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TREM2 and β-Catenin Regulate Bone Homeostasis by Controlling the Rate of Osteoclastogenesis

Karel Otero,*1 Masahiro Shinohara,†,1 Haibo Zhao,‡,§,1 Marina Cellar,* Susan Gilfillan,* Angela Colucci,* Roberta Faccio,¶ F. Patrick Ross,‖ Steve L. Teitelbaum,* Hiroshi Takayanagi,‖ and Marco Colonna*

TREM2 is an immunoreceptor expressed on osteoclasts (OC) and microglia that transmits intracellular signals through the adaptor DAP12. Individuals with genetic mutations inactivating TREM2 or DAP12 develop the Nasu–Hakola disease (NHD) with cystic-like lesions of the bone and brain demyelination that lead to fractures and presenile dementia. The mechanisms of this disease are poorly understood. In this study, we report that TREM2-deficient mice have an osteopenic phenotype reminiscent of NHD. In vitro, lack of TREM2 impairs proliferation and β-catenin activation in osteoclast precursors (OcP) in response to M-CSF. This defect results in accelerated differentiation of OcP into mature OC. Corroborating the importance of a balanced proliferation and differentiation of OcP for bone homeostasis, we show that conditional deletion of β-catenin in OcP also results in reduced OcP proliferation and accelerated osteoclastogenesis in vitro as well as osteopenia in vivo. These results reveal that TREM2 regulates the rate of osteoclastogenesis and provide a mechanism for the bone pathology in NHD. The Journal of Immunology, 2012, 188: 2612–2621.

Skeletal homeostasis is sustained by a balance between bone-forming osteoblasts (OB) and bone-resorbing osteoclasts (OC) (1). Whereas OB are of mesenchymal origin, OC originate from hematopoietic stem cells and belong to the myeloid lineage. OC differentiation and function is controlled by two major signals (2). M-CSF is required for the expansion of OC precursors (OcP) and their differentiation into mature OC. Receptor activator for NF-κB ligand (RANKL) is crucial for OC proliferation and differentiation into bone-resorbing OC (3). OC differentiation and function are also controlled by cell surface receptors, including MDL-1, TREM2, and OSCAR, which transmit intracellular signals through associated transmembrane adaptors containing ITAMs (4–11). These adaptors, including FcRγ and DAP12, recruit the protein tyrosine kinase Syk, which triggers phosphorylation events ultimately leading to Ca2+ mobilization and activation of the transcription factors NFAT, NF-κB, and AP-1 (12, 13). Moreover, recent evidence indicates that the receptor for M-CSF, although not physically associated with DAP12, uses this adaptor to transmit certain intracellular signals, particularly to activate Syk and β-catenin (14, 15).

FcRγ- and DAP12-associated receptors have a critical role in bone homeostasis. Mice lacking FcRγ and DAP12 have markedly reduced OC function, resulting in osteopetrosis with increased bone mass and elimination of bone marrow space (16–18). In humans, inactivating mutations of either TREM2 or DAP12 result in a disorder known as Nasu–Hakola disease (NHD) or polycystic lipomembranous osteodysplasia with sclerosing leuкоencephalopathy (19, 20). NHD patients have progressive loss of white matter in the brain, leading to severe presenile dementia. Moreover, they have bone lesions in the extremities that consist of areas of osteoporosis that contain poorly defined cavities filled with heterogeneous lipid material (21). These lesions often lead to bone fractures following minimal trauma. Despite the established involvement of TREM2 or DAP12 mutations in NHD, the mechanism by which these molecules regulate bone remodeling in these patients remains poorly understood.

Because the TREM2/DAP12 receptor complex is expressed in OC, it has been suggested that NHD pathological bone lesions are most likely initiated by deregulated OC generation and/or function. However, in vitro and in vivo studies of TREM2 and DAP12 function in bone homeostasis have produced unexpected results that are inconsistent with the osteoporosis observed in NHD. In fact, DAP12 deficiency leads to defective OC development and function in vitro and mild osteopetrosis in vivo (16, 22). Similarly, peripheral blood monocytes from both DAP12- and TREM2-deficient patients are unable to effectively generate OC with bone resorptive function in vitro (21, 23). Moreover, downregulation of TREM2 expression by RNA interference in the murine monocytic cell line RAW264.7 results in defective differentiation of this cell line into OC with bone resorbing function (24). Based on these experiments, lack of TREM2/DAP12 should result in increased bone mass.

