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Osteomicrobiology: The influence of gut microbiota on bone in health and disease

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1. Introduction

Host cell and microbe interactions have evolved in practically every animal, with the cross-talk between microorganisms that inhabit the gut lumen with tissues of the gastrointestinal tract an example that is increasingly recognized as being critical to health and disease [1–3]. Microbial colonization of the gastrointestinal tract starts at birth, eventuating in a taxonomically diverse community by early adulthood [4]. Intestinal microbes flourish in an environment that is rich in nutrients, with specific taxa recognized as causative of conferring beneficial effects on the host, such as improved energy extraction from food, exclusion of pathogenic bacteria, and stimulation of tissue development [5,6]. Gut luminal bacteria also beneficially influence tissue homeostasis in the intestine by enhancing epithelial cell proliferation and survival, and strengthening barrier function [7–12]. Indeed, mice raised in germ-free conditions exhibit many functional weaknesses [13], and have impaired homeostasis [14]. These observations show that there is an active and dynamic association between microbes that reside within the gut and host cells.

Gut microbes have also been shown to modulate intestinal and systemic immune responses [15]. For instance, gut-resident microbes have a robust influence on the emergence and/or maintenance of CD4 + T cell subsets. Examples include the effects of specific bacteria on the emergence of Th17 cells [16] and the impact of *Bacteroides fragilis* in Th1 cells and Treg differentiation [17]. Indeed, abnormalities in gut microbial diversity ("dysbiosis") have been suggested to be sufficient to aggravate intestinal pathologies related to the immune system such as in inflammatory bowel disease [18]. However, a more intriguing paradigm is emerging evidence that the commensal microbes also influence immune responses distant from mucosal surfaces, including, but not limited to, the CNS, joints and lungs [19–23]. Relevant to this review is the observation that gut microbes influence systemic immune responses critical for bone homeostasis. For example, investigations have revealed that germ-free mice display increased bone mass due to the lack of immune cell activation [24], that low-dose antibiotic treatment increases bone density in young mice [25], and that probiotic treatment prevents ovariectomy (ovx) induced bone loss [26,27].

We propose to use the term "Osteomicrobiology", which was introduced by Ohlsson et al. [28], to refer to investigations on the role of microbes and microbiota in health and disease and the mechanisms by

Abbreviations: BM, bone marrow; GF, germ-free; Conv.R, conventionally raised; TNF, tumor necrosis factor α.

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which the microbiota regulates post-natal skeletal development, bone aging and pathologic bone loss. In this review we will discuss the establishment of the field of osteomicrobiology and how a weakness in gut epithelial permeability influences bone turnover rates, and review the effects of probiotics as a therapeutic approach to enhance bone formation.

2. Definitions and key investigative methodologies

The term 'microbiome' originated with Nobel prize winner Josh Lederberg, and refers to the collection of microorganisms, their genomes, and their interactions in a given environment. Nearly all environments harbor distinct microbiomes, including the microenvironments of the human body. While it has long been hypothesized that the human microbiome plays a key role in susceptibility to adverse health outcomes, it is only with recent technologies that comprehensive study is possible. Until recently, culture-based techniques were the coin of the realm in microbiology, and still remain a foundational technique in the field. However, many community members of the human microbiome cannot be cultured since it is simply not currently possible to recapitulate growth conditions in a laboratory setting [29]. Because of this, the diversity of the human microbiome was inevitably underestimated. Two key advances have enabled a more complete census of the human microbiome. The first was an application of PCR-based technology which exploited features of the 16 s rRNA gene: this gene, present in many microorganisms, is comprised of constant, conserved regions which flank variable regions. In 1985, it was shown that PCR primers which anneal to the constant regions could amplify the internal variable region from a diverse set of bacteria [30]. These variable regions could then be sequenced and matched to a database, to identify the organisms present. Recently, this DNA-based, culture independent method gained substantial discriminating power when coupled to next-generation sequencing technology, which has the ability to sequence a population of PCR amplicons in a single experiment with single-molecule resolution [31]. 16S-based sequencing is rapid and inexpensive enough to be applied to population-based samples, and thus can be applied to (for example) case-control studies, or can be used to record longitudinal changes in microbiomes over time. This has created an explosion of data characterizing the microbiome, human and otherwise, with a vast array of applications from forensic science to translational health opportunities and beyond.

