**C**) Migrated DC2.4 DCs stimulated with CCL19. (**D**) Migrated bone marrow–derived DCs stimulated with CCL21. Values are expressed as the mean ± SE, n = 4 to 6. *Significant difference ($P < 0.05$) between young mice and corresponding aged mice or between DCs migrated from treatment with CCL19 or CCL21 and vehicle. +Significant difference ($P < 0.05$) between wild-type mice and age-matched DC FOXO1 deletion mice. #Significant difference ($P < 0.05$) between DCs migrated from treatment with AGE and control BSA. This was done by 2 or 3 independent experiments.

(Caporaso et al. 2011). The trimmed reads were clustered into operational taxonomic units at 97% identity level over an alignment and assigned to the respective genus-level taxa. Alpha diversity and rarefaction plots were computed with Faith's phylogenetic diversity (PD_whole_tree) and Shannon index biodiversity. Rarefaction curves for phylogenetic diversity plateaued after 1,000 reads per sample, approximating at a saturation phase. The relative abundances of each taxon were then computed for each mock community samples at each taxonomic level.

Quantitative Real-time Polymerase Chain Reaction

The number of total bacteria and *P. gingivalis* colonization on the tooth surface was determined by quantitative real-time polymerase chain reaction of the 16S rRNA gene (total bacteria) and the *ISPg1* gene (*P. gingivalis*) in the ABI 7500 Fast System (Applied Biosystems, Foster City, CA, USA). The broth culture was quantified by colony-forming unit to construct a standard curve (McIntosh and Hajishengallis 2012). The primers sets used to enumerate total bacterial load and *P. gingivalis* copy number were as follows:

16S rRNA (universal; total bacterial load): 5′TCCTACGGGAGGCAGCAGT-3′, 5′-GGACTACCAGGGTATCTAATCCTGTT-3′

ISPg1 (*P. gingivalis*): 5′-CGCAGACGACAGAGAAGACA-3′, 5′-ACGGACAACCTGTTTTTGATAATCCT-3′

Bacterial Analysis

All the molar crowns were cut along the edge of alveolar bone crest, and bacteria were separated from the surface of tooth by bead beating (Polysciences, Philadelphia, PA, USA) in cell lysis buffer from the DNeasy kit (Qiagen, Valencia, CA, USA; Kumar et al. 2011). After a 60-s vortex, DNA present in the buffer was isolated with the DNeasy kit and quantitated with a spectrophotometer (Tecan, Männedorf, Switzerland). Amplification of the V4 region of 16S ribosomal DNA (16S rDNA; IDT, Coralville, IA, USA) was performed in 50-µL reactions and then purified with both Agencourt XP DNA purification beads (Beckman Coulter, Beverly, MA, USA) and QiaAmp DNA Mini Spin Columns (Qiagen). The sequencing was performed with paired-end MiSeq runs, and data were analyzed by Qiime software

Enzyme-linked Immunosorbent Assay

Serum was obtained by cardiac puncture at the time of euthanasia. Antibody (IgG1) against *P. gingivalis* was measured by ELISA as we previously described (Chae et al. 2002).

Results

Aging and DC FOXO1 Deletion Reduce the Number of DCs in Lymph Nodes

The number of DCs in cervical lymph nodes was ~50% less in aged mice compared with young mice ($P < 0.05$). Young and aged mice with DC FOXO1 deletion had a significantly lower number of DCs compared with the matched control group (P <

0.05), indicating a reduced host response in aged and DC FOXO1–deleted mice (Fig. 1A, B). To investigate a potential mechanism, we examined the impact of an AGE on DC migration since AGEs accumulate in aged individuals and have been linked to cellular deficits caused by aging (Gkogkolou and Böhm 2012). Chemokine stimulation induced a 2.7- to 6.4-fold increase in DC migration in freshly isolated DCs or in a DC cell line. Migration was reduced by up to 60% to 75% by incubation with AGE compared with unmodified albumin ($P < 0.05$; Fig. 1C, D).