In this study, we analyzed the bone phenotype of TREM2−/− mice and found that these animals exhibit osteoporosis and hence may...
provide a more accurate mouse model of the bone pathology of NHD. Mechanistically, we demonstrate that TREM2 deficiency results in a defective activation of β-catenin and proliferation of OcP, which accelerates their differentiation into functionally mature OC with bone resorbing capacity. Thus, TREM2 and β-catenin modulate bone resorption by controlling the rate of OC generation.

**Materials and Methods**

**Mice and analysis of bone phenotype**

Wild-type (WT), TREM2−/−, LysM-Cre, and β-cateninlox/lox mice were on a C57BL/6 background and were born and maintained under specific pathogen-free conditions in the animal care unit of Washington University School of Medicine according to guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Histomorphometric and microcomputed tomographic (μCT) examinations of the long bones were performed essentially as described (17, 25). Male and female mice were studied in all experiments obtaining similar results; data obtained from females are shown.

**Reagents**

Cell culture media were obtained from Invitrogen; FBS was purchased from HyClone. RANKL was provided by D. Fremont (Washington University, St. Louis, MO). Mouse M-CSF was from R&D Systems. Commercially available kits were used to measure the levels of collagen degradation products in cell culture supernatants (Nordic Bioscience Diagnostic), to make apoptosis of the cultures (In Situ Cell Death Detection Kit, Roche), and to stain osteoclastogenic cultures for tartrate-resistant acid phosphatase (TRAP) (Sigma-Aldrich).

**OcP culture and osteoclastogenesis**

Bone marrow cells were cultured for 12 h in Petri dishes to remove adherent cells. Nonadherent cells were transferred to new dishes and cultured in complete α-MEM (Invitrogen) containing 10% FBS (HyClone) and a 1:10 vol CMG 14-12 cell culture supernatant as source of M-CSF, as previously described (26). Alternatively, mouse rM-CSF (R&D Systems) was used at 100 ng/ml. At day 3, cells were considered as Ocp. To generate mature OC, OcP were cultured at a density of 3000 cells per well in 96-well plates or 15,000 cells per well in 24-well plates in complete α-MEM with 10 ng/ml M-CSF and 100 ng/ml RANKL for varying times, with media being changed every 2 d.

**RNA isolation and quantitative PCR**

Total RNA was extracted from osteoclastogenic cultures at different time points using TRIzol reagent (Invitrogen). After first-strand cDNA synthesis using a SuperScript III kit (Invitrogen), real-time quantitative PCR reactions were performed for *Nfatc1, Acp5, Csk, Calcr, and Cnd1* as previously described (14, 27). Relative quantification of target mRNA expression was calculated and normalized to the expression of cyclophilin and expressed as (mRNA of the target gene/mRNA of cyclophilin) × 10^5.

**TRAP activity assay**

A quantitative TRAP solution assay was performed by adding a colorimetric substrate, 5.5 mM P-nitrophenyl phosphate, in the presence of 10 mM sodium tartrate at pH 4.5. The reaction product was quantified by measuring optical absorbance at 405 nm.

**Proliferation assay**

M-CSF–stimulated cells were labeled with 50 μg/ml BrdU for 12 h, and BrdU incorporation was measured by flow cytometry using a FITC BrdU flow kit (BD Pharmingen). Cell growth of the 96-well plate cultures was measured by MTT incorporation. In brief, MTT (Sigma-Aldrich) was added to the cultures at varying times at 50 μg/ml. Cells were incubated at 37°C, 5% CO2 for 4 h, media was removed, and cells were lysed with 200 μl DMSO. The MTT metabolic product formazan was quantified by measuring optical absorbance at 560 nm and subtracting background at 670 nm.

**Immunofluorescence and bone resorptive pits stain**

Cells were removed from bone slices by mechanical agitation. Bone slices were incubated with peroxidase-conjugated wheat germ agglutinin (Sigma-Aldrich) for 1 h and stained with 3,3′-diaminobenzidine (Sigma-Aldrich).