To impose order on the chaos of this data explosion, and encourage the development of companion bioinformatics tools necessary for data processing and analysis, two consortia evolved. The European MetaHIT consortium was focused on the gut microbiome and its role in adverse health outcomes, including obesity and inflammatory bowel disease [32]. In the United States, the Human Microbiome Project (HMP) was supported by the NIH common fund. In contrast to the MetaHIT consortium, the HMP focused on characterizing the healthy human microbiome, and creating a publicly-available reference set of control data [33]. The HMP sought to characterize five major human microbiome communities: the airway, skin, oral, gut and vaginal microbiomes [34]. Landmark studies from the HMP confirmed that different human microenvironments harbor distinct, characteristic microbiomes that differ in alpha diversity and community membership [34].

Recently, it has been discovered that alterations in the human microbiome are associated with various disease states. For example, decreased abundance of lactobacillus in the vaginal microbiome is associated with bacterial vaginosis (BV) [35], and may also be associated with preterm labor [36]. Changes in the oral microbiome are associated with periodontal disease [37], and new associations with systemic disease such as rheumatoid arthritis [38] and cardiovascular disease are hypothesized. The skin microbiome is important in the development of atopic dermatitis, and in the delayed wound healing that is a complication of diabetes [39]. The gut microbiome the largest, richest, and most complex of the human microbiomes, and is correspondingly the most well characterized and most investigated for disease associations. Changes in the gut microbiome are associated with risk for and progression of inflammatory bowel disease [18], risk for colorectal cancer [40,41], obesity [42], glucose control and diabetes risk [43], and may even serve as a reservoir for antimicrobial resistance genes [44]. Together, information added from these investigations will support an improved appreciation the role of the quality of the microbiome diversity in the prediction of disease onset, in pathophysiology, and in assessing the efficacy of response to various therapeutic interventions. Information may also allow scientists to develop new microbiome-targeted therapeutic interventions strategies for these diseases.

3. The influence of the microbiome on bone mass

3.1. Bone mass in germ-free or antibiotic treated mice

Initial investigations by Sjogren et al. [24] revealed that germ-free mice have higher cortical and trabecular bone mass compared to control mice raised in conventional conditions. Indeed, differences in bone turnover indicators between control and germ-free mice were quite substantial. Germ-free mice had fewer CD4 + T cells and osteoclast precursors in the bone marrow, had lower levels of osteoclastogenic cytokines, and at 9 weeks of age, the trabecular bone mass of germ-free mice was 39% higher than that of controls. Importantly, the high bone mass and the immune abnormalities of germ-free mice were reversed by reconstitution of the gut microbiota with flora from conventionally raised microbial replete mice [24]. Corroborating these observations, our research group reported that 20-week-old female germ-free C57Bl/6 mice trended to have higher trabecular bone volume than isogenic age matched mice raised in conventional conditions [45], although the difference in trabecular bone between germ-free mice and control mice was not significant. Furthermore, germ-free mice had increased femoral cortical volume compared to conventionally raised mice [45]. Surprisingly, opposite effects were reported in a study conducted on 8-week-old germ-free BALB/c male mice. BALB/c germ-free mice were found to have lower cortical and trabecular volume as compared to conventionally raised mice [46], probably because BALB/c mice undergo suboptimal growth leading to substantially reduced body weight and bone length when raised in germ-free conditions. Together these data indicate that mouse strain, age, and sex are influencing variables when assessing the impact of the microbiome on bone mass.

It is also known that considerable variation exists in the microbiome of mice housed in different facilities and/or fed different types of chow [47–49]. Therefore, the diversity of the microbiome in the particular facility is a potential confounding factor to data interpretation when assessing the influences the microbiome on bone density. For example, at sacrifice the trabecular bone volume of the conventionally raised mice used in our studies was <50% of that of the BALB/c male mice used by Schwarzer et al. [46]. Further attesting to the relevance of local housing conditions, in preliminary studies we found C57Bl/6 mice purchased from Jackson Laboratory to have a higher bone volume than isogenic mice bought from Taconic Biosciences. However, following 4 weeks of co-housing in our animal facility, the bone volume of mice from Jackson Laboratory decreased to the level of that of Taconic Biosciences mice. Since mice are coprophagic, and transfer their microbiomes from mouse to mouse by this behavior, the bone density decrease observed in mice from Jackson Laboratory is perhaps due to colonization of Jackson Laboratory mice with fecal material from Taconic Biosciences mice. These observations clearly point to the critical need to account for reciprocal host-microbiome interactions in experimental approaches that investigate the microbiome and bone density.

Evidence for the critical influence of the gut microbiota on bone growth is supported by studies that employed antibiotic treatment of mice. Initial investigations revealed that short-term administration of sub therapeutic doses of antibiotics at weaning resulted in elevated levels of bone mass [25]. Corroborating results were generated in a study showing that a low-dose of penicillin from birth to weaning

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