Aging Enhances Activation of the Adaptive Immune Response

To investigate the impact of reduced DC function, we examined activation of the adaptive immune response following inoculation of bacteria. *P. gingivalis*–specific IgG1 in aged mice was 2 to 3 times higher than matched young mice ($P < 0.05$; Fig. 2A). In contrast, DC FOXO1 deletion had the opposite effect, reducing anti–*P. gingivalis* IgG1 in aged mice ($P < 0.05$). Similarly, the number of CD138+ plasma cells in the draining lymph nodes was 2.0-fold higher in aged mice in comparison with young mice (Fig. 2B), and the increase was reversed by FOXO1 deletion in DC. IL-12 production in the germinal centers of lymph nodes increased ~30% in aged mice, which was blocked by FOXO1 deletion in DCs (Fig. 2C). The results indicate that aging results in an increased adaptive immune response, whereas DC FOXO1 deletion does not. This suggests that factors other than age-associated DC function play a major role in the response of aged mice to oral inoculation of bacteria.

Aging Increases Colonization of P. gingivalis *and Reduces Bacterial Diversity*

Aging increased *P. gingivalis* levels in the dental biofilm by ~4.7-fold. However, deletion of FOXO1 in DCs in aged mice reduced the amount of *P. gingivalis* associated with teeth ($P < 0.05$; Fig. 3A). Aged mice had 9 times more bacteria in their dental biofilm than young mice ($P < 0.05$). FOXO1 ablation in DCs also increased the amount of bacteria, although to a smaller extent than aging (Fig. 3B).

Phylogenetic and Shannon index were calculated to elucidate differences in bacterial diversity associated with aging. Aged mice had less diversity in comparison with young mice ($P < 0.05$; Fig. 3C, D). To further analyze changes in the dental biofilm, bacteria were examined at the taxonomic levels of family or genus (Fig. 3E). Among these bacteria, *Ruminococcaceae* and *Lactobacillus* have been reported to be commensal bacterial in the periodontium or gastrointestinal tract (Szkaradkiewicz et al. 2011; Biddle et al. 2013). *Bacillus, Staphylococcus, Prevotella, Pseudomonas, Acinetobacter, Fusobacterium*, and *Porphyromonas* have been reported to have pathogenic potential in the periodontium and gut (Szkaradkiewicz et al. 2011; Hajishengallis 2014; Rolny et al. 2014; Souto et al. 2014). The proportion of the bacterial community composed of *Ruminococcaceae* and *Lactobacillus* in aged mice was reduced

