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Short communication

Regulation of bone mass by the gut microbiota is dependent on NOD1 and NOD2 signaling

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ABSTRACT

Germ-free (GF) mice have increased bone mass that is normalized by colonization with gut microbiota (GM) from conventionally raised (CONV-R) mice. To determine if innate immune signaling pathways mediated the effect of the GM, we studied the skeleton of GF and CONV-R mice with targeted inactivation of MYD88, NOD1 or NOD2. In contrast to WT and *Myd88*$^{-/-}$ mice, cortical bone thickness in mice lacking *Nod1* or *Nod2* was not increased under GF conditions. The expression of *Tnfα* and the osteoclastogenic factor *Rankl* in bone was reduced in GF compared to CONV-R WT mice but not in *Nod1*$^{-/-}$ or *Nod2*$^{-/-}$ mice indicating that the effect of the GM to increase *Tnfα* and *Rankl* in bone and to reduce bone mass is dependent on both NOD1 and NOD2 signaling.

1. Introduction

We have shown that absence of GM in germ-free (GF) mice results in increased bone mass associated with altered immune status in bone and reduced bone resorption and that colonization of GF mice with a normal GM results in a normalisation of bone mass and immune status in bone [1]. Antibiotic administration increases bone density in young mice supporting a role of the GM in the regulation of bone mass [2]. In addition, probiotics limit bone-loss following ovariectomy (ovx) and GF mice are protected from trabecular bone-loss induced by sex-steroid depletion [3–6]. It has been proposed that the GM influences bone mass via an effect on the immune system but the possible role of different innate immune signaling pathways for the effect of the GM on bone mass is unknown.

In the gut the innate immune system recognizes bacteria and other infectious agents by pattern recognition receptors such as toll-like receptors (TLRs) situated on the cell-surface. There are alternative signaling pathways but most TLR signaling is mediated via the adaptor protein MYD88 to activate MAP kinase and nuclear factor kappa B-driven pro-inflammatory signaling [7]. Bacterial recognition also takes place in the cytoplasm by the Nod-like receptors (NLRs), NOD1 and NOD2. They bind bacterial peptidoglycan and after that a common protein kinase, RIP2, is recruited which in turn activates the NFκB signaling pathway leading to expression of genes such as cytokines and chemokines. Although the NOD1 and NOD2 proteins elicit a similar inflammatory response their location and function differ. *Nod1* is expressed in most cell types and induces pro-inflammatory signaling after detection of diaminopimelic acid-type peptidoglycan found mainly in Gram-negative bacteria [8]. *Nod2* is broadly expressed in cells of myeloid origin and to some extent in lymphoid and non-hematopoietic cells, including intestinal epithelial stem cells [9,10]. NOD2 detects all types of peptidoglycans found in Gram-positive and Gram-negative bacteria. The outcome of the activation of both TLRs and NLRs is an inflammatory response [7].

To determine the possible role of different innate immune signaling pathways for the effect of the GM on levels of the pro-inflammatory cytokine *Tnfα* in bone and on bone mass, we evaluated the skeleton in GF and CONV-R mice with targeted inactivation of *Myd88*, *Nod1* or *Nod2*.

2. Material and methods

2.1. Mice

At the gnotobiotic facility at the University of Gothenburg, female GF C57Bl6/J mice were maintained in flexible plastic film isolators

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under a strict 12-h-light cycle (lights on at 06:00 h). Sterility was routinely confirmed by culturing and PCR analysis from faeces using universal bacterial primers amplifying the 16S rRNA gene (8F, AGAGTTTGATCCTGGCTCAG; 338R, TGCTGCCTCCCGTAGGAGT). Age-matched female CONV-R C57Bl6/J mice were transferred to identical isolators at weaning. Both groups of mice were fed an autoclaved chow diet (Labdiet, St. Louis, MO) ad libitum. The *Myd88*$^{-/-}$ mice were initially purchased (Jackson Laboratory, Bar Harbor, ME, USA). The *Myd88*$^{-/-}$ were backcrossed at least eight generations to C57Bl6/J and the last two crossings were performed using mice from our colony. GF and CONV-R mice were separated by a maximum of three generations. The study protocols were approved by the University of Gothenburg Animal Studies Committee.

