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Alveolar bone loss in T helper 1/T helper 2 cytokine-deficient mice

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Background and Objective: The role of cytokines in bone loss is important in the context of periodontitis, where inflammation-induced bone destruction is a major manifestation. Numerous cytokines have been implicated as mediators of bone resorption. The purpose of this study was to observe the impact of targeted gene deletion of T helper 1 (Th1) and T helper 2 (Th2) cytokines on naturally occurring alveolar bone loss in genetically modified mice.

Material and Methods: Alveolar bone loss was measured histomorphometrically in interleukin-4, interleukin-10, interleukin-12p40, interferon-γ (IFN-γ) and tumor necrosis factor (TNF) knockout mice at 6, 16 and 30 wk of age.

Results: Both Th1 (interleukin-12p40, IFN-γ, TNF) and Th2 (interleukin-10, interleukin-4) knockout mice exhibited significantly more alveolar bone loss than their respective wild-type control mice ($p < 0.001$). Interleukin-10–/– and interleukin-12p40–/– mice exhibited a three-fold increase in alveolar bone loss at 30 wk of age, whereas bone loss in IFN-γ–/–, TNF–/– and interleukin-4–/– mice was 1.5- to two-fold higher compared with wild-type control mice.

Conclusion: The results of the present study indicate that both Th1 and Th2 cytokines play an important role in maintaining alveolar bone homeostasis. The kinetics of alveolar bone loss seen in cytokine gene knockout mice indicates that bone loss is age dependent and late in onset.

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The role of cytokines in bone loss is important in the context of periodontitis, where inflammation-induced bone destruction is a major manifestation. Numerous cytokines have been implicated as mediators of bone resorption (1,2). It has been hypothesized that the spectrum of cytokines which are produced in the surrounding microenvironment determines the development of normal bone remodeling or pathologic bone loss (3).

Cytokines such as tumor necrosis factor (TNF) have been shown to be powerful bone resorptive mediators both *in vitro* and *in vivo* (4,5). TNF stimulates bone resorption by inducing the proliferation, differentiation and activation of osteoclasts (5).

In contrast, cytokines such as interleukin-4 and interleukin-10 have been suggested to have protective properties. Interleukin-4 has been shown to inhibit osteoclastogenesis via multiple mechanisms, including inhibition of nuclear factor-κB signaling (6). Studies in interleukin-10 knockout mice have shown elevated levels of interleukin-1 and TNF, highlighting its significant role in regulating pro-inflammatory cytokine levels *in vivo* (7–9).

The effects of interferon-γ (IFN-γ) on bone remodeling have, to date, been contradictory. IFN-γ has been shown to inhibit bone resorption by preventing proliferation and differentiation of committed precursors to mature osteoclasts (10,11). Conversely, IFN-γ decreases the amount of trabecular bone and mineralization in osteoporotic mice (12).

Spontaneously occurring alveolar bone loss has been shown in animals with various genetic deficiencies (13–15). HLA-B27 transgenic rats are known to suffer increased alveolar bone loss (13,15) and other severe

inflammatory reactions, such as spontaneous arthritis and colitis (16,17). Similarly, studies in interleukin-10 knockout mice have demonstrated significantly greater natural alveolar bone loss relative to their wild-type counterparts (18).

Studies on the response of alveolar bone to the presence or absence of T helper 1 (Th1) and T helper 2 (Th2) cytokines may provide insights into the mechanisms that regulate alveolar bone loss in periodontal disease. The purpose of this study was to observe the impact of targeted gene deletion of Th1 and Th2 cytokines on naturally occurring alveolar bone loss in genetically modified mice. This was achieved by measuring bone loss in interleukin-4, interleukin-10, interleukin-12p40, IFN-γ and TNF knockout mice at 6, 16 and 30 wk of age.

Material and methods

Mice

Specific pathogen-free female cytokine knockout mice and their respective controls, 6–8 wk of age, were used in this experiment. Mice were obtained from various sources and bred at the Herston Medical Research Centre, Brisbane Australia, with the genotypes checked regularly by polymerase chain reaction. Animal experiments were approved by the Animal Experimentation Ethics Committee of the University of Queensland, and carried out in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 1997. Mice were housed in filter-top cages in a PC2 facility, and provided with food and water *ad libitum*. All mice were fed the same fixed-formulation autoclavable diet consisting of wheat, lupins, barley, soya meal, fish meal, mixed vegetable oils, canola oil, salt, calcium carbonate, dicalcium phosphate, magnesium oxide, and a vitamin and trace mineral premix (Specialty Feeds, Glen Forrest, WA, Australia).

