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Review

Gut microbiome and bone

Lidia Ibáñez[a,b,c], Matthieu Rouleau[a,b], Abdelilah Wakkach[a,b], Claudine Blin-Wakkach[a,b,*]

[a] CNRS UMR7370, LP2M, faculté de médecine, 28, avenue de Valombrose, 06107 Nice cedex 2, France
[b] Université Nice–Sophia-Antipolis, 06100 Nice, France
[c] Department of Pharmacy, Cardenal Herrera-CEU University, 46115 Alfara del Patriarca, València, Spain

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ABSTRACT

The gut microbiome is now viewed as a tissue that interacts bidirectionally with the gastrointestinal, immune, endocrine and nervous systems, affecting the cellular responses in numerous organs. Evidence is accumulating of gut microbiome involvement in a growing number of pathophysiological processes, many of which are linked to inflammatory responses. More specifically, data acquired over the last decade point to effects of the gut microbiome on bone mass regulation and on the development of bone diseases (such as osteoporosis) and of inflammatory joint diseases characterized by bone loss. Mice lacking a gut microbiome have bone mass alteration that can be reversed by gut recolonization. Changes in the gut microbiome composition have been reported in mice with estrogen-deficiency osteoporosis and have also been found in a few studies in humans. Probiotic therapy decreases bone loss in estrogen-deficient animals. The effect of the gut microbiome on bone tissue involves complex mechanisms including modulation of $CD4^+$T cell activation, control of osteoclastogenic cytokine production and modifications in hormone levels. This complexity may contribute to explain the discrepancies observed betwwen some studies whose results vary depending on the age, gender, genetic background and treatment duration. Further elucidation of the mechanisms involved is needed. However, the available data hold promise that gut microbiome manipulation may prove of interest in the management of bone diseases.

1. Introduction

The 10^{14} microorganisms in the gut microbiome (GM) continuously engage in a dynamic dialog with the host cells [1]. The GM is now viewed as an organ that contributes to digestive function, transforming complex food components such as fibers and carbohydrates into absorbable nutrients including short-chain fatty acids [2]. Commensal bacteria produce factors that modulate the host immune responses, help preserve gut barrier integrity, defend against pathogenic microorganisms and contribute to immune system development and regulation. These effects are particularly important for the mucosal immune system in the gut, which maintains tolerance toward food allergens and the GM while ensuring protection against pathogenic microorganisms. Thus, the GM regulates the development and function of lymphoid cells, the polarization of gut T cells, most notably Th17 cells, and the production of cytokines [3,4].

The composition of the GM can vary over the life span depending on age, genetic factors, diet, medication intake and host immune status. The interactions of the GM with the host may be altered by dysbiosis, which is defined as adverse changes in bacterial composition, diversity, and function. When dysbiosis occurs, the GM loses its protective capabilities, the gut barrier is impaired and the host fails to effectively control the dissemination of GM components into the tissues. The resulting stimulation of the immune system can lead to a variety of diseases. Dysbiosis is associated not only with Crohn's disease, irritable bowel syndrome and celiac disease, but also with metabolic, cardiovascular and neurodegenerative diseases, as well as with inflammatory rheumatic diseases [5–7]. In recent years, the effect of the GM on bone tissue has been evaluated in animals lacking GM (axenic mice), in animals given antibiotics or probiotics to modify the GM and in humans. Although the results of these studies are somewhat conflicting, they establish the GM as a major regulator of bone mineral density (BMD), chiefly via effects on the immune system.

* Corresponding author. CNRS UMR7370, LP2M, faculté de médecine, 28, avenue de Valombrose, 06107 Nice cedex 2, France.
E-mail address: blin@unice.fr (C. Blin-Wakkach).

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2. Osteoimmune interactions and bone resorption