Female GF WT control, *Nod1*$^{-/-}$ and *Nod2*$^{-/-}$ mice on a C57Bl6/J background were maintained at the gnotobiotic facility at Institut Pasteur. *Card4/Nod1*-deficient (*Nod1*$^{-/-}$) mice were initially generated by Millennium Pharmaceuticals Boston, MA, USA. *Card15/Nod2*-deficient C57Bl/6J (*Nod2*$^{-/-}$) mice were provided by J.-P. Hugot (Hôpital Robert Debré, Paris, France) [11]. The *Nod1*$^{-/-}$ and *Nod2*$^{-/-}$ mice were backcrossed at least 8 times to C57Bl6/J. All mice were kept under specific-pathogen-free conditions, and all animal experiments were approved by the committee on animal experimentation of the Institut Pasteur and by the French Ministry of Agriculture.

Blood was collected from the axillary vein under anesthesia with Ketalar®/Domitor® vet and the mice were subsequently killed by cervical dislocation. Tissues for RNA preparation were immediately removed and snap frozen in liquid nitrogen for later analysis. Bones were excised and fixed in 4% paraformaldehyde.

2.2. RNA Isolation and Real Time PCR (RT-PCR)

Total RNA was prepared from bone using RNeasy Mini Kit (Qiagen, CA, USA). The RNA was reverse transcribed into cDNA using High-Capacity cDNA Reverse Transcription Kit (#4368814, Applied Biosystems, Stockholm, Sweden). RT-PCR analyses were performed using predesigned RT-PCR assays from Applied Biosystems for the analysis of Tumor necrosis factor alpha (*Tnfα*, Mm00443258_m1), receptor activator of nuclear factor-κB ligand (*Rankl*, Mm00441908_m1 and cathepsin K (*Ctsk*, Mm00484036_m1). The mRNA abundance of each gene was adjusted for the expression of *18S* (4308329) ribosomal RNA.

2.3. High-resolution μCT

High-resolution μCT analyses were performed on the distal femur by using an 1172 model μCT (Bruker micro-CT, Aartselaar, Belgium). The femurs were imaged with an X-ray tube voltage of 50 kV and current of 201 μA, with a 0.5-mm aluminium filter. The scanning angular rotation was 180° and the angular increment 0.70°. The voxel size was 4.48 μm isotropically. The NRecon (version 1.6.9) was employed to perform the reconstruction following the scans. Cortical measurements were performed in the diaphyseal region of femur starting at a distance of 3.59 mm from the growth plate and extending a further longitudinal distance of 134.5 μm in the proximal direction.

2.4. Statistical analysis

We used GraphPad Prism for all statistical analysis. Results are presented as the means ± SEM. Between-group differences were calculated using unpaired t-tests, GF vs. CONV-R. A two-tailed $p \leq 0.05$ was considered significant.

3. Results

We have previously shown that 7-week-old C57bl6/J mice raised GF have increased cortical bone area of the femur compared to CONV-R mice [1]. In a study by Li J-Y et al. similar results, with increased cortical area and thickness of GF compared to CONV-R mice were found in the femur of 20-week-old C57bl6/J mice [6]. In the present study, we used 9-week-old C57bl6/J mice from the gnotobiotic facility at the Pasteur Institute, France and 10-week-old C57bl6/J mice from the gnotobiotic facility at the University of Gothenburg, Sweden, to determine the possible role of different innate immune signaling pathways for the effect of the GM on bone mass. Similar to earlier studies, we found that WT mice raised GF in the Pasteur Institute had increased cortical thickness of the femur diaphysis compared to CONV-R mice ($p < 0.01$; Fig. 1A). We hypothesized that the GM might regulate bone mass via TLR signaling and we, therefore, studied mice lacking MYD88, which mediates most TLR signaling in the innate immune system. *Myd88*$^{-/-}$ mice had a pronounced increase in cortical bone mass when they were raised GF compared to CONV-R, demonstrating that the effect of the GM on bone mass is independent of the adaptor protein MYD88 ($p < 0.01$; Fig. 1B). In contrast, mice with a targeted inactivation of *Nod1* or *Nod2* displayed no increase in cortical bone mass in the GF state, demonstrating that the effect of GM on cortical bone mass is dependent on both NOD1 and NOD2 signaling (Fig. 1C, D).