Knockout (–/–) mice used in this study included interleukin-10 (19), interleukin-12p40 (20,21), IFN-γ (22) and TNF (23) on the C57BL/6J background, and interleukin-4 (24) on the BALB/c background.

Histomorphometric bone analysis

Mice were killed using CO_2 inhalation at baseline (6–8 wk old), 70 d later (16–18 wk old) and 6 mo later (30–32 wk old). Skulls were dissected, and jaws defleshed after treatment in 6% Triton-100 at 85°C for 3 h. Subsequently, the samples were immersed overnight in 3% H_2O_2, followed by a 20-s wash with 1% NaOCl, air-dried and stained with 0.5% eosin for 5 min followed by 1% methylene blue for 1 min, in order to delineate the cemento–enamel junction (CEJ) more clearly.

Alveolar bone loss was measured morphometrically, according to the method of Tatakis & Guglielmoni, with minor modifications (13). The area of bone loss (mm^2) was calculated as the sum of the exposed molar root surface on all three molars, in both the maxilla (buccal and palatal) and mandible (lingual). Blinded measurements were performed using a dissecting microscope and a computer-assisted image analysis system (Axiovision; Carl Zeiss Vision, Berlin, Germany), by a sole blinded operator. The area of interest was highlighted by accurately circumscribing the area bounded by the CEJ and the alveolar bone crest. These represent both the occlusal and apical boundaries of each molar, respectively. The mesial and distal boundaries were represented by the mesial and distal line angles of each molar, respectively. A scale was obtained using a 1-micrometer microscopic slide, mounted at the same magnification as the jaw specimens. Intra-observer reproducibility was determined to be ≈95%.

Statistics

Quantitative data were analysed using the statistical features of GRAPHPAD PRISM Version 2.01 (GraphPad Inc., San Diego, CA, USA). Student's *t*-test and one-way analysis of variance (ANOVA) were used with $p < 0.05$, unless otherwise stated.

Results

Alveolar bone loss kinetics

Interleukin-10–/– and interleukin-12p40–/– mice exhibited a three-fold increase in alveolar bone loss at 30 wk of age when compared with knockout mice at 6 and 16 wk of age ($p < 0.001$) (Fig. 1A,B).

TNF–/–, IFN-γ –/– and interleukin-4–/– mice also displayed increased bone loss at 30 wk of age compared with mice at 6 and 16 wk of age ($p < 0.01$), although the magnitude of bone loss was less than that seen in interleukin-10–/– and interleukin-12p40–/– mice at 30 wk (Fig. 1C,D,E). There was no significant increase in bone loss between mice of 6 and 16 wk of age for any of the knockout mouse strains (Fig. 1).

Alveolar bone loss in aged knockout mice

All knockout mice exhibited increased bone loss, as determined by area of root exposure compared with wild-type control mice at 30 wk of age (Fig. 2). Interleukin-10–/–, interleukin-12p40–/–, IFN-γ–/– and TNF–/– mice exhibited significantly more alveolar bone loss than C57BL/6J wild-type mice ($p < 0.001$) (Fig. 2A). Interleukin-4–/– mice displayed increased alveolar bone loss compared with BALB/c wild-type control mice ($p < 0.001$) (Fig. 2B). The extent of alveolar bone loss seen in interleukin-10–/– and interleukin-12 p40–/– mice was three-fold higher compared with wild-type C57BL/6J mice, and is clearly demonstrated in Fig. 3.