The immune system is central to control the BMD under abnormal conditions. The receptor activator of NFκB ligand (RANKL) is the main cytokine involved in osteoclast differentiation. RANKL is produced by mesenchymal cells, osteoblasts and osteocytes in the bone marrow. During inflammation, activated $CD4^+$ T cells are also a source of RANKL [8,9], as well as of other cytokines including interleukin (IL)-17 and tumor necrosis factor alpha (TNF-α), which stimulate osteoclastogenesis [10,11]. Changes in T cell activation levels therefore affect osteoclast differentiation [12,13]. Among helper $CD4^+$ T cell subsets, only Th17 cells have been found to promote osteoclastogenesis in vitro [14]. In vivo, Th17 cells are associated with enhanced osteoclast differentiation in mice and humans during inflammatory states [13,15,16]. Crosstalk between Th17 cells and osteoclasts has also been demonstrated in Crohn's disease in both mice and humans [16,17]. Th17 cells activated at sites of bowel inflammation produce high levels of the osteoclastogenic factors RANKL, IL-17 and TNF-α [17]. They migrate to the bone marrow, where they strongly enhance osteoclast differentiation by producing cytokines and upregulating RANKL expression by mesenchymal cells [17]. Th17 cells also upregulate the expression by mesenchymal cells of chemokines (MCP1, MIP1α) that attract the monocytic precursors of osteoclasts, increasing their recruitment to the bone marrow [17]. The peripheral blood of patients with Crohn's disease contains Th17 cells that exhibit the same osteoclastogenic properties and therefore probably contribute to the bone loss often seen in this disease [16,17]. Th17 cells activated in the gut are thus crucially involved in inflammatory bone destruction.

Interestingly, Th17 cells are lacking in the gut of axenic mice. Introducing certain bacterial species into the gut efficiently induces Th17 cell differentiation, demonstrating the importance of the GM in the emergence of these cells [3,4]. Th17 cells are characterized by considerable phenotypic and functional plasticity [18]. They not only protect against pathogenic bacteria [19] but also contribute to many chronic inflammatory diseases, including those that are associated with bone destruction, such as Crohn's disease [20], arthritis [21], spondyloarthritis [22] and psoriasis [23]. These data support the existence of a link between the GM and bone resorption via the emergence of Th17 cells.

3. Gut microbiome and bone formation

Several clinical studies reported about two decades ago pointed to an association between excessive bacterial proliferation in the gut and decreased BMD [24]. Patients with small intestinal bacterial overgrowth syndrome have low BMD values and osteomalacia and some of them have high levels of the proinflammatory cytokines TNF-α and IL-1, as well as increased osteoclast activation. Another feature of this syndrome is the development of nutritional deficiencies due to nutrient consumption by the gut bacteria. Thus, insufficient intestinal absorption of calcium, phosphate and vitamin D contribute to the bone manifestations of the syndrome [24]. Consequently, these studies cannot provide evidence of a direct link between the GM and the bone phenotype.

Convincing evidence of GM involvement in bone tissue development was obtained only a decade later, via studies of axenic mice, which have no GM. Given the effect of the GM on metabolism, absence of a GM from birth induces a number of physiological and metabolic alterations. The absorption of calories, vitamins and nutrients is decreased; height and weight gains are delayed, and many organs are abnormally small [25]. Another consequence is