**Immunoprecipitation and immunoblotting**

OcP were kept without M-CSF for 4 h in medium containing or not 10% FCS, harvested with PBS/3 mM EDTA, carefully counted, and replated onto tissue culture plates. Cells previously starved in medium containing serum or without it were stimulated with M-CSF or RANKL, respectively (both at 100 ng/ml), for the appropriate times, lysed, and immunoblotted as previously described (14). For immunoprecipitation of Syk, 5 × 10^6 cells were immunoprecipitated with anti-Syk Ab (N-19) and protein A-Sepharose (GE Healthcare) and subsequently immunoblotted. Abs directed to the following molecules were used (catalog nos. are in parentheses): phospholiophosphate C (PLC)γ2 (3872), phospho-PLCγ2 (3874), phospho-c-Cbl (3555), phospho-Shc (2434), phospho-ERK (9101), p38 (9212), phospho-p38 (9211), Akt (9272), phospho-Akt (9271), JNK (9252), phospho-JNK (9251), phospho-p65 (3033), phospho-c-Jun (2361), IκBα (9242), phospho-IκBα (5A5), Syk (2712), β-catenin (9562), and GAPDH (14C10) (Cell Signaling Technology); phospho-tyroside (4G10; Upstate Biotechnology); ERK2 (C-14), β-actin (C-11), Syk (N-19), and lamin B (C-20) (Santa Cruz Biotechnology); phospho-Pyk2 (44-632) (BioSource International); and Pyk2 (610548) (BD Transduction Laboratories).

**Statistical analyses**

Statistical significance was determined using GraphPad Prism version 4.0c. Statistical differences were determined by a two-tailed Student t test (between two groups) and a one-way ANOVA (among multiple groups). The p values were defined as *p < 0.05 and **p < 0.01.

**Results**

**TREM2−/− mice are osteopenic**

To elucidate the role of TREM2 in bone homeostasis, we analyzed the bone characteristics of TREM2−/− mice. There were no gross abnormalities in skeletal development, but μCT analyses of the long bones revealed osteopenia, reduced bone volume and trabecular number, as well as increased trabecular separation in comparison with WT mice (Fig. 1A). Notably, we observed by histomorphometric analysis more OC and larger OC surface and eroded area in TREM2−/− mice (Fig. 1B). In contrast, OB and dynamic bone formation parameters were very similar in TREM2−/− and WT mice (Fig. 1C). Thus, the osteopenic phenotype was not due to a reduction in OB numbers or activity. Collectively, these results indicate that during in vivo bone remodeling, TREM2 deficiency led to a decrease in bone mass most likely due to enhanced OC formation and, consequently, augmented bone resorption.

**TREM2 deficiency accelerates OC formation in vitro**

To examine the impact of TREM2 deficiency on osteoclastogenesis, we cultured TREM2−/− and WT bone marrow cells depleted of stromal cells with M-CSF for 72 h to generate OcP. OcP were then cultured with M-CSF and RANKL to induce the differentiation of OcP into OC, identified as cells that contain more than three nuclei and express TRAP. We found that TREM2−/− OcP differentiated into multinucleoc OC more rapidly than did WT OcP (Fig. 2A, 2B). Consistent with this result, TREM2−/− cultures showed more expression of OC-specific genes *Nfatc1, Acp5, Csk, and Calcr* as compared with WT cultures 2 d after addition of RANKL (Fig. 2C). This difference was transient; after 4 d culture with RANKL, there were no differences in expression of OC-specific genes. TRAP activity was also increased in TREM2−/− cells after 3 d culture (Fig. 2D). OC cultured in vitro on plastic dishes are short lived and die by apoptosis. We found that TREM2−/− OC not only formed earlier but also died earlier (Fig. 2E). We conclude that TREM2 deficiency causes accelerated osteoclastogenesis in vitro.
We also examined the ability of TREM2−/− OcP to differentiate into OC when cultured on a mineralized substrate. OC generated by this method are more similar to OC in vivo, as their actin cytoskeleton forms typical actin rings. Similar to cultures generated on tissue culture plastic, many more OC were formed on bone slices from TREM2−/− OcP compared with WT cells as early as 4 d culture, although no differences in numbers were noted after 7 d culture (Fig. 2F). Accelerated osteoclastogenesis was associated with increased numbers of resorption pits after 4 d, but not after 7 d culture (Fig. 2G). Bone degradation, measured as the accumulation of type I collagen fragments in the culture media, was also significantly higher in the TREM2−/− cultures at early time points (Fig. 2H). Therefore, TREM2 deficiency also causes accelerated differentiation of OC on a mineralized substrate. Taken together, these results indicate that TREM2 negatively regulates OC formation at an early stage, but has no impact on the function of mature OC.