Discussion

Bone remodeling is tightly regulated by numerous factors, including cytokines, hormones and growth factors (3). Cytokines appear to have a crucial role in both normal and pathologic bone cell function (3). The results of the present study indicate that both Th1 (interleukin-12p40, IFN-γ, TNF) and Th2 (interleukin-10, interleukin-4) cytokines play an important role in maintaining alveolar bone homeostasis. The

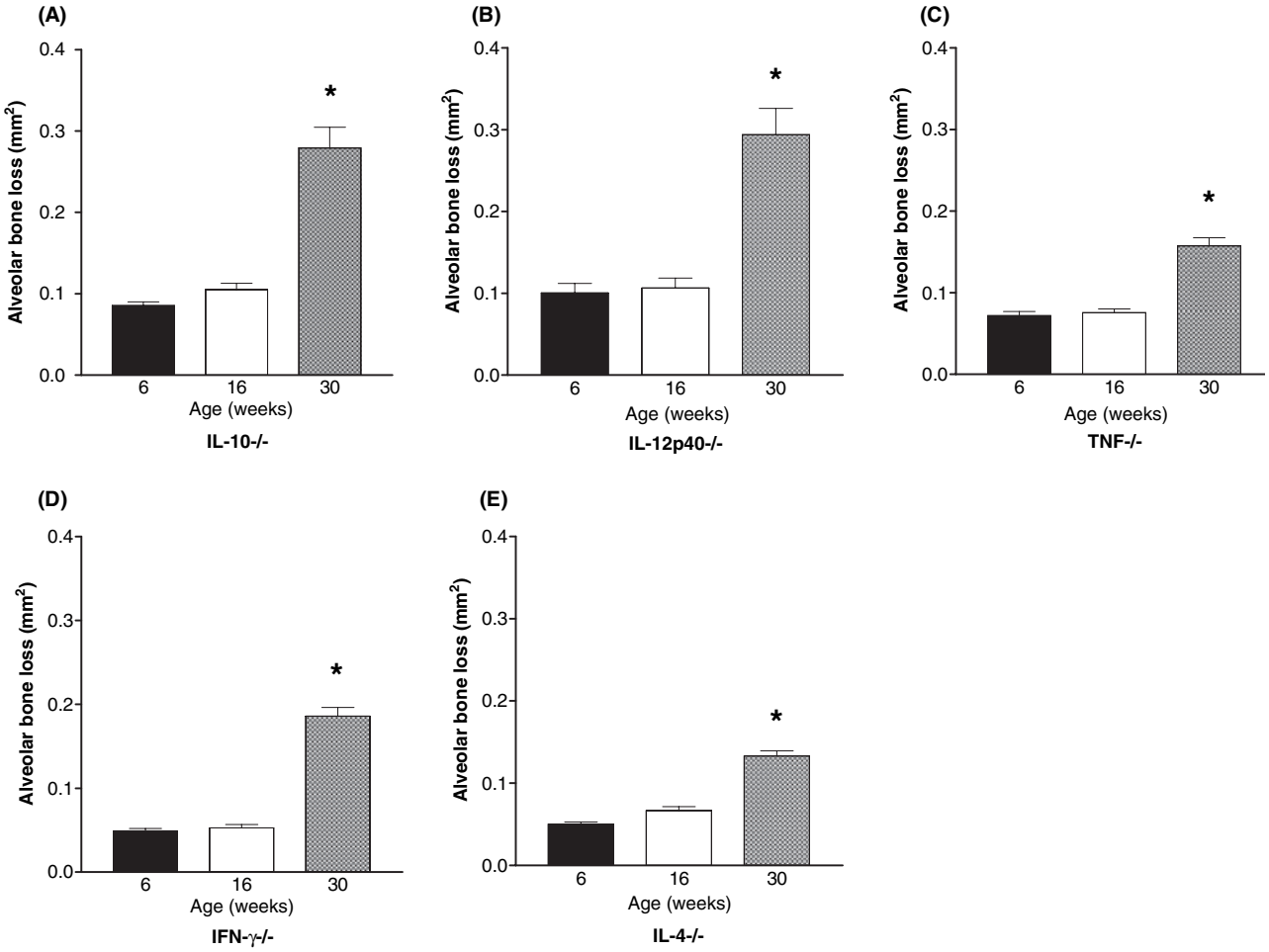

Fig. 1. Kinetics of alveolar bone loss (mm^2) (expressed as mean ± standard error of the mean) in (A) interleukin-10–/–, (B) interleukin-12p40–/–, (C) TNF–/–, (D) IFN-γ–/– and (E) interleukin-4–/– mice, at 6, 16 and 30 wk of age. *Significantly greater alveolar bone loss in the 30-wk-old mice compared with the 6- and 16-wk-old mice ($p < 0.001$) ($n = 15$ mice per group). IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor.

findings of the present study, in conjunction with studies reporting increased spontaneous alveolar bone loss in HLA-B27 transgenic rats (15) and P/E-selectin-deficient mice (14), point to multiple mechanisms for increasing the host's susceptibility to spontaneous alveolar bone loss.