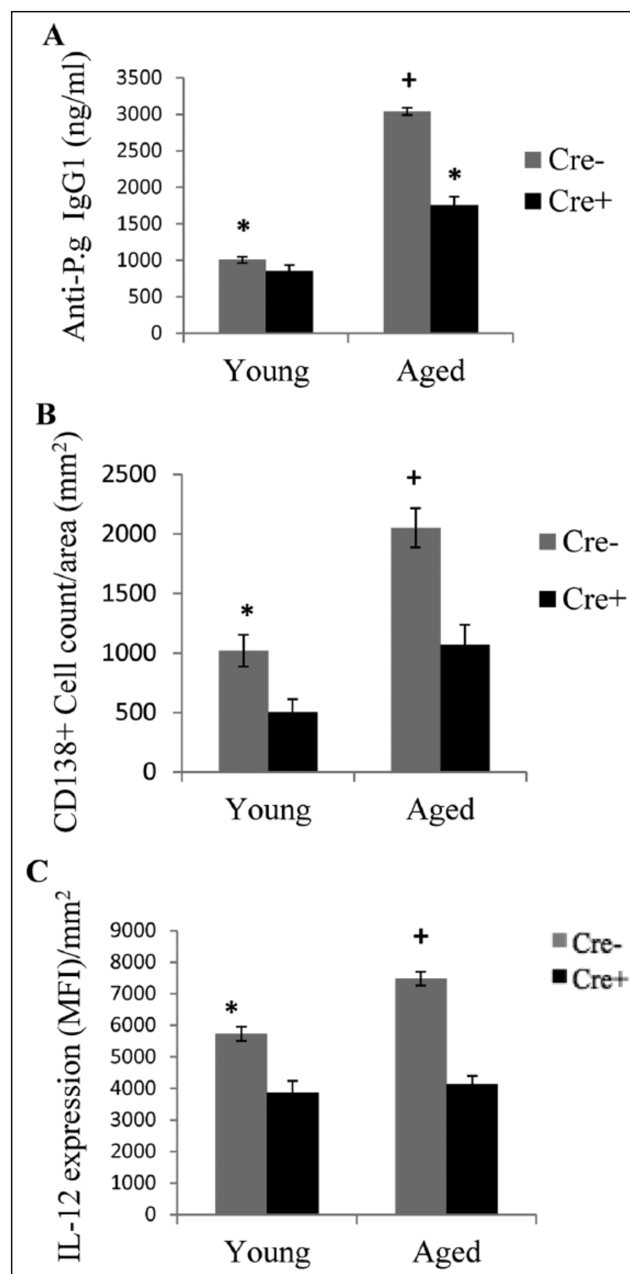

Figure 2. Aging enhances activation of the adaptive immune response. Serum antibody (IgG1) to *Porphyromonas gingivalis* was measured by ELISA. Paraffin sections of neck lymph nodes from young or aged dendritic cell (DC) FOXO1-deleted mice or control littermates mice were stained with CD138-specific antibody for plasma cells or IL-12 by immunofluorescence. Matched control antibody was negative for immunofluorescent staining. (**A**) Serum anti–*P. gingivalis* IgG1 antibody measured by ELISA. (**B**) Quantification of CD138-positive immunostaining in lymph nodes. (**C**) Quantification of IL-12-positive immunostaining in lymph nodes. Values are expressed as the mean ± SE, n = 4 to 6. *Significant difference ($P < 0.05$) between young mice and corresponding aged mice. $^{+}$Significant difference ($P < 0.05$) between wild-type mice and age-matched DC FOXO1-deleted mice. n = 5 or 6 mice per group.

in comparison to young mice, while the proportion of bacteria with pathogenic characteristics such as *Staphylococcus* increased in aged mice. Thus, the aged mice exhibited less

