Editorial

A bone to pick with Fc gamma receptors

Christian M. Karsten[1], Jörg Köhl[1,2]

[1]Institute for Systemic Inflammation Research, University of Lübeck, 23562 Lübeck, Germany; [2]Division of Immunobiology and the Center for Systems Immunology, Cincinnati Children's Hospital and University of Cincinnati, College of Medicine, Cincinnati, OH 45229, USA

Correspondence to: Jörg Köhl. Institute for Systemic Inflammation Research, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany. Email: joerg.koehl@uksh.de.

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Bone formation is a dynamic process, in which the bone structure is constantly remodeled. Osteoclasts and osteoblasts play critical but opposing roles in bone formation and resorption. While osteoclasts promote bone resorption, osteoblasts drive bone formation. Both processes are intertwined and tightly regulated to ensure the integrity of the bony skeleton. In particular, bone mass, strength and mineral homeostasis depend on balanced osteoclast and osteoblast function. Enhanced osteoclast activity leads to massive bone loss as exemplified in autoimmune diseases like rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Osteoclasts and osteoblasts originate from different precursors. Whereas osteoblasts derive from mesenchymal stem cells, osteoclasts originate from multinucleated progenitors of the monocyte/macrophage family. Two critical factors that regulate osteoclastogenesis are macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor kappa B (NF-κB) (RANK) ligand (RANKL). RANKL is expressed by T cells, endothelial cells and osteoblasts. Although activation of the RANKL pathway is essential to initiate osteoclastogenesis, an immunoreceptor tyrosin based activation motif (ITAM) co-stimulatory pathway is required for calcium-mediated activation of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), which serves as an important factor in osteoclast differentiation (1). The ITAM co-stimulatory pathway is activated in response to ligation of immunoglobulin-like receptors such as osteoclast-associated receptor (OSCAR), triggering receptor expressed on myeloid cells (TREM-2) or paired Ig-like receptor A (PIR-A), and the phosphorylation of the adaptor molecules containing ITAM motifs. Such ITAM motif-containing proteins are DNAX activation protein of 12 kDa (DAP12) and the Fc-receptor γ subunit (FcRγ) Importantly, the γ-chain not only facilitates FcγR signaling but is also required for the transport of IgG Fc receptors to the cell surface (2,3). The critical role of FcRγ and DAP12 for osteoclast activation was first demonstrated in mice suffering from severe osteopetrosis when both factors were lacking. Importantly, the phenotype was less pronounced in mice lacking only DAP12, whereas FcRγ-deficient mice showed no disease phenotype (2). These data suggest that FcRγ plays an important role in osteoclastogenesis in concert with DAP12.

In mice, four different FcγRs have been described: FcγRI, FcγRIIB, FcγRIII and FcγRIV (4). FcRγ acts as the common subunit of the activating FcγRs, whereas the only inhibitory FcγR, FcγRIIB, signals independent of FcRγ. Despite the availability of knockout-mice for the distinct FcγRs, a detailed understanding of the individual roles of IgG Fc receptors in osteoclastogenesis has been lacking. Negishi-Koga *et al*. provide now detailed insights into the role of activating and inhibitory FcγRs in bone homeostasis at steady state and under inflammatory conditions (5).

The first and surprising finding was that naive FcγRIII-deficient mice showed an osteoporotic phenotype, which was associated with an increased number of osteoclasts. In contrast, osteoblast numbers were not altered. *In vitro* experiments confirmed this observation. FcγRIII-deficient bone marrow-derived monocyte/macrophage cells (BMM) stimulated with RANKL and M-CSF showed enhanced osteoclast formation. These results suggest that FcγRIII has an inhibitory effect on osteoclastogenesis under homeostatic conditions, even when IgG1 immune complexes (IC), the dominant ligand of FcγRIII, are missing. The authors explain this unexpected suppressive role of FcγRIII on

osteoclast activity by a sequestering effect of FcRγ that is associated with the highly expressed FcγRIII under physiologic conditions. In the absence of FcγRIII, more FcRγ is available resulting in increased surface expression of OSCAR and PIR-A. As a consequence, mice lacking FcγRIII showed hyperactivation of PLCγ2 and increased intracellular calcium release. In support of this finding, the authors showed that FcγRIII is inversely correlated with the surface expression of OSCAR and PIR-A.