Interleukin-10 inhibits the macrophage-dependent development of Th1 cells (25) and has a major role in regulating *in vivo* levels of pro-inflammatory cytokines, such as interleukin-1 and TNF (26). Interleukin-10 has also been shown to be a potent inhibitor of osteoclast formation *in vitro* (27). The results of our study are consistent with others in implicating interleukin-10 as a major regulator of bone homeostasis (18,28). The ability of interleukin-10 to suppress pro-inflammatory cytokine synthesis may contribute to the increased alveolar bone loss seen in interleukin-10 knockout mice (28), as these same cytokines have been implicated in alveolar bone resorption (29,30). Interleukin-1 and TNF production has been shown to be elevated in the absence of interleukin-10 (8,31).

The inhibitory effect of interleukin-10 on osteoclast formation and pro-inflammatory cytokine production suggests that the action of interleukin-10 on alveolar bone is via increased resorption as opposed to decreased bone formation. In support of this hypothesis, the bone resorption marker type 1 collagen C-telopeptide, has been found to be significantly elevated in interleukin-10–/– mice (28), although another study, showing osteopaenia of both cancellous and cortical bone in interleukin-10–/– mice, suggests that this was caused by suppressed bone formation with no evidence of increased resorption (32).

Interleukin-10–/– mice also suffer from altered immune responses, leading to chronic inflammation with continuous over-production of cytokines such as TNF, interleukin-1 or IFN-γ, and have been shown to develop enteric colitis (19). Interleukin-10 has also been shown to be involved in the development and progression of arthritis (8,9,33). An interesting finding in interleukin-10–/– mice is that the development of inflammatory bowel disease is dependent on the presence of a normal gut flora (34). Moreover,

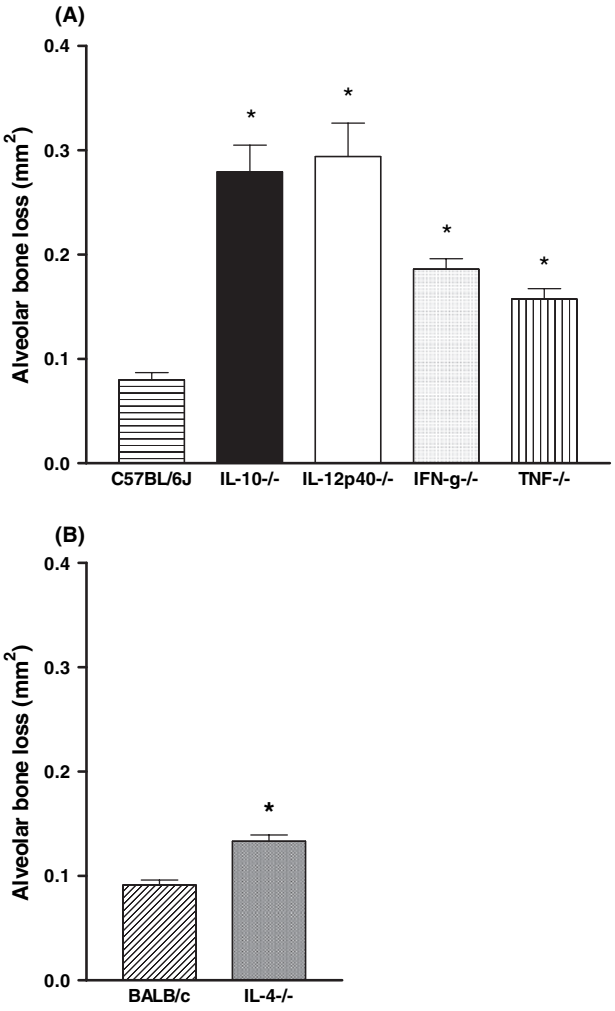

Fig. 2. Alveolar bone loss (mm^2) (expressed as mean ± standard error of the mean) in cytokine knockout mice at 30 wk of age in (A) interleukin-10–/–, interleukin-12p40–/–, IFN-γ–/– and TNF–/– mice compared with C57BL/6J control mice, and in (B) interleukin-4–/– mice compared with BALB/c mice ($n = 15$ mice per group). *Significantly greater alveolar bone loss in interleukin-10–/–, interleukin-12p40–/–, IFN-γ–/– and TNF–/– mice compared with C57BL/6 J control mice ($p < 0.001$), and in interleukin-4–/– compared with BALB/c mice ($p < 0.001$). IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor.

interleukin-10–/– mice with colitis have significantly lower bone mass and reduced bone formation when compared with interleukin-10–/– mice without colitis (32). It remains to be proven, however, whether the severe alveolar bone loss seen in interleukin-10–/– mice is dependent on the presence of commensal oral flora. Similarly to previous studies, the oral environment of the animals used in this experiment was not manipulated (18,28).