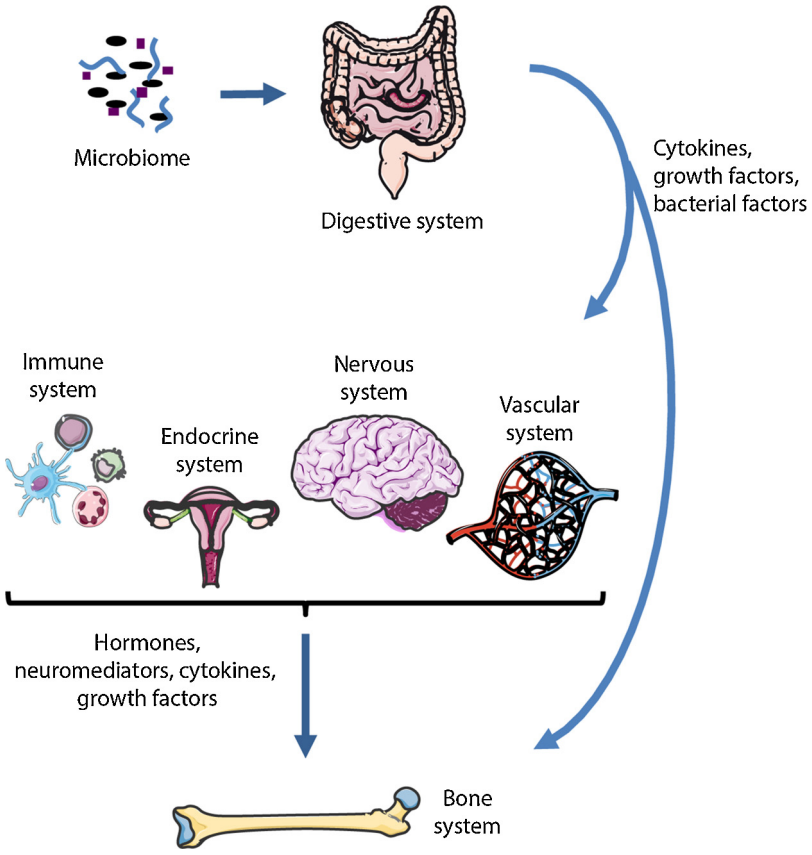

Fig. 1. Link between the gut microbiome and bone. The gut microbiome contributes to preserve gut barrier integrity and digestive system maturity. GM alterations lead to increased dissemination of bacteria-derived compounds and to changes in the expression of cytokines and growth factors. As a result, impairments occur in the responses of the immune, endocrine, vascular and nervous systems, all of which contribute to regulate bone cell differentiation and/or function.

immaturity of the immune, vascular, endocrine, intestinal and nervous systems, all of which are involved in regulating bone mass (Fig. 1) [26,27].

Female C57Bl/6J mice lacking a GM from birth have higher BMD values in both trabecular and cortical bone compared to conventionally raised mice[28]. These high BMD values reflect decreases in the number of osteoclast precursors and mature osteoclasts, which translate into diminished bone resorption. Bone formation is unchanged. Additional contributors to the reduction in bone resorption include decreases in $CD4^+$ T cell frequency and in the expression levels of TNF-α and IL-6 in the bone marrow [28]. Colonization of axenic mice with GM from conventionally raised mice corrects the BMD and immune parameter values, confirming that the GM controls osteoclast differentiation during skeletal growth [28]. Among the receptors involved in recognition of bacteria by the immune system, the nucleotide-binding oligomerization domain proteins NOD1 and NOD2 bind bacterial peptidoglycans and activate the NFκB pathway. In knockout mice for both *NOD1* and *NOD2*, changes in the GM have no effect on BMD or on TNF-α and RANKL expression, demonstrating that NOD1 and NOD2 play a critical role in the GM effects on bone [29].

However, GM effects on bone development are more complex than appears to the casual eye. A study in axenic juvenile male Balb/c mice reported in 2016 produced contradictory results, with delays in most of the main growth parameters and shorter femurs compared to conventionally raised controls [30]. The discrepancy between the results of the two studies may be ascribable to differences in the genetic background, gender, or age of the mice. The C57Bl/6J and Balb/C strains differ regarding GM diversity, IgA levels (which modulate GM diversity) and the T cell profile [31,32]. Treatment duration is also of the utmost importance. Colonization of axenic mice by GM from normal mice is followed by an increase in both bone resorption and bone formation. The bone resorption enhancement, however, is transient, resolving after 4 weeks. Thus, the GM exerts an acute catabolic effect on bone tissue [33]. In the longer term, bone formation predominates, leading to increased skeletal growth and bone mass correction, indicating a long-term anabolic effect of the GM on bone tissue [33]. This anabolic effect coincides with an increase in the production of insulin growth factor (IGF)-1. IGF-1 levels in the serum and bone marrow are low in axenic mice and in mice given antibiotics. They increase after gut recolonization or ingestion of a diet enriched in short-chain fatty acids, which are produced when GM bacteria break down polysaccharides [33]. Given the major role for IGF-1 in skeletal development [34], control of IGF-1 production is among the mechanisms that may explain the effect of the GM on bone growth.

Another major issue is the influence of gender. Male and female C57Bl/6J mice with significant GM alterations induced by low-dose penicillin treatment started at birth exhibit different phenotypes [35]. The females, but not the males, have higher adulthood BMD values compared to untreated animals [35]. In addition, the BMD increase seen after gut recolonization of axenic mice is more marked in the long-term in males than in females [33]. Interestingly, the GM in male and female mice is similar before puberty but differs in adulthood, and the differences contribute to increase the susceptibility to certain diseases, such as type I diabetes in females [36]. Gender-related differences in the GM have also been reported in humans [37,38]. Thus, reciprocal interactions between the GM and sex hormones influence the composition of the GM and the response of host tissues, including bone, to the GM [39].