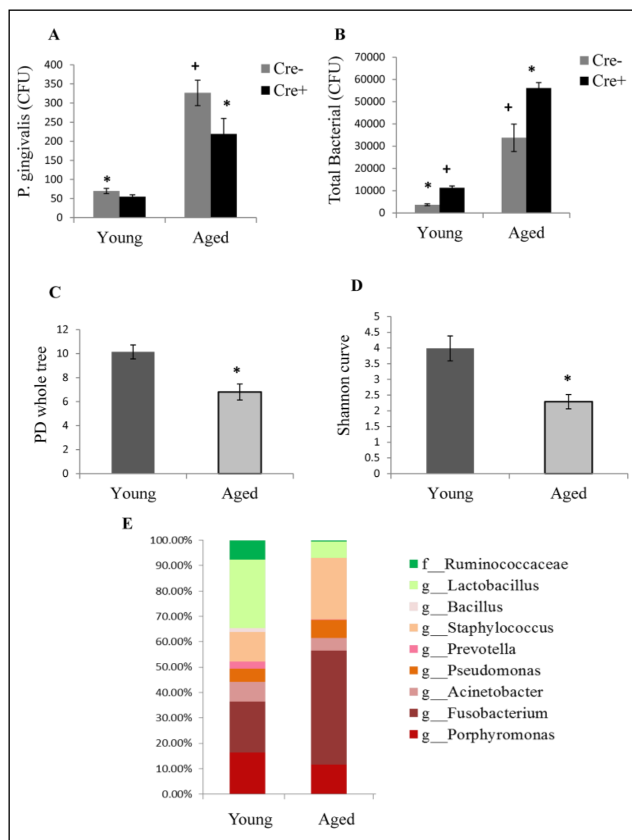

Figure 3. Aging increases colonization of *Porphyromonas gingivalis* and reduces bacterial diversity. Bacteria were separated from the surface of tooth crown, and the number of *P. gingivalis* and total bacteria were determined with quantitative real-time polymerase chain reaction (PCR). DNA collected from bacteria on tooth surfaces was sequenced with the MiSeq Sequencing System. (**A**) *P. gingivalis* on the tooth surface was determined by quantitative real-time PCR of the *ISPg1* gene. CFU, colony-forming units. (**B**) Total oral bacteria loading on the tooth surface was determined by quantitative real-time PCR of the 16S rRNA gene. (**C**) Alpha diversity and rarefaction plots were computed with Faith's phylogenetic diversity (PD_whole_tree). (**D**) Alpha diversity and rarefaction plots were computed with Shannon index biodiversity. (**E**) Bar charts represent the relative abundance of the main microbial phyla related to the periodontal diseases in mice subjected to different treatment. Phylogenetic orders are represented by different colors: Green indicates commensal bacteria, including *Ruminococcaceae* and *Lactobacillus*; yellow and red indicate pathogenic bacteria, including *Bacillus, Staphylococcus, Prevotella, Pseudomonas, Acinetobacter, Fusobacterium*, and *Porphyromonas*. Values are expressed as the mean ± SE, *n* = 4 to 6. *Significant difference ($P < 0.05$) between young mice and corresponding aged mice. +Significant difference ($P < 0.05$) between wild-type mice and age-matched DC FOXO1 deletion mice.

diversity, reflected by an increased proportion of bacteria with higher pathogenic potential.

Aging Increases Susceptibility to Periodontal Disease

The amount of periodontal bone present in young mice was 51% greater than aged mice ($P < 0.05$). DC FOXO1 deletion further reduced the amount of bone by ~40% in aged mice ($P < 0.05$; Fig. 4A, B) and caused greater bone loss in young mice ($P <$ 0.05). The number of osteoclasts, which reflects recent bone loss, followed a similar pattern. Aged mice had ~60% more osteoclasts per millimeter compared with the corresponding young mice ($P < 0.05$). Reduced DC function increased osteoclasts by ~65% in aged mice ($P < 0.05$; Fig. 5A, B) and in young mice ($P < 0.05$). The effect on gingival inflammation was assessed by measuring TNF expression. The number of cells that expressed TNF increased ~2-fold in aged mice versus young mice. DC-specific deletion of FOXO1 further increased TNF in aged mice by ~30% increase ($P < 0.05$; Fig. 5C, D).