Interleukin-12 and IFN-γ are integral regulators of the immune system and promote the development of Th1 cells whilst suppressing the Th2 phenotype (20,35,36). Like many cytokines, IFN-γ and interleukin-12 also play a role in bone metabolism (11,37–40). The results of our study indicate that alveolar bone destruction is significantly increased in the absence of either interleukin-12p40 or IFN-γ. The increased levels of bone loss seen in interleukin-12p40–/– mice compared with IFN-γ–/– mice may be explained by the fact that the former are deficient in both interleukin-12 and IFN-γ, whereas the latter are only deficient in IFN-γ. To our knowledge, this is the first study to assess bone loss in interleukin-12p40–/– mice, and highlights the importance of this Th1 cytokine in natural bone metabolism.

In vitro, interleukin-12 has been shown to inhibit osteoclast formation in a dose-dependent manner, and this inhibition is believed to be T-cell dependent (40). As interleukin-12 is known to induce the production of IFN-γ by T cells and natural killer cells (41,42), it is not surprising then that an IFN-γ-dependent pathway is implicated as the mediator of this inhibition. IFN-γ has well known inhibitory actions on osteoclastic formation and is certainly believed to play a role in the interleukin-12 inhibition of bone resorption (39,43). Despite this, however, IFN-γ-independent pathways have also been suggested as mechanisms for interleukin-12 inhibition of osteoclastic bone resorption (40,43).

The actions of IFN-γ on osteoclastic bone resorption may be mediated by both its early effects on osteoclastic development (11,38), and later by its actions on mature osteoclast activity (44). IFN-γ has been shown to suppress osteoclastogenesis strongly by interfering with the receptor activator of nuclear factor κB ligand/receptor activator of nuclear factor κB (RANKL/RANK) signaling pathway (11,38). IFN-γ induces rapid degradation of the RANK adaptor protein, TNF receptor-associated factor 6, resulting in strong inhibition of RANKL transcription (11).

TNF is one of the most potent osteoclastogenic cytokines produced in inflammation. It is also an important cytokine in the pathogenesis of chronic inflammatory diseases, such as periodontal disease and arthritis (30,45,46). In a nonhuman primate periodontal model, the use of TNF and interleukin-1 blockers resulted in significant reductions in osteoclast formation and alveolar bone loss (30). TNF and interleukin-1 have an important role in promoting osteoclast recruitment, activation and osteolysis in pathological states (45). The results of our study support the fact that TNF is a potent mediator of bone resorption. Although TNF gene deletion resulted in more bone loss than that seen in wild-type mice,