4. Gut microbiome and bone destruction

Potential effects of the GM on bone mass during estrogen-deficient osteoporosis have been investigated. At the menopause, the drop in estrogen production leads to a decline in bone mass due to a combination of diminished bone formation and increased bone resorption. In mice with osteoporosis induced by ovariectomy, osteoclast activity is enhanced due to increased production of RANKL and TNF-α in the bone marrow [40]. The production of these two factors is mediated by $CD4^+$ T cells [40]. Thus, mice deficient in $CD4^+$ T cells do not lose bone after being ovariectomized [41]. Similarly, compared to premenopausal women and to postmenopausal women without osteoporosis, patients with postmenopausal osteoporosis exhibit RANKL and TNF-α overproduction by peripheral blood $CD4^+$ T cells [42].

The central role for the GM in controlling lymphocyte activation [4] and its reciprocal interactions with sex hormones [36] have prompted studies in mice designed to assess the effect of the GM on the BMD decrease induced by estrogen deprivation. In several studies of ovariectomized mice, a protective effect against bone loss was obtained by the administration of probiotics such as *Lactobacillus (L) reuteri*, *L. paracasei*, *L. plantarum*, *Bifidobacterium (B) longum*, and mixtures of several species *(B. breve*, *B. longum*, *B. infantis*, *L. acidophilus, L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*) [43–45]. This finding was confirmed in a model of osteoporosis induced by the gonadotropin-releasing hormone agonist leuprolide, which suppresses the production of sex hormones, including estrogens, in the long term. The estrogen deficiency led to bone loss in conventionally raised mice but not in axenic mice [46]. In conventionally raised mice, estrogen deficiency increased gut barrier permeability and the proportion of $CD4^+$ T cells producing RANKL, IL-17, and TNF-α, thereby enhancing osteoclastogenesis (Fig. 2) [46]. Axenic mice exhibited none of these modifications [46]. In a small preliminary study of normal controls, patients with osteopenia, and patients with osteoporosis (6 per group), differences in the GM were found across the three groups. This finding requires confirmation in a larger sample size but suggests a role for dysbiosis in human osteoporosis [47].

GM alterations have a major impact in inflammatory rheumatic diseases accompanied by bone loss. Mice and rats whose GM is lacking or altered by antibiotics have decreased susceptibility to arthritis and spondyloarthritis [21,48]. Gut dysbiosis has been reported in patients with rheumatoid arthritis or spondyloarthritis [49–51]. In addition, in rats with collagen-induced arthritis, the administration of probiotics protects against inflammation and bone loss [52]. Thus, the effect of the GM on bone is not confined to conditions characterized by hormonal changes and can involve a wide variety of mechanisms.

Recent data indicate that osteoporosis and inflammatory joint diseases share a common immune component. The gut barrier is a crucial player in host-GM interactions. Dysbiosis is associated with gut barrier alterations that promote the dissemination of bacteria and of the factors they produce. The gut barrier is altered in both rheumatic diseases and estrogen deficiency [46,53,54]. In both situations, the alterations are accompanied with enhanced $CD4^+$ T cell activation and increased production of the proinflammatory and osteoclastogenic cytokines IL-17, TNF-α, IL-1β and RANKL [44–46].

By modulating the gut immune response, dysbiosis also alters monocyte and lymphocyte migration to tissues, including the bone marrow. In axenic mice, the numbers of monocytes and osteoclastic precursors in the bone marrow are diminished but return to normal after gut colonization with GM from control mice [28,55]. In addition, changes in the GM translate into alterations in monocyte trafficking [56]. In the context of Crohn's disease, which is associated with severe bone loss and dysbiosis, the Th17 cells can migrate to the bone marrow and induce the recruitment of osteoclastic precursors, thereby prompting a massive increase in osteoclastogenesis [17]. Whereas osteoclasts from normal mice induce the generation of regulatory T cells (Tregs), osteoclasts generated during inflammatory states activate $CD4^+$ T cells that produce TNF-α