TREM2 deficiency does not augment M-CSF and RANK signaling

It has been reported that engagement of DAP12-associated receptors with low-avidity ligands induces a partial phosphorylation of DAP12, leading to DAP12 recruitment of inhibitory mediators, such as the protein tyrosine phosphatases Src homology region 2 domain-containing phosphatase 1 (SHIP) or the lipid phosphatase SHIP (29). The abnormal recruitment of phosphatases in place of the canonical recruitment of the protein tyrosine kinase Syk may result in inhibition of heterologous receptor signaling pathways. Thus, we tested the hypothesis that TREM2 delivers inhibitory signals during bone homeostasis and that accelerated osteoclastogenesis of TREM2−/− OcP depends on the release of M-CSF and/or RANKL signaling from TREM2-mediated inhibition. TREM2−/− and WT OcP were cultured in M-CSF for 72–96 h, deprived of M-CSF for few hours, and then left untreated or stimulated with M-CSF for increasing periods of time. Cell lysates were analyzed for phosphorylation of several downstream effectors, including the MAPK ERK, JNK, and p38, the adaptors c-Cbl and Shc, as well as Syk, PLCγ2, and Akt. M-CSF–induced phosphorylation of most mediators was very similar in TREM2−/− and WT OcP, with the exception of a slight but consistent increase of ERK phosphorylation in TREM2−/− OcP, reminiscent of what was previously observed in DAP12-deficient macrophages (14) (Fig. 3A). Notably, phosphorylation of Syk, a known target of DAP12 that has an essential role in osteoclastogenesis (18), was reduced in TREM2−/− OcP (Fig. 3B). Additionally, phosphorylation of Pyk2, a downstream target of Syk, was slightly but reproducibly reduced in TREM2−/− OcP compared with WT OcP (Fig. 3C). These results suggest that TREM2 does not recruit tyrosine phosphatases. Lack of TREM2 did not abolish basal Syk phosphorylation, most likely because TREM2-
deficient OC expressed other DAP12-associated receptors, such as Sirpβ1 and Clec5A, which can transduce ITAM signals (Supplemental Fig. 1).

We also examined activation of signaling mediators in TREM2−/− and WT OcP cultured in M-CSF for 72–96 h and stimulated with RANKL. RANK-induced activation of PLCγ2, MAPKs, p65, c-Jun, and IκBα were comparable in TREM2−/− and WT cells (Fig. 3D). We conclude that TREM2 does not elicit inhibitory signals through DAP12 and that accelerated osteoclastogenesis in TREM2−/− mice is not due to an increase of M-CSF or RANK signaling.

TREM2 deficiency impairs M-CSF–induced activation of β-catenin and OcP proliferation

We previously reported that M-CSF signaling promotes expression and nuclear translocation of β-catenin and that DAP12 enhances M-CSF–induced activation of β-catenin (14). Thus, we asked whether TREM2 deficiency affects this pathway. After stimulation with M-CSF, TREM2−/− and WT OcP were examined for expression of β-catenin in the total cell lysates (Fig. 3E). We found that basal β-catenin levels were similar in TREM2−/− and WT OcP, whereas basal β-catenin levels were similar in TREM2−/− and WT OcP, M-CSF–induced expression of β-catenin in the total cell lysates as well as in isolated cytosolic and nuclear extracts. Whereas basal β-catenin levels were similar in TREM2−/− and WT OcP, M-CSF–induced ex-
expression of β-catenin in total cell lysates, cytosolic accumulation, and nuclear translocation of β-catenin were all reduced in the absence of TREM2 (Fig. 3E). Additionally, TREM2−/− OC expressed less cyclin D1 mRNA, a known β-catenin target gene, than did WT OC (Supplemental Fig. 1).