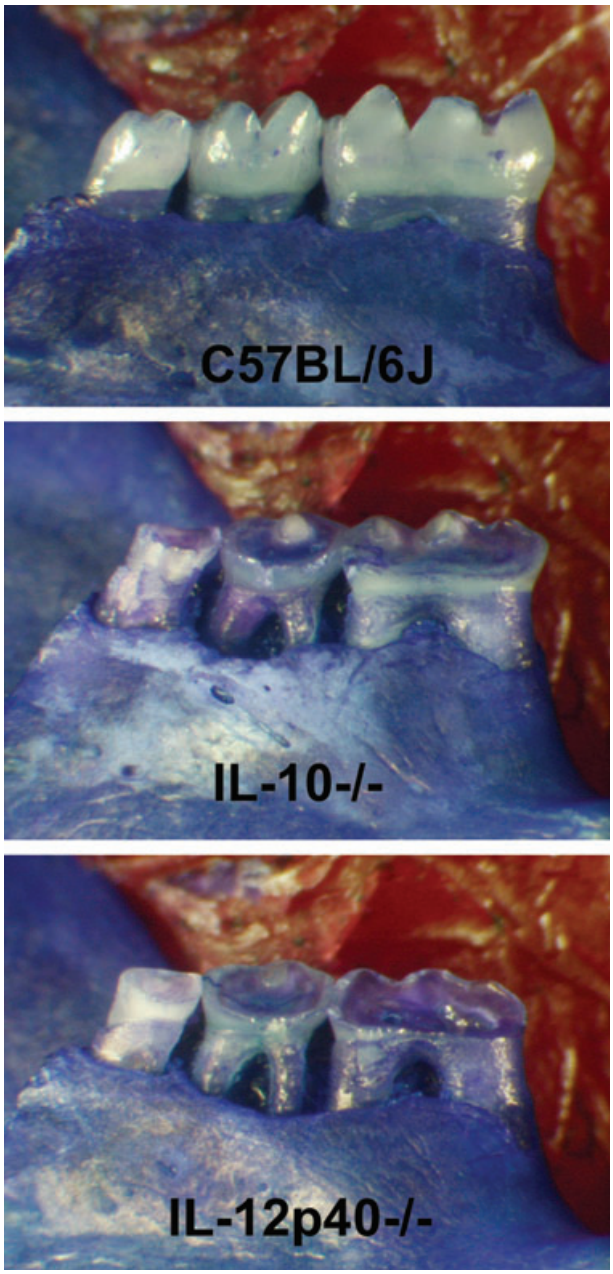

Fig. 3. Alveolar bone loss on mandibular lingual aspect of (A) C57BL/6J, (B) interleukin-10–/– and (C) interleukin-12p40–/– mice at 30 wk of age. Representative images show extensive areas of bone loss, especially surrounding the second and third molar teeth, with significant furcation involvement in knockout mice compared with the wild-type mice. Correlation of these images with area of alveolar bone loss, as seen in Fig. 2, is evident. IL, interleukin.

TNF–/– mice displayed the least amount of natural bone loss compared with the other cytokine knockout mice. This finding also illustrates the point that cytokines identified as having an important role in pathological states may only have a minor role in natural bone remodeling.

Interleukin-4 is a potent inhibitor of osteoclast formation both *in vitro* and *in vivo*. Several mechanisms have been proposed to explain the actions of interleukin-4 (47), and these include both direct inhibitory actions on osteoclast formation and indirect actions via osteoblasts or through the action of T cells (48). Although there was increased bone loss in interleukin-4 knockout mice, deletion of the interleukin-4 gene resulted in the least amount of alveolar bone loss compared with other cytokine gene deletions, suggesting that interleukin-4 may have a less significant impact on alveolar bone homeostasis compared with the other cytokines examined.

The kinetics of alveolar bone loss seen in cytokine knockout mice in this study indicates that bone loss was clearly late in onset. This confirms results seen in similar knockout models (13,15,28), and further highlights the importance of age matching test and control groups when carrying out research in animal knockout models.

Although we did not assess the natural oral flora in these mice, it is unlikely that this would have a substantial effect on the level of bone loss. All mice used in this study were housed in the same specific pathogen-free facility, bred in the same manner, and fed the same diet. As in interleukin-10–/– mice, it remains to be proven whether alveolar bone loss is dependent on the presence of a commensal oral flora. Furthermore, the excessive levels of tooth wear seen in the interleukin-10 and interleukin-12p40 knockout mice compared with the C57BL/6J wild-type controls, although possibly related to differences in the oral microflora, are more likely to be related to the mineral content of the dentition itself, or to qualitative/quantitative changes in saliva. This will require further analysis in the future to determine the exact mechanism.

Although naturally occurring alveolar bone loss differs from that seen during periodontitis, the relevance of this model to periodontal disease lies in the importance of acknowledging the host's increasing susceptibility to spontaneous alveolar bone loss. Identifying the contribution of Th1 and Th2 cytokines to natural alveolar bone loss will aid in understanding their role in periodontal disease. Determining natural alveolar bone loss in these mice is a prerequisite to investigating the effect of periodontopathic bacteria on bone loss in murine models. Further studies are planned to evaluate the

effect of different periodontopathic bacteria on bone loss in these knockout mice.