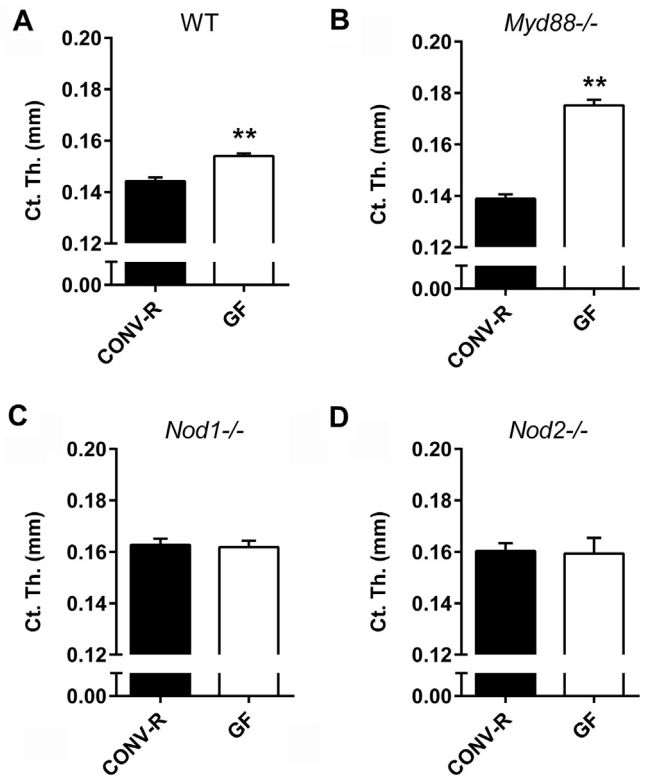

Fig. 1. The increased cortical bone mass in germ-free mice is dependent on NOD1 and NOD2 signaling. Cortical thickness (Ct Th) was measured at 9–10 weeks of age by μCT in the mid-diaphyseal region of femur in mice raised either conventionally (CONV-R) or germ-free (GF); (A) WT mice, (B) *MyD88*$^{-/-}$ mice, (C) *Nod1*$^{-/-}$ mice and (D) *Nod2*$^{-/-}$ mice. Values are given as mean ± SEM, (n = 4–6). ** $p \leq 0.01$. Student's *t* test, GF vs. CONV-R.

We have earlier shown that the increased bone mass in GF compared to CONV-R mice is associated with increased levels of pro-inflammatory cytokines and increased formation of bone resorbing osteoclasts [1]. To investigate the mechanism for the effect of the GF state on cortical bone in the different mouse-models, we measured expression of pro-inflammatory cytokines in bone. The expression of *Tnfα*, an inflammatory cytokine produced by immune cells that promotes osteoclastogenesis, was decreased in femur of GF compared to CONV-R WT mice but not in GF compared to CONV-R mice deficient of NOD1 or NOD2, demonstrating that the stimulatory effect of the GM