Because β-catenin plays an essential role in proliferation, we measured proliferation and cell growth in TREM2−/− and WT OcP cultures by BrdU incorporation and MTT assays, respectively. TREM2−/− OcP showed a significant reduction of proliferation (Fig. 3F). As previously reported, M-CSF alone promoted cell proliferation of WT OcP, which was reduced by addition of RANKL (30, 31) (Fig. 3G). Notably, TREM2−/− OcP cultured with M-CSF alone or both M-CSF and RANKL proliferated significantly less than did WT OcP (Fig. 3G).
conclude that TREM2 is essential for enhancing M-CSF–induced proliferation of OcP, possibly by facilitating the activation of β-catenin. OcP, similar to other precursor cells, undergo cell cycle arrest prior to differentiation into mature OC. Accordingly, it is conceivable that TREM2 deficiency may induce an earlier cell cycle arrest, which favors OcP to differentiate more rapidly into mature OC.

Mice lacking β-catenin in the OcP compartment are osteopenic

The osteopenic phenotype observed in TREM2−/− mice associated with the reduced activation of β-catenin suggested the intriguing possibility that β-catenin could be essential for regulating osteoclastogenesis. To investigate the effect of β-catenin in OC differentiation in a cell-autonomous fashion, we generated mice with

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**FIGURE 4.** β-catenin–deficient OcP proliferate less to M-CSF and exhibit accelerated osteoclastogenesis. (A) Immunoblot analysis of β-catenin and actin (loading control) in OcP total protein lysates from control LysM-Cre+/+ β-catenin+/+ (βcat+/+) and LysM-Cre+/+ β-cateninfl/fl (βcatΔΔ) mice. (B) Defect of M-CSF–induced proliferation in β-catenin–deficient OcP. Proliferation of βcat+/+ and βcatΔΔ OcP was measured 12 h after incubation with BrdU in the presence of 100 ng/ml M-CSF. (C and D) OcP from βcat+/+ and βcatΔΔ mice were cultured with 10 ng/ml M-CSF and 100 ng/ml RANKL to generate multinuclear OC. Development of OC was monitored at different time points by TRAP staining. (C) Representative images of TRAP-stained cultures. Original magnification ×200. (D) The number of TRAP-positive cells containing three or more nuclei was scored (total). Additionally, the nuclei in each OC was enumerated as follows: 3–10, 6–10, and >10 nuclei per TRAP-positive cell. (E) Effect of β-catenin deficiency on the expression of the osteoclastic differentiation markers Nfatc1 (encoding NFATc1), Acp5 (encoding TRAP), Ctsk (encoding cathepsin K), and Calcr (encoding calcitonin receptor) by quantitative PCR. (F) OC differentiation induced on bone slices. (F) After 4 and 7 d differentiation, cultures were fixed and stained with phalloidin-FITC to visualize the OC actin rings. Original magnification ×200. (G) Bone resorption pits were revealed by lectin staining of the bone slices (brown reaction product). Original magnification ×200. Results are representative of at least three separate experiments. *p < 0.05, **p < 0.01.
a selective deletion of β-catenin in OcP. Mice harboring a floxed β-catenin allele (βcat<sup>f/f</sup>) were crossed with mice expressing the Cre recombinase under the control of regulatory elements of the LysM gene (LysM<sup>Cre/Cre</sup>). LysM<sup>Cre/</sup>βcat<sup>f/f</sup> mice were then crossed with LysM<sup>Cre/Cre</sup> and the LysM<sup>Cre/Cre</sup>βcat<sup>f/f</sup> mice obtained were intercrossed to generate LysM<sup>Cre/Cre</sup>βcat<sup>f/f</sup> β-catenin-deficient mice (βcat<sup>Δ/Δ</sup>). LysM<sup>Cre/Cre</sup>βcat<sup>f/+</sup> littermates served as controls.