In conclusion, the results of the present study indicate that both Th1 (interleukin-12p40, IFN-γ, TNF) and Th2 (interleukin-10, interleukin-4) cytokines play an important role in maintaining alveolar bone homeostasis. Deletion of key cytokines that polarize the Th1 (interleukin-12) or Th2 (interleukin-10) pathways appear to exert the most significant effect on natural bone loss. The kinetics of alveolar bone loss seen in these cytokine gene knockout mice indicates that bone loss is age-dependent and late in onset. The results of this study will be beneficial in understanding the effects of periodontopathic bacteria on alveolar bone loss in models utilizing these gene-targeted mice.

Acknowledgements

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References

1. Baker PJ. The role of immune responses in bone loss during periodontal disease. *Microbes Infect* 2000;**2:**1181–1192.
2. McCauley LK, Nohutcu RM. Mediators of periodontal osseous destruction and remodeling: principles and implications for diagnosis and therapy. *J Periodontol* 2002;**73:**1377–1391.
3. Horowitz M, Lorenzo J. Local regulators of bone. In: Bilezikian J, Raisz L, Rodan G, eds. *Principles of Bone Biology*. San Diego: Academic Press, 2002: 961–977.
4. Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. *J Bone Miner Res* 1997;**12:**869–879.
5. Manolagas SC. Role of cytokines in bone resorption. *Bone* 1995;**17:**63S–67S.
6. Abu-Amer Y. IL-4 abrogates osteoclastogenesis through STAT6-dependent inhibition of NF-kappab. *J Clin Invest* 2001;**107:**1375–1385.
7. Berg DJ, Kuhn R, Rajewsky K, Muller W, Menon S, Davidson N. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxic tolerance. *J Clin Invest* 1995;**96:**2339–2347.
8. Cuzzocrea S, Mazzon E, Dugo L *et al*. Absence of endogeneous interleukin-10 enhances the evolution of murine type-II collagen-induced arthritis. *Eur Cytokine Netw* 2001;**12:**568–580.
9. Puliti M, Von Hunolstein C, Verwaerde C, Bistoni F, Orefici G, Tissi L. Regulatory role of interleukin-10 in experimental group B streptococcal arthritis. *Infect Immun* 2002;**70:**2862–2868.
10. Takahashi N, Mundy GR, Kuehl TJ, Roodman GD. Osteoclast like formation in fetal and new born long term baboon marrow cultures is more sensitive to 1,25-di-hydroxyvitamin D_3 than adult long term marrow cultures. *J Bone Miner Res* 1987;**2:**311–317.
11. Takayanagi H, Ogasawara K, Hida S *et al*. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. *Nature* 2000;**408:**600–605.
12. Mann GN, Jacobs TW, Buchinsky FJ *et al*. Interferon-gamma causes loss of bone volume in vivo and fails to ameliorate cyclosporin a-induced osteopenia. *Endocrinology* 1994;**135:**1077–1083.
13. Tatakis DN, Guglielmoni P. HLA-B27 transgenic rats are susceptible to accelerated alveolar bone loss. *J Periodontol* 2000;**71:**1395–1400.
14. Niederman R, Westernoff T, Lee C *et al*. Infection-mediated early-onset periodontal disease in P/E-selectin-deficient mice. *J Clin Periodontol* 2001;**28:**569–575.
15. May NY, Tatakis DN. Accelerated alveolar bone loss in male HLA-B27 transgenic rats: Adult onset. *J Periodont Res* 2004;**39:**33–36.
16. Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell* 1990;**63:**1099–1112.
17. Taurog JD. Arthritis in HLA-B27 transgenic animals. *Am J Med Sci* 1998;**316:**250–256.
18. Al-Rasheed A, Scheerens H, Rennick DM, Fletcher HM, Tatakis DN. Accelerated alveolar bone loss in mice lacking interleukin-10. *J Dent Res* 2003;**82:**632–635.
19. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993;**75:**263–274.
20. Magram J, Sfarra J, Connaughton S *et al*. IL-12-deficient mice are defective but not devoid of type 1 cytokine responses. *Ann N Y Acad Sci* 1996;**795:**60–70.
21. Magram J, Connaughton SE, Warrier RR *et al*. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. *Immunity* 1996;**4:**471–481.
22. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* 1993;**259:**1739–1742.
23. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary b cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 1996;**184:**1397–1411.
24. Noben-Trauth N, Kohler G, Burki K, Ledermann B. Efficient targeting of the IL-4 gene in a BALB/c embryonic stem cell line. *Transgenic Res* 1996;**5:**487–491.
25. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989;**170:**2081–2095.
26. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991;**147:**3815–3822.
27. Owens JM, Gallagher AC, Chambers TJ. IL-10 modulates formation of osteoclasts in murine hemopoietic cultures. *J Immunol* 1996;**157:**936–940.
28. Al-Rasheed A, Scheerens H, Srivastava AK, Rennick DM, Tatakis DN. Accelerated alveolar bone loss in mice lacking interleukin-10: late onset. *J Periodont Res* 2004;**39:**194–198.
29. Tatakis DN. Interleukin-1 and bone metabolism: a review. *J Periodontol* 1993;**64:**416–431.
30. Assuma R, Oates T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *J Immunol* 1998;**160:**403–409.
31. Berg DJ, Davidson N, Kuhn R *et al*. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) Th1-like responses. *J Clin Invest* 1996;**98:**1010–1020.
32. Dresner-Pollak R, Gelb N, Rachmilewitz D, Karmeli F, Weinreb M. Interleukin 10-deficient mice develop osteopenia, decreased bone formation, and mechanical fragility of long bones. *Gastroenterology* 2004;**127:**792–801.