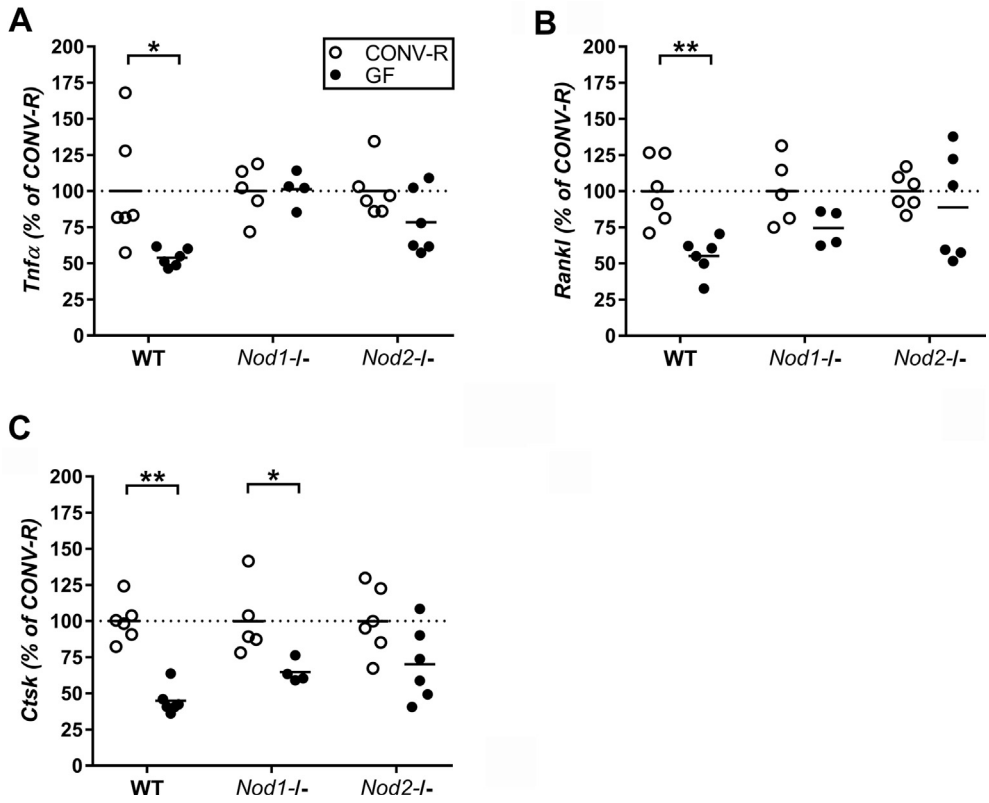

Fig. 2. The effect of the gut microbiota on expression of *TNFα* and *Rankl* in bone is dependent on NOD1 and NOD2 signaling. QRT-PCR analysis of gene expression in bone (A) Tumor Necrosis Factor alpha (*TNFα*), (B) Receptor activator of nuclear factor kappa-B ligand (*Rankl*) and (C) Cathepsin K (*Ctsk*). CONV-R = conventionally raised mice, GF = germ-free mice. Values are expressed as percentage of CONV-R and given as mean ± SEM, (n = 4–6). ** $p \leq 0.01$. Student's *t* test, GF vs. CONV-R.

on the levels of *Tnfα* in bone is dependent on both NOD1 and NOD2 signaling (Fig. 2A). In addition, WT mice raised GF had decreased expression of the osteoclast activating gene *Rankl* compared to CONV-R WT mice (Fig. 2B). In contrast, neither NOD1 nor NOD2 deficient mice had decreased *Rankl* expression in the GF compared to the CONV-R state, suggesting that the effect of the GM on osteoclastogenesis is blunted in the absence of NOD1 or NOD2 (Fig. 2B). For WT mice, the expression of the osteoclast-specific gene marker Cathepsin K (*Ctsk*) was markedly reduced in GF mice compared to CONV-R mice, indicating less bone resorption ($p < 0.01$; Fig. 2A). This reduction was partly blunted in *Nod1*$^{-/-}$ and *Nod2*$^{-/-}$ mice (Fig. 2C).

In summary, the effect of the GM to increase *Tnfα* and the osteoclastogenic factor *Rankl* in bone and to reduce cortical bone thickness is dependent on both NOD1 and NOD2 signaling.