In autoimmune disorders, auto-antibodies form either soluble or cell-bound IC, serving as ligands for FcγRs. Depending on their subclass, they bind with different affinities to the four FcγRs. IgG1 antibodies are the most dominant subclass in mice. IgG1 IC bind predominantly to FcγRIIB and FcγRIII, both of which are expressed on osteoclasts. Importantly, the affinity of IgG1 IC for FcγRIIB is tenfold higher than for FcγRIII. Consequently, IgG1-mediated osteoclast activation is mainly regulated by the relatively low binding affinity to activating FcγRIII and the high binding affinity of IgG1 IC to inhibitory FcγRIIB resulting in an A/I ratio of 0.1 (4). In line with these considerations, FcγRIIB-deficient mice suffer from enhanced inflammation in various IgG1-mediated autoimmunity models (6), which are often associated with enhanced bone resorption. Accordingly, the authors demonstrate that IgG1 IC induce osteoclast formation in cells from mice lacking the inhibitory FcγRIIB but not in wildtype cells. This phenotype could be rescued by the additional deletion of FcRγ or reduction of FcγRIII expression by shRNA, demonstrating a critical role for FcγRIIB and FcRγ activation downstream of FcγRIII for bone homeostasis under inflammatory conditions. The *in vivo* relevance of IgG1 IC for the regulation of bone resorption was underscored by experiments, in which the authors injected IgG1 IC locally into the calvarial bone. Recapitulating the *in vitro* findings, bone loss occurred only in FcγRIIB-deficient but not in wild type mice. Of note, the impact on bone dynamics was not associated with any signs of cellular inflammation.

During the course of experimental RA or SLE, auto-antibodies of the IgG2a/c and IgG2b subclasses develop that can bind with high to moderate affinities to FcγRI, FcγRIV and FcγRIII, when complexed with their antigens. Importantly, the A/I ratios of IgG2a/c and IgG2b are 70 or 7 and thus much higher than the A/I ratio for IgG1. Consistent with this notion, inflammation models using IgG switch variants confirmed the higher inflammatory potency of IgG2a/c or IgG2b as compared with IgG1 antibodies (7). Here, the authors demonstrated that IgG2a/c and IgG2b antibodies induce strong osteoclast formation even in wild type cells that was markedly suppressed in response to shRNA-mediated knock-down of FcγRI or FcγRIV. The *in vivo* relevance of this observation was highlighted using sera from mice suffering from collagen-induced arthritis (CIA). Such sera induced strong osteoclastogenesis, whereas sera from control mice did not. Depletion of IgG abrogated this effect. Mechanistically, BMM from CIA mice upregulated activating FcγRIII and FcγRIV and downregulated inhibitory FcγRIIB.

This reciprocal regulation of activating and inhibitory FcγRs may result from classical and/or alternative pathway activation of the complement system by IgG2a/c and IgG2b IC. In fact, the cleavage fragment of the complement component 5 (C5), i.e., C5a, can set the threshold for FcγR activation by upregulation of activating and downregulation of inhibitory FcγRs on macrophages in the lung (8) and the peritoneum (9) through activation of C5a receptor 1 (C5aR1). Of note, C5aR1 is expressed in osteoclasts and drives osteoclastogenesis in response to C5a, even in the absence of RANKL and M-CSF (10), suggesting that IgG2a/c IC can induce osteoclast formation directly through the activation of FcγRI, FcγRIII and/or FcγRIV and indirectly through the activation of the complement system.