33. Walmsley M, Katsikis PD, Abney E *et al*. Interleukin-10 inhibition of the progression of established collagen-induced arthritis. *Arthritis Rheum* 1996;**39:**495–503.
34. Sellon RK, Tonkonogy S, Schultz M *et al*. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998;**66:**5224–5231.
35. O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 1998;**8:**275–283.
36. Macatonia SE, Hsieh CS, Murphy KM, O'Garra A. Dendritic cells and macrophages are required for Th1 development of CD4+ T cells from alpha beta TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-gamma production is IFN-gamma-dependent. *Int Immunol* 1993;**5:**1119–1128.
37. Garcia-Lopez S, Meikle MC, Villanueva RE *et al*. Mechanical deformation inhibits IL-10 and stimulates IL-12 production by mouse calvarial osteoblasts in vitro. *Arch Oral Biol* 2005;**50:**449–452.
38. Kotake S, Nanke Y, Mogi M *et al*. IFN-gamma-producing human T cells directly induce osteoclastogenesis from human monocytes via the expression of RANKL. *Eur J Immunol* 2005;**35:**3353–3363.
39. Nagata N, Kitaura H, Yoshida N, Nakayama K. Inhibition of RANKL-induced osteoclast formation in mouse bone marrow cells by IL-12: involvement of IFN-gamma possibly induced from non-T cell population. *Bone* 2003;**33:**721–732.
40. Horwood NJ, Elliott J, Martin TJ, Gillespie MT. IL-12 alone and in synergy with IL-18 inhibits osteoclast formation in vitro. *J Immunol* 2001;**166:**4915–4921.
41. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;**392:**245–252.
42. Kobayashi M, Fitz L, Ryan M *et al*. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 1989;**170:**827–845.
43. Yamada N, Niwa S, Tsujimura T *et al*. Interleukin-18 and interleukin-12 synergistically inhibit osteoclastic bone-resorbing activity. *Bone* 2002;**30:**901–908.
44. Kamolmatyakul S, Chen W, Li YP. Interferon-gamma down-regulates gene expression of cathepsin k in osteoclasts and inhibits osteoclast formation. *J Dent Res* 2001;**80:**351–355.
45. Romas E, Gillespie MT, Martin TJ. Involvement of receptor activator of NFkappab ligand and tumor necrosis factor-alpha in bone destruction in rheumatoid arthritis. *Bone* 2002;**30:**340–346.
46. Keystone EC. Tumor necrosis factor-alpha blockade in the treatment of rheumatoid arthritis. *Rheum Dis Clin North Am* 2001;**27:**427–443.
47. Riancho JA, Zarrabeitia MT, Gonzalez-Macias J. Interleukin-4 modulates osteoclast differentiation and inhibits the formation of resorption pits in mouse osteoclast cultures. *Biochem Biophys Res Commun* 1993;**196:**678–685.
48. Mirosavljevic D, Quinn JM, Elliott J, Horwood NJ, Martin TJ, Gillespie MT. T-cells mediate an inhibitory effect of interleukin-4 on osteoclastogenesis. *J Bone Miner Res* 2003;**18:**984–993.