Immunoblot analysis of OcP derived in vitro from βcat<sup>Δ/Δ</sup> bone marrow cells confirmed complete deletion of β-catenin expression (Fig. 4A). CD11b, F4/80, MHC class II, and CD115 were equally expressed in βcat<sup>Δ/Δ</sup> and βcat<sup>+</sup/+ OcP, suggesting that β-catenin deficiency does not affect the differentiation of bone marrow precursor cells into OcP (data not shown). However, βcat<sup>Δ/Δ</sup> OcP proliferated less than did βcat<sup>+</sup/+ OcP in response to M-CSF (Fig. 4B). Moreover, βcat<sup>Δ/Δ</sup> OcP formed mature OC more rapidly than did βcat<sup>+</sup/+ OcP during culture with M-CSF and RANKL (Fig. 4C, 4D). Mature, terminally differentiated βcat<sup>Δ/Δ</sup> OC had no obvious defects in size or morphology. Consistent with accelerated osteoclastogenesis, acquisition of the OC-specific genes Nfatc1, Acp5, Ctsk, and Calcr was more rapid in βcat<sup>Δ/Δ</sup> OC as compared with βcat<sup>+</sup/+ OC at early time points during culture (Fig. 4E). When OcP were differentiated on bone slices, βcat<sup>Δ/Δ</sup> OcP formed more OC compared with βcat<sup>+</sup/+ cells after 4 d culture, although no differences in numbers were found after 7 d (Fig. 4F). Accelerated osteoclastogenesis of βcat<sup>Δ/Δ</sup> OC was associated with increased numbers of resorption pits after 4 d, but not after 7 d culture (Fig. 4G). No major differences in the activation of classical downstream mediators were observed following analysis M-CSF– and RANKL-induced signals in βcat<sup>Δ/Δ</sup> and βcat<sup>+</sup/+ OcP (Supplemental Fig. 2). Taken together, these results indicate that β-catenin has a cell-autonomous function at the early stages of OC generation, sustaining M-CSF–induced proliferation of OcP, but preventing their differentiation into mature OC. In contrast, β-catenin has no impact on the function of mature OC once they are generated.

Consistent with the inhibitory role of β-catenin in the in vitro OC differentiation, μCT and histomorphometry analysis revealed
a decrease in trabecular bone mass in βcatΔΔ mice compared with control mice (Fig. 5A). TRAP staining of long bone sections demonstrated significant increases in OC numbers, OC surface, and eroded surface areas in βcatΔΔ mice (Fig. 5B, 5C). No differences in OB and dynamic bone formation parameters were noted following bone morphometric analysis (Fig. 5D), indicating that β-catenin deletion in the βcatΔΔ mice does not affect OB numbers or function. We conclude that β-catenin in the OC lineage plays an essential role in controlling early OC differentiation and bone homeostasis.

Simultaneous heterozygosity for TREM2- and β-catenin-null alleles results in osteoporosis

TREM2 and β-catenin deficiency cause similar bone phenotypes, and M-CSF induction of β-catenin is reduced in TREM2−/− OcP, suggesting a link between TREM2 and β-catenin. To corroborate this possibility, we generated mice double heterozygous for TREM2 and β-catenin (βcatΔ/Δ Trem2−/+ in the LysMCreCre background and analyzed their bone phenotypes. μCT analysis of the long bones showed that these mice had a significant reduction of bone volume and trabecular number, as well as a marked increase in trabecular separation, compared with age-matched single heterozygous littermates, which, in turn, were not different from control WT littermates (Fig. 6). Importantly, the osteopenic phenotype of LysMCreCre βcatΔ/Δ Trem2−/+ mice phenocopies the TREM2−/− and the βcatΔΔ mutant phenotype. These results provide a genetic basis in support of a coordinate action of TREM2 with β-catenin to modulate bone homeostasis.

Discussion

Our study unveils a TREM2/β-catenin pathway that regulates bone mass by regulating the rate of OC generation. Mechanistically, TREM2 and β-catenin augment the M-CSF–induced proliferation of OcP, retarding their differentiation into mature OC. Ablation of either TREM2 or β-catenin inhibits the proliferation of OcP, accelerating their differentiation into bone-resorbing OC, which ultimately cause osteoporosis. The possibility that TREM2 and β-catenin act along the same pathway is supported not only by the similar osteoporotic phenotypes of TREM2−/− and βcatΔΔ mice, but also by genetic evidence that simultaneous heterozygosity for TREM2- and β-catenin–null alleles results in osteoporosis, whereas no phenotype is observed in mice heterozygous for either of these alleles.