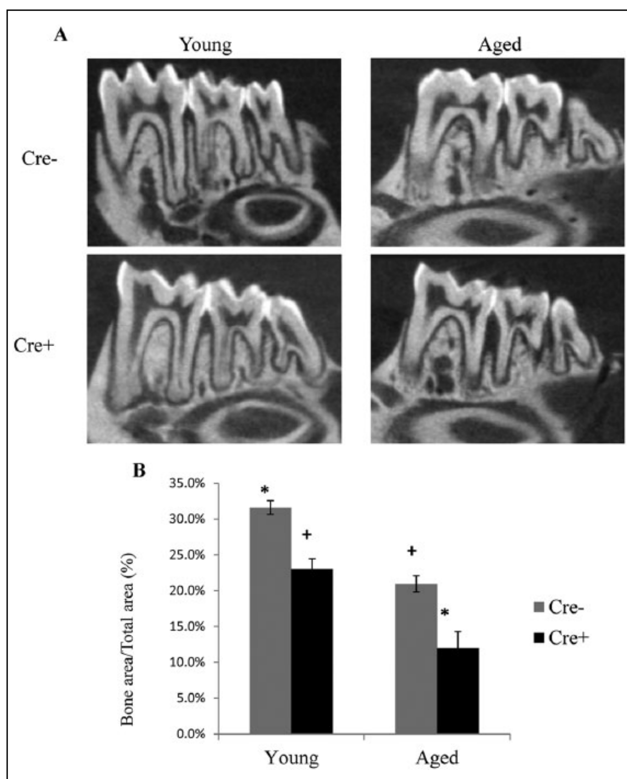

Figure 4. Aging reduces the amount of alveolar bone. Maxillary teeth were scanned by micro–computed tomography. (**A**) Micro-CT of maxilla. (**B**) Remaining bone area/total area between the first and second molars. Values are expressed as the mean ± SE, *n* = 5 or 6 mice per group. *Significant difference ($P < 0.05$) between young mice and corresponding aged mice. +Significant difference ($P < 0.05$) between wild-type mice and age-matched dendritic cell FOXO1 deletion mice.

Discussion

We assessed the effect of aging on the host response in the gingiva and draining lymph nodes by comparing aged and young mice after oral infection. Aged mice and DC FOXO1–deleted mice both had decreased numbers of DCs in cervical lymph nodes. Surprisingly, aging increased the adaptive immune response to oral infection, reflected by increased antibody to *P. gingivalis*, increased IL-12, and greater numbers of plasma cells in lymph nodes. In contrast, DC FOXO1 deletion reduced these parameters in aged mice, suggesting that the changes in the host response in aged mice was not strictly tied to age-related changes in DCs. This was investigated further by

demonstrating that aged mice had greater colonization by *P. gingivalis*. This increased colonization may be associated with reduced bacterial diversity observed in aged compared with young mice and may in turn enhance susceptibility to periodontal disease. The latter was demonstrated by greater levels of TNF in the periodontium of aged mice following oral inoculation and increased numbers of osteoclasts with reduced levels of bone.

Aged mice had reduced DCs in cervical lymph nodes following challenge with *P. gingivalis*/*F. nucleatum*. To investigate a potential mechanism, we examined DCs in vitro and found that an AGE that binds to RAGE (Nedić et al. 2013) and is increased with aging (Nedić et al. 2013) significantly reduces DC migration. Thus, a potential mechanism for reduced DC numbers in lymph nodes may involve the effect of AGE on DC migration. Aging increased the number of osteoclasts in mice challenged with *P. gingivalis*/*F. nucleatum* compared with young mice. These mice also exhibited overall higher levels of bacteria. The reduced numbers of DCs in draining lymph nodes were observed with DC-specific deletion of FOXO1. It is possible that long-term reduction of DCs in lymph nodes of aged mice and mice with FOXO1 deletion results in greater accumulation of bacteria. This speculation is supported by findings that depletion of DCs increases bacterial levels in the kidneys and lungs (Schindler et al. 2012).

