ARTICLE OPEN

Toll-like receptor 9 deficiency induces osteoclastic bone loss via gut microbiota-associated systemic chronic inflammation

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Toll-like receptors (TLRs) play pivotal roles in inflammation and provide important links between the immune and skeletal systems. Although the activation of TLRs may affect osteoclast differentiation and bone metabolism, whether and how TLRs are required for normal bone remodeling remains to be fully explored. In the current study, we show for the first time that TLR9$^{-/-}$ mice exhibit a low bone mass and low-grade systemic chronic inflammation, which is characterized by the expansion of CD4^{+} T cells and increased levels of inflammatory cytokines, including TNFα, RANKL, and IL1β. The increased levels of these cytokines significantly promote osteoclastogenesis and induce bone loss. Importantly, TLR9 deletion alters the gut microbiota, and this dysbiosis is the basis of the systemic inflammation and bone loss observed in TLR9$^{-/-}$ mice. Furthermore, through single-cell RNA sequencing, we identified myeloid-biased hematopoiesis in the bone marrow of TLR9$^{-/-}$ mice and determined that the increase in myelopoiesis, likely caused by the adaptation of hematopoietic stem cells to systemic inflammation, also contributes to inflammation-induced osteoclastogenesis and subsequent bone loss in TLR9$^{-/-}$ mice. Thus, our study provides novel evidence that TLR9 signaling connects the gut microbiota, immune system, and bone and is critical in maintaining the homeostasis of inflammation, hematopoiesis, and bone metabolism under normal conditions.

Bone Research (2022)10:42 ; https://doi.org/10.1038/s41413-022-00210-3

INTRODUCTION

The close relationship between inflammation and bone metabolism has long been appreciated. Osteoclastic bone resorption stimulated by activated T cells and macrophages is the main cause of bone loss in inflammatory conditions such as RA and infectious diseases. Inflammatory cytokines, including TNFα and IL17 secreted by activated T cells and macrophages, work in concert to promote inflammation and osteoclastogenesis.[1] Additionally, inflammation caused by sepsis leads to the inhibition of osteoblastic bone formation.[2] Osteoclasts and immune cells are closely related. Osteoclasts are multinucleated cells formed by the fusion of monocyte–macrophage precursors and share many similarities with innate immune cells, including monocytes, macrophages, and dendritic cells, in terms of their origin and function. Since bone marrow is the primary site of hematopoiesis, harboring hematopoietic stem and progenitor cells (HSPCs) and mature immune cells (including B cells, macrophages and T cells), osteoclasts and immune cells share the same microenvironment and interact with each other in bone marrow. Osteoclasts have been shown to process, present and cross-present antigens, resulting in T cell activation. They also produce cytokines and immunomodulatory factors that affect immune responses.[3] Reciprocally, activated T and B cells produce RANKL, which is one of the key signals driving osteoclast differentiation and activation. Moreover, OPG (the decoy receptor of RANKL) is highly expressed on B lymphocytes, and T cells are key regulators of OPG production in B cells and basal bone turnover.[4] The imbalanced RANKL/OPG ratio caused by dysregulated T and B cell function contributes to the bone loss that is associated with HIV infection and induced by combination antiretroviral therapy.[5] Furthermore, inflammatory cytokines, including TNFα, IL6 and IL1β, secreted by immune cells are potent proresorptive factors leading to bone loss in many inflammatory conditions, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and HIV infection.[1,5,6]

In addition to classic inflammatory diseases such as RA, low-grade inflammation has been observed to play important roles in bone loss during aging and metabolic disorders.[7–9] Studies have shown that inhibiting the production of the proinflammatory secretome by senescent cells prevents age-associated osteoporosis in mice.[8] Inflammation related to T cell activation is an important cause of hyperparathyroidism- and estrogen deficiency-induced osteoporosis.[7,9] Furthermore, increasing evidence suggests that alterations in the gut microbiota modulate inflammation and play a central role in estrogen deficiency-induced bone loss.[10,11] The gut microbiome may affect bone physiology through different mechanisms; nevertheless, the modulation of the host immune system is thought to be an important pathway linking the gut microbiota and bone.[11]

Toll-like receptors (TLRs) play a pivotal role in innate immune responses against microbes through the recognition of pathogen-associated molecular patterns.[12] TLR9 senses unmethylated CpG DNA from bacteria and viruses to initiate type I interferon (IFN)

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Received: 1 July 2021 Revised: 6 February 2022 Accepted: 13 March 2022
Published online: 27 May 2022