TREM2 may enhance M-CSF–induced activation of β-catenin by facilitating the recruitment of DAP12 to the receptor for M-CSF. In turn, DAP12 may activate Syk and Pyk2, which promote phosphorylation and nuclear translocation of β-catenin (14). Accordingly, we found that TREM2 deficiency impaired the M-CSF–induced phosphorylation of Syk and, in part, Pyk2. Conversely, basal phosphorylation of Syk was preserved. M-CSF–induced activation of Syk was proposed to mediate OC function (14, 15). However, the reduction of this signaling pathway in TREM2−/− mice resulted in a selective defect in OcP proliferation, whereas OC differentiation was accelerated and OC function was preserved. Therefore, we speculate that differentiation and particularly cytoskeleton rearrangement of OC may be independent of M-CSF–induced Syk activation, depending rather on the basal activation of Syk by signals emanating from integrins or other ITAM-signaling receptors.

![FIGURE 6. TREM2 and β-catenin function together to maintain OC numbers and bone homeostasis. We generated mice with the genotype LysM-CreCre with the allelic combinations β-catenin+/+Trem2+/+ (Control), β-catenin+/−Trem2−/+ (βcatΔ/Δ), β-catenin+/+ Trem2−/− (Trem2−/−), and β-catenin−/−Trem2−/− (βcatΔΔ). Femurs were analyzed by μCT. Longitudinal (upper panels) and axial (lower panels) views of the metaphyseal region are shown. The parameters are based on the μCT analysis of the metaphyseal region of mice at the age of 8 wk. Graphs show means ± SEM (n = 5). **p < 0.01.](http://www.jimmunol.org/)
The bone phenotype observed in TREM2−/− and BcatΔΔ mice more closely resembles the bone phenotype of NHD. NHD patients have osteoporosis whether the disease is caused by inactivating mutations of TREM2 or the associated adaptor DAP12. However, in the mouse, only TREM2−/− mice have an osteoprotic phenotype, whereas DAP12−/− mice exhibit a mild osteoprotic phenotype. The disparate impacts of DAP12 deficiency in humans and mice most likely reflect a difference in the spectrum of receptors involved in osteoclastogenesis and their capacity to signal through DAP12 or other ITAM-containing subunits such as FcRγ. In humans, the TREM2/DAP12 complex may selectively enhance OCP proliferation, whereas OCP differentiation into mature OC may be mediated by FcRγ-associated receptors. Thus, both TREM2 and DAP12 deficiencies would result in accelerated differentiation of OC and osteoporosis in vivo. In the mouse, DAP12 may control OCP proliferation through TREM2 as well as differentiation of OC through other receptors. Thus, whereas deficiency of TREM2 selectively affects OCP proliferation, deficiency of DAP12 may affect both OCP proliferation and mature OC functions, resulting in a mild osteoporosis, which becomes more dramatic in mice lacking both DAP12 and FcRγ (17).

Our demonstration that TREM2 deficiency accelerates osteoclastogenesis in cultures of bone marrow cells in vitro is at odds with previous studies showing that human blood monocytes lacking TREM2 have a reduced capacity to generate functional OC in vitro (21, 23). Similarly, RNA interference knockdown of TREM2 in the murine macrophage cell line RAW264.7 inhibited in vitro osteoclastogenesis (24). It is possible that human monocytes and mouse RAW264.7 cells, although capable of generating OC in vitro, differ from the physiological OCP in their spectrum of DAP12 and FcRγ-associated receptors, such that the lack or blockade of a functional TREM2/DAP12 complex has wider impact on human monocytes and RAW264.7 cell-derived OC than in OCP-derived OC. In support of this hypothesis, several reports have shown that modulation of TREM2 function in human monocytes has a major impact on DAP12/ITAM signaling (32–34). Alternatively, cultures of purified human monocytes and RAW264.7 cells may lack ligands capable of engaging DAP12 and/or FcRγ-associated receptors. Regardless of the cause, TREM2−/− bone marrow cultures and TREM2−/− mice seem to provide the best models for in vitro and in vivo studies of NHD bone pathology.