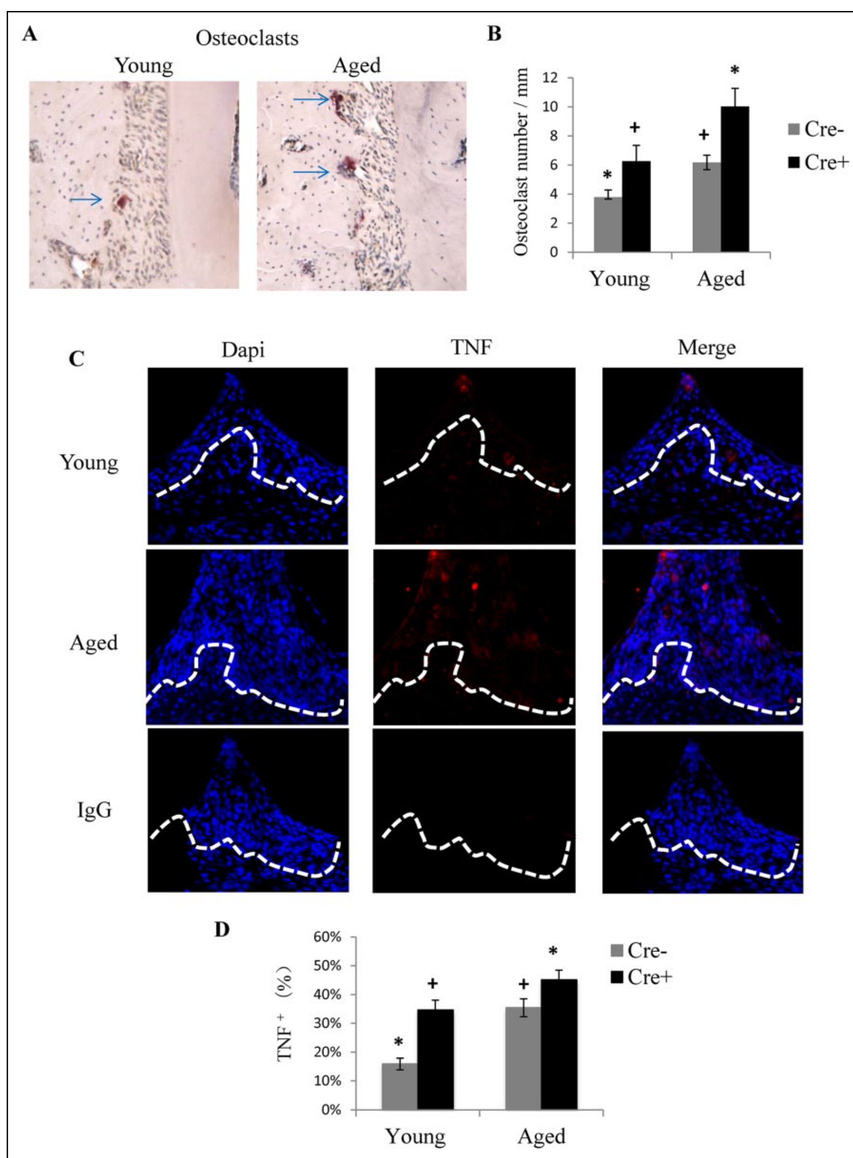

Figure 5. Aging increases susceptibility to periodontal disease. Serial paraffin sections that included maxillary first and second molars and alveolar bone were prepared. Osteoclast number was analyzed by tartrate-resistant acid phosphatase (TRAP) staining, and TNF expressed in the gingival epithelium was determined by immunofluorescence. (**A**) Histomorphometric analysis of osteoclast number analyzed by TRAP staining (blue arrow indicates the osteoclast). (**B**) Number of osteoclasts per bone length was measured in alveolar bone adjacent to the second molar. (**C**) Images of TNF-positive staining in gingival epithelium between the first and second molars. The upper part of the white line indicates epithelium, and the lower part indicates connective tissue. (**D**) The percentage TNF-positive cells was measured in gingival epithelium by immunofluorescence. Values are expressed as the mean ± SE, n = 5 or 6 per group. *Significant difference ($P < 0.05$) between young mice and corresponding aged mice. +Significant difference ($P < 0.05$) between wild-type mice and age-matched dendritic cell FOXO1 deletion mice.

Several aspects of the adaptive immune response were greater in aged mice than in young mice despite the reduced number of DCs found in lymph nodes. The changes followed closely an increase in *P. gingivalis* in aged mice. Aging was linked to an increase in antibody production to *P. gingivalis*, which coincided with a higher number of plasma cells in cervical lymph nodes of aged versus young mice and higher levels of IL-12 production in the germinal centers of lymph nodes. These changes are not simply related to age-reduced DC function, since FOXO1 deletion partially rescued the impact of aging on each.

