Unlocking the bone: Fcγ-receptors and antibody glycosylation are keys to connecting bone homeostasis to humoral immunity

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Abstract: Bone tissue is characterized by a constant remodeling process mediated by bone resorbing osteoclasts and bone forming osteoblasts. During autoantibody mediated autoimmune diseases, such as inflammatory arthritis, this balance is disturbed and the de novo generation of osteoclasts through cross-linking of activating Fcγ-receptors (FcγRs) expressed on osteoclasts results in excessive bone erosions and joint destruction. A recent study by Negishi-Koga and colleagues now provides conclusive evidence, that FcγRs may also play a crucial role for bone homeostasis during the steady state, further highlighting the tight interactions between the bone and immune system.

Keywords: Bone; osteoclast; glycosylation; Fcγ-receptors (FcγRs); IgG

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For a long time the bone has been considered to merely represent a niche for allowing early immune system development. This simple model, however, is subject to change and the term osteoimmunology has been coined to reflect the multitude of interactions between bone cells and cells of the innate and adaptive immune system (1). In fact, one of the two cell types critical for bone homeostasis, the bone resorbing osteoclasts are derived from hematopoietic precursor cells, whereas bone forming osteoblasts derive from the mesenchymal lineage. Moreover, the bone is not only a reservoir of calcium but also a storage place for cytokines such as the transforming growth factor β (TGF-β) for example (1,2). Furthermore, it is clear that the bone can react to inflammatory stimuli, best exemplified by bone loss and joint destruction during inflammatory arthritis (3). In this autoimmune disease autoantibodies are triggering a cascade of proinflammatory events via complement activation and crosslinking of activating Fcγ-receptors (FcγRs) widely expressed on innate immune effector cells such as mast cells, monocytes, neutrophils and tissue resident macrophages (4). These pro-inflammatory effects of autoantibodies are counterbalanced by the co-expression of the inhibitory FcγRIIB, which sets a threshold for cell activation (5,6). While the capacity of autoantibodies or immune complexes to recruit innate immune effector cells via activating FcγRs is firmly established, the impact of immune complexes to modulate bone homeostasis through activating FcγRs has remained unclear. Thus, the process of de novo generation of osteoclasts during joint inflammation, which ultimately drives bone erosions and joint destruction, was thought to largely depend on the pro-inflammatory cytokine milieu present in the inflamed joint (7). Indeed mice deficient in the FcR common γ-chain (FcRγ), which is essential for mediating activating signaling pathways upon crosslinking of activating FcγRs, had a normal bone morphology, suggesting that activating FcγRs did not play a major role in this process (8). In contrast, the lack of DAP12 resulted in an osteopetrotic phenotype, due to a block in osteoclast development, which was further enhanced by the additional loss of the FcRγ-chain. Further studies demonstrated that receptors such as the osteoclast-associated receptor (OSCAR) or TREM2, which associate with the FcRγ-chain or DAP12, respectively, may play the dominant role in this process during the steady state (8,9).

Upon inflammation, however, an important function of autoantibody mediated crosslinking of activating FcγRs expressed on osteoclasts and their myeloid precursor cells was noted recently (10). Thus, an osteoclast specific deletion

of FcγRIV resulted in a protection from autoantibody induced bone erosions and osteoclast generation in inflamed joints *in vivo*, providing strong evidence that activating signals transmitted through the common FcRγ-chain act as essential co-stimulatory signals in concert with pro-inflammatory cytokines to allow effective osteoclastogenesis during inflammation. Of note, the Ly6C high inflammatory monocyte subset was identified to be the precursor of osteoclasts generated during inflammation offering a novel therapeutic avenue to prevent autoantibody induced bone loss (10,11). While these results strongly argued for a critical role of FcγR as a molecular link between the bone and humoral immune system, it remained unclear if this is specific for inflammatory disease states or also relevant under steady state conditions.