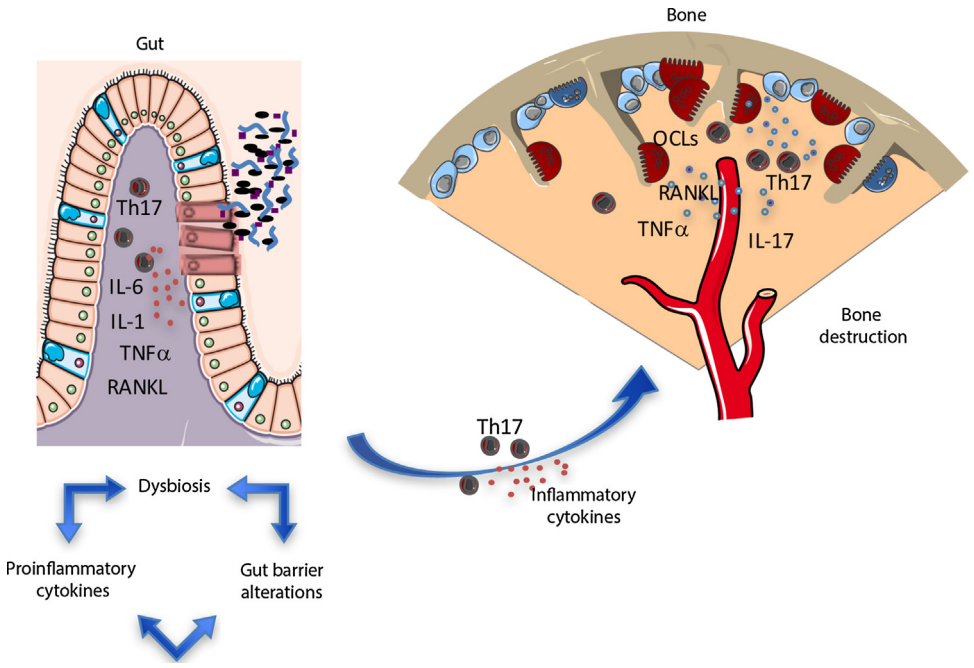

Fig. 2. Gut microbiome alterations and osteoclast differentiation. Alterations in the gut microbiome result in Th17 cell stimulation and in increased production of the osteoclastogenic cytokines TNF-α, IL-17 and RANKL in the gut. Th17 cells migrate to the bone marrow, where increases in the same cytokines enhance the differentiation of osteoclasts, thereby diminishing bone mineral density. OCLs: osteoclasts.

[57]. This difference is ascribable to the cellular origin of the osteoclasts and reflects differences in osteoclastic precursor recruitment under normal conditions versus during dysbiosis-related inflammatory states.

5. Future directions

A growing number of studies indicate a major role for the GM in regulating bone mass both during growth and during disease states. The GM can act in combination with other factors including the diet, genetic susceptibility, lifestyle and medications. Another mechanism of action of the GM is a direct effect due to the dissemination of bacteria [58] or of the factors they produce, which then activate the inflammatory responses in various tissues including the bone marrow. However, the mechanisms involved are complex and further investigations are needed to clarify and to control them.

Restoring a balanced GM is now being considered as a therapeutic tool for various diseases. Methods of modifying the GM include dietary changes and supplementation with probiotics such as short-chain fatty acids, oligosaccharides, carbohydrates and dietary fiber. These supplements are metabolized by certain bacterial strains, whose growth they promote, thereby modifying the composition of the GM. The modified GM stimulates antiinflammatory responses and promotes the intestinal absorption of calcium, thereby increasing BMD, an effect demonstrated in mice given short-chain fatty acids by gavage [33]. Oligosaccharides from human milk have shown similar beneficial effects on bone [59]. Probiotics also have a substantial effect on BMD. Various strains of *Lactobacillus* and *Bifidobacterium* have antiinflammatory effects, enhance vitamin D absorption and diminish osteoclast differentiation, thereby protecting against the bone mass loss induced by ovariectomy in mice [43–45]. The effects of probiotics in humans are being evaluated in several clinical trials registered on ClinicalTrials.gov, but the results are not yet available.

Another approach is GM transplantation, which has been widely used in mice to demonstrate that the GM was involved in many disease states, including those affecting the bone [28,33,46]. In humans, GM transplantation has been used successfully to treat bowel diseases such as colitis due to antibiotic-resistant bacteria [60]. Clinical studies are under way to evaluate the efficacy of GM transplantation in rheumatic diseases. However, no effects on bone have been reported. Interest is growing in GM manipulation as a therapeutic tool and additional research is therefore needed to elucidate the mechanisms involved and to evaluate the efficacy of this approach in bone diseases. The beneficial effects of GM manipulation in preclinical models suggest promise for the treatment of bone disease.

Disclosure of interest

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The other authors declare that they have no competing interest.

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