The increase in antibody levels in aged mice is likely to reflect greater exposure of aged mice to bacterial stimulus since the amount of *P. gingivalis* recovered on the tooth surface of aged mice was higher than young mice. The difference in *P. gingivalis* colonization may reflect altered bacteria-bacteria interactions in aged and young mice. It is noteworthy that the aged mice had reduced bacterial diversity that may increase subsequent colonization of inoculated *P. gingivalis*. The reduced microbial diversity that we found in aged mice was linked to a reduced proportion of commensal bacteria and an increased proportion of bacteria known to have pathogenic characteristics. Similar changes have been noted in the gut during aging (Power et al. 2014), and an increase in anaerobes has been reported with aging in the oral cavity (Shoemark and

Allen 2015). Human studies report that individuals with periodontitis have higher microbial diversity than healthy controls (Griffen et al. 2012). This finding does not necessarily disagree with our results, since we are comparing young and old animals while Griffen and coworkers (2012) compared similarly aged individuals with and without periodontitis.

In conclusion, we found that aging reduced numbers of DCs in regional lymph nodes similar to mice with impaired DC function. However, aged mice had a greater adaptive immune response to inoculated *P. gingivalis*, which is likely due to greater accumulation of *P. gingivalis* following inoculation. High levels of *P. gingivalis* were not strictly due to an impaired adaptive immunity, since it was not observed in mice with FOXO1 deletion in DCs but may be linked to altered bacteria-bacteria interactions in aged mice, which had reduced bacterial diversity. Ultimately the changes in the aged mice led to increased osteoclast numbers when challenged by inoculation of oral pathogens, indicative of enhanced susceptibility to periodontitis.

Author Contributions

Y. Wu, G. Dong, contributed to data analysis and interpretation, drafted and critically revised the manuscript; W. Xiao, E. Xiao, F. Miao, A. Syverson, N. Missaghian, R. Vafa, A.A. Cabrera-Ortega, contributed to data analysis, critically revised the manuscript; C. Rossa Jr, contributed to data analysis and interpretation, critically revised the manuscript; D.T. Graves, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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References

Arizon M, Nudel I, Segev H, Mizraji G, Elnekave M, Furmanov K, Eli-Berchoer L, Clausen BE, Shapira L, Wilensky A, et al. 2012. Langerhans cells down-regulate inflammation-driven alveolar bone loss. Proc Natl Acad Sci U S A. 109(18):7043–7048.

Benakanakere M, Abdolhosseini M, Hosur K, Finoti LS, Kinane DF. 2015. TLR2 promoter hypermethylation creates innate immune dysbiosis. J Dent Res. 94(1):183–191.

Biddle A, Stewart L, Blanchard J, Leschine S. 2013. Untangling the genetic basis of fibrolytic specialization by lachnospiraceae and ruminococcaceae in diverse gut communities. Diversity. 5(3):627–640.

Bodineau A, Folliguet M, Séguier S. 2009. Tissular senescence and modifications of oral ecosystem in the elderly: risk factors for mucosal pathologies. Curr Aging Sci. 2(2):109–120.

Brown J, Wang H, Suttles J, Graves DT, Martin M. 2011. mTORC2 negatively regulates the toll-like receptor 4-mediated inflammatory response via FoxO1. J Biol Chem. 286(52):44295–44305.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A. 108 Suppl 1:4516–4522.

Chae P, Im M, Gibson F, Jiang Y, Graves DT. 2002. Mice lacking monocyte chemoattractant protein 1 have enhanced susceptibility to an interstitial polymicrobial infection due to impaired monocyte recruitment. Infect Immun. 70(6):3164–3169.

Cutler CW, Teng YT. 2007. Oral mucosal dendritic cells and periodontitis: many sides of the same coin with new twists. Periodontology. 45:35–50.

Dong G, Wang Y, Xiao W, Pacios Pujado S, Xu F, Tian C, Xiao E, Choi Y, Graves DT. 2015. FOXO1 regulates dendritic cell activity through ICAM-1 and CCR7. J Immunol. 194(8):3745–3755.

Dye BA. 2012. Global periodontal disease epidemiology. Periodontol. 58(1):10–25.