4. Discussion

In the present study, the possible role of different innate immune signaling pathways for the effect of the GM on bone mass was evaluated using mice with targeted inactivation of *Myd88*, *Nod1* or *Nod2*. The mice of the different genotypes (WT, *Nod1*$^{-/-}$, *Nod2*$^{-/-}$ and *Myd88*$^{-/-}$) were killed on separate occasions and, therefore, we could not make a direct comparison of the skeletal phenotype between the different genotypes. The aim of this study was to determine if the difference in bone mass between mice raised with or without gut microbiota is dependent on the presence of NOD1, NOD2 or MYD88. We, therefore, only presented comparisons within each genotype, between the CONV-R and GF mice. We found that the effect of the GM to increase *Tnfα* and the osteoclastogenic factor *Rankl* in bone and to reduce cortical bone thickness is dependent on both NOD1 and NOD2 signaling.

We, and others, have previously shown that GF mice have increased cortical bone mass compared to CONV-R mice [1,6]. In addition, antibiotic administration increases bone mass in young mice [2]. Similar as in the present study, the mice in these studies were females on a C57bl6/J background. However, Schwarzer M. et al. observed that male GF mice on a BALB/c background had a general growth defect reflected by reduced body weight gain and decreased longitudinal bone growth associated with reduced cortical thickness compared to CONV-R mice [12]. A recent study by Yan J. et al., found that the short-term effects of the GM one month after colonization of GF mice was a reduction of bone mass associated with increased bone resorption while the long-term effect of colonization was increased skeletal growth [13]. Thus, the initial effect of colonization seems to be increased bone resorption resulting in bone loss but this is overcome by a later GM-mediated increase in bone growth. Taken together, this indicates that sex, genetic background, length of colonization and possibly different animal facilities and type of chow may influence the result when assessing the effect of the GM on bone mass. We do not have the GM composition of the CONV-R mice of the different genotypes used in this study. Although, we have tried to minimize the effect of housing and diet by comparing mice raised in the same animal facility, backcrossed to C57Bl6/J and fed the same chow, there are studies indicating that lack of Nod1 or Nod2 in naive mice induces changes in GM composition[1,2]. However, other studies have been unable to support these findings[3,4]. We cannot exclude the possibility that the GM composition differs in the CONV-R mice of the different genotypes and that the absence of an effect of the GM on cortical bone in NOD1 and NOD2 deficient mice may be an indirect effect of changes in GM composition compared to WT mice.

In the present study, we show that young adult WT and MYD88 deficient GF mice have increased cortical bone mass compared with corresponding CONV-R mice and that this effect of the GM is dependent on intact NOD1 and NOD2 signaling. RANKL is a major stimulator of osteoclastogenesis and bone resorption and TNFα promotes osteoclast formation indirectly by enhancing *Rankl* expression by marrow stromal cells and osteoblasts and by direct stimulation of osteoclast precursors

exposed to permissive levels of RANKL [14]. We show that the effect of the GM on cortical bone was associated with decreased *Rankl* and *Tnfα* expression in bone. In addition, mice deficient in NOD1 or NOD2 had unchanged cortical thickness when comparing GF mice with CONV-R mice. In line with a lack of an effect on bone mass by the GM, the expression of *Rankl* and *Tnfα* in bone was not affected by the GM in the NOD1 or NOD2 deficient mice.