Convincing evidence for the latter scenario was now provided by a study from Negishi-Koga and colleagues (12). By studying bone density in mice deficient for the low affinity activating FcγRIII, which is broadly expressed on innate immune cells including osteoclast precursor cells, immature and to a lesser extent on mature osteoclasts, they show that the absence of this receptor results in a osteoporotic phenotype, characterized by a lower bone density and a higher number of osteoclasts. Thus surprisingly, the presence of the activating FcγRIII seemed to have an inhibitory effect on osteoclastogenesis *in vivo*. By using a set of elegant biochemical experiments the authors demonstrate that the loss of FcγRIII results in a higher expression of other pro-osteoclastogenic cell surface receptors associated with the FcRγ-chain, including OSCAR and PIR-A, which may explain the increased level of osteoclast generation and lower bone density. This type of compensatory mechanism is consistent with other studies showing that deletion of one activating FcγR may lead to the upregulation of other activating Fc-receptors, which are coexpressed on the same cell (13,14). More expectedly, the deletion of the inhibitory FcγRIIB resulted in a similar osteoporotic phenotype due to an increase in osteoclast numbers. Here, *in vitro* experiments with osteoclast cultures in the presence of mouse serum containing or lacking IgG antibodies could clearly demonstrate that this inhibitory effect of FcγRIIB on osteoclastogenesis was mediated through serum IgG [or rather minimal amounts of immune complexes constantly present in the serum (15)]. In mice, IgG1 is the dominant serum IgG subclass, which has a much higher affinity for the inhibitory FcγRIIB compared to its activating counterpart FcγRIII, suggesting that during the steady state FcγRIIB expressed on osteoclast precursor cells, such as inflammatory monocytes, provides a negative feedback loop to prevent spontaneous osteoclastogenesis (10,16). In FcγRIIB deficient mice this process is further enhanced by the fact that FcγRIIB is also regulating IgG production in B cells and humoral tolerance (17). Thus, enhanced production of immune complexes and the lack of negative regulation on osteoclast precursor cells may contribute to the lower bone mass in mice lacking this receptor (12). Consistent with the differential binding of mouse IgG subclasses to the individual activating Fcγ-receptors, the authors could demonstrate that IgG1 immune complex mediated osteoclastogenisis was solely dependent on FcγRIII and strongly regulated by the inhibitory FcγRIIB, whereas IgG2a and IgG2b immune complexes induced osteoclastogenesis via FcγRI and FcγRIV, which was not influenced by the absence of FcγRIIB (10,12,16). Further strengthening their observation, the local injection of IgG2a but not IgG1 immune complexes resulted in an increased number of osteoclasts and local bone loss. In FcγRIIB deficient mice, however, the local or systemic injection of IgG1 immune complexes resulted in bone loss, strongly supporting a model in which the inhibitory FcγRIIB sets a threshold for preventing excessive osteoclastogenesis and bone loss during the steady state. More excitingly, Negishi-Koga and another study by Harre and colleagues published in the same issue of *Nature Communications* noted that sialic acid containing IgG glycovariants within the serum or autoantibody preparation had an inhibitory effect on the osteoclastogenic activity of IgG. Thus, desialylation strongly increased the IgG-dependent osteoclast development, fully consistent with other studies which have also noticed the potent immunomodulatory function of this IgG glycovariant in a variety of model systems (18-23).

The final question addressed by the authors was how inflammation impacts this threshold set by the inhibitory FcγRIIB. This is critical, as it is well known that pro-inflammatory cytokines, such as TNFα or IFNγ can downmodulate FcγRIIB expression on innate immune effector cells, while upregulating expression of activating FcγRs (24). To analyze this, osteoclasts were generated from mice upon induction of collagen induced arthritis, indeed demonstrating that osteoclasts generated under inflammatory conditions displayed a lower level of inhibitory and an increased amount of activating FcγR expression. Consistent with their previous results, IgG1 immune complexes were now more potent in stimulating osteoclastogenesis *in vitro*, due to the lower level of negative regulation through FcγRIIB.

Taken together, this study in combination with the study of Harre and colleagues and a previous report by our group firmly establishes the important role of FcγRs on osteoclasts as the link between the bone and humoral immune system (10,12,25,26). Of note, the threshold set by activating and inhibitory FcγR expression seems to be a crucial to determine under which conditions (amount of immune complexes, pro-inflammatory environment) (auto)antibodies in the form of immune complexes will be able to induce osteoclastogenesis and bone loss. As always, new insights into a field not only answer but also trigger new questions. For example, certain FcγRIIB allelic variants, such as the FcγRIIB-I232T allele, which loses its inhibitory signaling capacity, have been described to be associated with the development or severity of human autoimmune diseases such as systemic lupus erythematosus (27,28). One may expect that this FcγRIIB allelic variant may also be associated with a more severe osteoporosis if present in patients with arthritis.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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