Another important part of IgG Fc that the authors identified as a regulator of osteoclast activation is the glycan fraction within the CH2 region of the heavy chain. IgG Fc harbors a complex biantennary glycan structure at Asn297 that either terminates with N-acetyl-glucosamine, galactose or sialic acid. IgG lacking Fc-sialylation bind with higher affinity to activating FcγR than their sialylated counterpart (11). Interestingly, the authors identified a higher frequency of desialylated IgG in sera from FcγRIIB-deficient mice as compared with wildtype controls. The purified IgG from FcγRIIB-deficient mice stimulated osteoclastogenesis more efficiently than those from wild type mice. This is an important finding, as sera from RA patients suffering from acute flares show a high frequency of auto-antibodies lacking terminal sialylation and galactosylation (12). In fact, the decrease in terminal Fc-glycosylation precedes the onset of RA, suggesting that bone resorption may already start prior to clinical signs of autoimmune disease. In addition to terminal sialic acid, galactose may also impact on bone homeostasis. Highly galactosylated IgG1 IC suppress C5a-mediated cell activation through a pathway that cross-links FcγRIIB and the C-type lectin receptor dectin-1 (13),

thereby potentially interfering with the direct and indirect effects of C5a on osteoclastogenesis, as outlined above. In light of these findings, the impact of the glycan composition on bone resorption needs to be considered in the design of therapeutic antibodies that are administered in autoimmune diseases or cancer. In these settings, IC formation might provide ligands for osteoclasts and induce therapy-related bone loss, in particular after long-term administration.

In addition to specific immunotherapy by IgG antibodies, patients suffering from autoimmune diseases are often treated with intravenous immunoglobulin (IVIG). IVIG is composed of pooled serum of many thousand donors. The IgG fraction is the main component of IVIG. The findings of Negishi-Koga *et al*. suggest that IVIG treatment will reduce the bone loss in autoimmune disease. Among the many proposed immunomodulatory pathways of IgG within IVIG, C5a scavenging, modulation of activating and inhibitory FcγR expression, blockade of activating FcγRs and saturation of neonatal FcR may limit auto-antibody-induced increase in osteoclastogenesis (14).

Finally, it will be important to delineate how the data obtained in the mouse system translate into the human situation. First findings are promising and support the view that the observations by Negishi-Koga *et al*. may also apply to the regulation of human bone homeostasis. For example, RA patients carrying the high affinity FcgRIIIA158V allele suffer from more severe bone erosion when compared to patients carrying the less affine FcgRIIIA158F allele (15). Also, the levels of IgG- or citrullinated peptide-specific antibodies in RA patients correlate with the incidence and the extent of bone destruction (16). However, as not only the IgG subclasses and FcγR composition differ between mice and humans but also the potency of individual IgG subclasses to activate the complement system, future research will need to address in more detail the impact of the different IgG subtypes and their Fc-glycan structures on the multiple FcγR-complement axes and also the activation of C-type lectin receptors.

In summary, Negishi-Koga *et al*. identified an unexpected inhibitory role for FcγRIII in osteoclastogenesis under physiological conditions and provide important novel insights into FcγR-mediated mechanisms that lead to bone resorption in IC-mediated diseases (5). Their data provide evidence that the IgG isotype determines the activation of the downstream pathways that eventually result in osteoclast differentiation and activation. For IgG1 IC driven osteoclast activation, the A/I ratio between FcγRIII and FcγRIIB is critical. In contrast, IgG2a/c or IgG2b IC mediate osteoclast activation mainly through FcγRI and FcγRIV aggregation. Further, the complement-activating properties of IgG isotypes and their Fc-glycan composition need to be taken into account, as they drive important feedback loops that impact on FcγR expression, define the binding affinity of IgG Fc to FcγRs and can activate osteoclasts independent of FcγRs through complement and C-type lectin receptors.

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Footnote

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