The effects of NOD2 on bone resorption have been studied in a model of microbial-induced periodontitis. Bone resorption and osteoclastogenesis were significantly reduced in NOD2 deficient mice [15,16]. In the study by Souza et al., bone marrow-derived macrophages from NOD2 deficient mice formed fewer osteoclasts than cells from WT mice when grown *in vitro* in the presence of heat-killed gram negative bacteria, indicating that bacteria induced bone resorption is dependent on NOD2 signaling [15]. Supporting this finding, the NOD2 ligand muramyl dipeptide has been shown to enhance osteoclastogenesis induced by LPS and inflammatory cytokines in co-cultures of osteoblasts and hematopoietic cells by increasing the *Rankl* mRNA expression in osteoblasts [17]. Mechanical unloading, such as in a microgravity environment in space or during prolonged bed rest, leads to a decrease in bone mass because of the suppression of bone formation and the stimulation of bone resorption and interestingly, NOD2 deficient mice had less unloading-induced cortical bone loss compared to WT controls in a recent study [18]. These studies suggest that NOD2 supports bone resorption and loss in response to challenges such as bacteria or unloading. On the other hand, in a model of microbial-induced periodontitis using heat-killed Gram-negative bacteria to stimulate only NOD1, mice deficient in NOD1 exhibited increased bone resorption and number of osteoclasts [19]. Furthermore, simultaneous stimulation of osteoclast precursor cells with RANKL and NOD1 agonist inhibited osteoclast differentiation *in vitro* suggesting a bone-sparing effect of NOD1 [20]. In the present study, neither NOD1 nor NOD2 deficient mice exhibited increased cortical thickness or decreased *Rankl* and *Tnfα* expression in bone when raised in a GF environment. Taken together this suggests that both NOD1 and NOD2 modulate the effects on bone mass in response to external stimuli such as the GM. Our hypothesis is that in CONV-R WT mice, bone mass was affected due to bacterial ligand stimulation of the NODs that in turn affects osteoclastogenesis. In the GF mice no such stimulation will occur due to the absence of ligands. In the NOD1 and NOD2 deficient mice there will be no stimulation and no changes in bone mass in either the GF or the CONV-R mice due to absence of the receptors NOD1 or NOD2. Thus for the difference in cortical bone mass between CONV-R and GF mice, both NOD1 and NOD2 are essential, indicating no redundancy between the two receptors for this effect

In summary, the effect of the GM to increase *Tnfα* and the osteoclastogenic factor *Rankl* in bone and to reduce cortical bone thickness is dependent on both NOD1 and NOD2 signaling.