Garlet GP. 2010. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J Dent Res. 89(12):1349–1363.

Gkogkolou P, Böhm M. 2012. Advanced glycation end products: key players in skin aging? Dermatoendocrinol. 4(3):259–270.

Graves DT, Li J, Cochran DL. 2011. Inflammation and uncoupling as mechanisms of periodontal bone loss. J Dent Res. 90(2):143–153.

Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, Podar M, Leys EJ. 2012. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J. 6(6):1176–1185.

Hajishengallis G. 2010. Too old to fight? Aging and its toll on innate immunity. Mol Oral Microbiol. 25(1):25–37.

Hajishengallis G. 2014. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol. 35(1):3–11.

Hovav AH. 2014. Dendritic cells of the oral mucosa. Mucosal Immunol. 7(1):27–37.

Kumar PS, Matthews CR, Joshi V, de Jager M, Aspiras M. 2011. Tobacco smoking affects bacterial acquisition and colonization in oral biofilms. Infect Immun. 79(11):4730–4738.

Liu R, Bal HS, Desta T, Krothapalli N, Alyassi M, Luan Q, Graves DT. 2006. Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. J Dent Res. 85(6):510–514.

McIntosh ML, Hajishengallis G. 2012. Inhibition of *Porphyromonas gingivalis*–induced periodontal bone loss by CXCR4 antagonist treatment. Mol Oral Microbiol. 27(6):449–457.

Nedić O, Rattan SI, Grune T, Trougakos IP. 2013. Molecular effects of advanced glycation end products on cell signalling pathways, ageing and pathophysiology. Free Radic Res. 47 Suppl 1:28–38.

Power SE, O'Toole PW, Stanton C, Ross RP, Fitzgerald GF. 2014. Intestinal microbiota, diet and health. Br J Nutr. 11(3):387–402.

Rolny IS, Minnaard J, Racedo SM, Pérez PF. 2014. Murine model of *Bacillus cereus* gastrointestinal infection. J Med Microbiol. 63(Pt 12):1741–1749.

Salih DA, Brunet A. 2008. FoxO transcription factors in the maintenance of cellular homeostasis during aging. Curr Opin Cell Biol. 20(2):126–136.

Schindler D, Gutierrez MG, Beineke A, Rauter Y, Rohde M, Foster S, Goldmann O, Medina E. 2012. Dendritic cells are central coordinators of the host immune response to Staphylococcus aureus bloodstream infection. Am J Pathol. 181(4):1327–1337.

Shoemark DK, Allen SJ. 2015. The microbiome and disease: reviewing the links between the oral microbiome, aging, and Alzheimer's disease. J Alzheimers Dis. 43(3):725–738.

Souto R, Silva-Boghossian CM, Colombo AP. 2014. Prevalence of *Pseudomonas aeruginosa* and *Acinetobacter* spp. in subgingival biofilm and saliva of subjects with chronic periodontal infection. Braz J Microbiol. 45(2):495–501.

Szkaradkiewicz AK, Karpinski TM, Zeidler A, Wyganowska-Swiatkowska M, Szkaradkiewicz A. 2011. Protective effect of oral *Lactobacilli* in pathogenesis of chronic periodontitis. J Physiol Pharmacol. 6(2):685–689.

Wilensky A, Segev H, Mizraji G, Shaul Y, Capucha T, Shacham M, Hovav AH. 2014. Dendritic cells and their role in periodontal disease. Oral Dis. 20(2):119–126.

Xiao W, Dong G, Pacios S, Alnammary M, Barger LA, Wang Y, Wu Y, Graves DT. 2015. FOXO1 deletion reduces dendritic cell function and enhances susceptibility to periodontitis. Am J Pathol. 185(4):1085–1093.

Zhang C, Ponugoti B, Tian C, Xu F, Tarapore R, Batres A, Alsadun S, Lim J, Dong G, Graves DT. 2015. FOXO1 differentially regulates both normal and diabetic wound healing. J Cell Biol. 209(2):289–303.