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References

[1] K. Sjogren, C. Engdahl, P. Henning, U.H. Lerner, V. Tremaroli, M.K. Lagerquist, F. Backhed, C. Ohlsson, The gut microbiota regulates bone mass in mice, J. Bone Miner. Res. 27 (2012) 1357–1367.
[2] I. Cho, S. Yamanishi, L. Cox, B.A. Methe, J. Zavadil, K. Li, Z. Gao, D. Mahana, K. Raju, I. Teitler, H. Li, A.V. Alekseyenko, M.J. Blaser, Antibiotics in early life alter the murine colonic microbiome and adiposity, Nature 488 (2012) 621–626.
[3] R.A. Britton, R. Irwin, D. Quach, L. Schaefer, J. Zhang, T. Lee, N. Parameswaran, L.R. McCabe, Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model, J. Cell. Physiol. 229 (2014) 1822–1830.
[4] C. Ohlsson, C. Engdahl, F. Fak, A. Andersson, S.H. Windahl, H.H. Farman, S. Moverare-Skrtic, U. Islander, K. Sjogren, Probiotics protect mice from ovariectomy-induced cortical bone loss, PLoS ONE 9 (2014) e92368.
[5] K. Parvaneh, M. Ebrahimi, M.R. Sabran, G. Karimi, A.N. Hwei, S. Abdul-Majeed, Z. Ahmad, Z. Ibrahim, R. Jamaluddin, Probiotics (Bifidobacterium longum) increase bone mass density and upregulate Sparc and Bmp-2 genes in rats with bone loss resulting from ovariectomy, Biomed Res Int 2015 (2015) 897639.
[6] J.Y. Li, B. Chassaing, A.M. Tyagi, C. Vaccaro, T. Luo, J. Adams, T.M. Darby, M.N. Weitzmann, J.G. Mulle, A.T. Gewirtz, R.M. Jones, R. Pacifici, Sex steroid deficiency-associated bone loss is microbiota dependent and prevented by probiotics, J. Clin. Invest. (2016).
[7] T.A. Kufer, P.J. Sansonetti, Sensing of bacteria: NOD a lonely job, Curr. Opin. Microbiol. 10 (2007) 62–69.
[8] T.B. Clarke, K.M. Davis, E.S. Lysenko, A.Y. Zhou, Y. Yu, J.N. Weiser, Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity, Nat. Med. 16 (2010) 228–231.
[9] G. Nigro, R. Rossi, P.H. Commere, P. Jay, P.J. Sansonetti, The cytosolic bacterial peptidoglycan sensor Nod2 affords stem cell protection and links microbes to gut epithelial regeneration, Cell Host Microbe 15 (2014) 792–798.
[10] Y. Ogura, N. Inohara, A. Benito, F.F. Chen, S. Yamaoka, G. Nunez, Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB, J. Biol. Chem. 276 (2001) 4812–4818.
[11] F. Barreau, U. Meinzer, F. Chareyre, D. Berrebi, M. Niwa-Kawakita, M. Dussaillant, B. Foligne, V. Ollendorff, M. Heyman, S. Bonacorsi, T. Lesuffleur, G. Sterkers, M. Giovannini, J.P. Hugot, CARD15/NOD2 is required for Peyer's patches homeostasis in mice, PLoS ONE 2 (2007) e523.
[12] M. Schwarzer, K. Makki, G. Storelli, I. Machuca-Gayet, D. Srutkova, P. Hermanova, M.E. Martino, S. Balmand, T. Hudcovic, A. Heddi, J. Rieusset, H. Kozakova, H. Vidal, F. Leulier, Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition, Science 351 (2016) 854–857.
[13] J. Yan, J.W. Herzog, K. Tsang, C.A. Brennan, M.A. Bower, W.S. Garrett, B.R. Sartor, A.O. Aliprantis, J.F. Charles, Gut microbiota induce IGF-1 and promote bone formation and growth, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) E7554–E7563.
[14] J. Lam, S. Takeshita, J.E. Barker, O. Kanagawa, F.P. Ross, S.L. Teitelbaum, TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand, J. Clin. Invest. 106 (2000) 1481–1488.
[15] J.A. Souza, M.C. Medeiros, F.R. Rocha, S.G. de Aquino, M.J. Avila-Campos, L.C. Spolidorio, D.S. Zamboni, D.T. Graves, C.J. Rossa, Role of NOD2 and RIP2 in host-microbe interactions with Gram-negative bacteria: insights from the periodontal disease model, Innate Immun. 22 (2016) 598–611.
[16] T.P. Prates, T.M. Taira, M.C. Holanda, L.A. Bignardi, S.L. Salvador, D.S. Zamboni, F.Q. Cunha, S.Y. Fukada, NOD2 contributes to Porphyromonas gingivalis-induced bone resorption, J. Dent. Res. 93 (2014) 1155–1162.
[17] S. Yang, N. Takahashi, T. Yamashita, N. Sato, M. Takahashi, M. Mogi, T. Uematsu, Y. Kobayashi, Y. Nakamichi, K. Takeda, S. Akira, H. Takada, N. Udagawa, K. Furusawa, Muramyl dipeptide enhances osteoclast formation induced by lipopolysaccharide, IL-1 alpha, and TNF-alpha through nucleotide-binding oligomerization domain 2-mediated signaling in osteoblasts, J. Immunol. 175 (2005) 1956–1964.
[18] J.S. Sankaran, B. Li, L.R. Donahue, S. Judex, Modulation of unloading-induced bone loss in mice with altered ERK signaling, Mamm. Genome 27 (2016) 47–61.
[19] J.A. Chaves de Souza, S.C. Frasnelli, F.A. Curylofo-Zotti, M.J. Avila-Campos, L.C. Spolidorio, D.S. Zamboni, D.T. Graves, C. Rossa Jr., NOD1 in the modulation of host-microbe interactions and inflammatory bone resorption in the periodontal disease model, Immunology 149 (2016) 374–385.
[20] T. Kishimoto, T. Kaneko, T. Ukai, M. Yokoyama, R. Ayon Haro, Y. Yoshinaga, A. Yoshimura, Y. Hara, Peptidoglycan and lipopolysaccharide synergistically enhance bone resorption and osteoclastogenesis, J. Periodontal Res. 47 (2012) 446–454.