Although the role of β-catenin in OB development and function is well established (35–38), little is known about its function in OC. A recent study proposed that β-catenin regulates osteoclastogenesis in a dosage-dependent fashion such that β-catenin can have either an activating or inhibitory function (39). Our study suggests that activation of the TREM2/β-catenin pathway in OCPs may reduce osteoclastogenesis. Thus, from a therapeutic perspective, activation of the β-catenin pathway in bone cells could be a valuable strategy in the treatment of osteoporosis, as it would uniquely combine simultaneous stimulation of OB with inhibition of osteoclastogenesis. However, systemic activation of β-catenin may have important drawbacks. It has been shown that continuous activation of β-catenin in the hematopoietic system causes excessive proliferation and exhaustion of the hematopoietic progenitor pool, leading to a failure in the generation of myeloid and lymphoid lineages (40, 41). Moreover, β-catenin is key to balancing tolerance and immunity through the control of dendritic cell function (42, 43). Because of TREM2-restricted expression, activating β-catenin downstream of TREM2 may provide a good strategy to selectively activate β-catenin in the OC lineage for increasing bone mass in osteoporosis.

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Disclosures

The authors have no financial conflicts of interest.

References


Corrections


The original article contains errors in the legend for Fig. 2 and in Fig. 4C and 4F. In the legend for Fig. 2B, the first range for the nuclei in each osteoclast was enumerated incorrectly and should have been indicated as 3–5, not 3–10. In Fig. 4, the panels corresponding to osteoclasts generated from βcat\( ^{Δ/Δ} \) mice after 4 days of culture (Fig. 4C) and 7 days of culture (Fig. 4F) are duplicated. The corrected images are shown here, along with the rest of Fig. 4. The first range for the nuclei in each osteoclast was enumerated incorrectly in the legend for Fig. 4D and should have been indicated as 3–5, not 3–10. The corrected legend is shown below. The original description of the results does not change. The authors deeply regret this oversight.

**FIGURE 4.** β-catenin–deficient OcP proliferate less to M-CSF and exhibit accelerated osteoclastogenesis. (A) Immunoblot analysis of β-catenin and actin (loading control) in OcP total protein lysates from control LysM-Cre\(^{−/−}\) β-catenin\(^{+/+}\) (βcat\(^{+/+}\)) and LysM-Cre\(^{−/−}\) β-catenin\(^{Δ/Δ}\) (βcat\(^{Δ/Δ}\)) mice. (B) Defect of M-CSF–induced proliferation in β-catenin–deficient OcP. Proliferation of βcat\(^{+/+}\) and βcat\(^{Δ/Δ}\) OcP was measured 12 h after incubation with BrdU in the presence of 100 ng/ml M-CSF. (C and D) OcP from βcat\(^{+/+}\) and βcat\(^{Δ/Δ}\) mice were cultured with 10 ng/ml M-CSF and 100 ng/ml RANKL to generate multinuclear OC. Development of OC was monitored at different time points by TRAP staining. (C) Representative images of TRAP-stained cultures. Original magnification ×200. (D) The number of TRAP-positive cells containing three or more nuclei was scored (total). Additionally, the nuclei in each OC was enumerated as follows: 3–5, 6–10, and >10 nuclei per TRAP-positive cell. (E) Effect of β-catenin deficiency on the expression of the osteoclastic differentiation markers Nfatc1 (encoding NFATc1), Acp5 (encoding TRAP), Ctsk (encoding cathepsin K), and Calcr (encoding calcitonin receptor) by quantitative PCR. (F and G) OC differentiation induced on bone slices. After 4 and 7 d differentiation, cultures were fixed and stained with phalloidin-FITC to visualize the OC actin rings. Original magnification ×200. (G) Bone resorption pits were revealed by lectin staining of the bone slices (brown reaction product). Original magnification ×200. Results are representative of at least three separate experiments. *\( p < 0.05 \), **\( p < 0.01 \).

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**Figure S1.** TREM2 deficiency reduces the expression of Cyclin D1 and does not modulate the expression of other DAP12-associated receptors *in vitro*.

OcP generated from WT and TREM2−/− mice were cultured *in vitro* with 10 ng/ml M-CSF and 100 ng/ml RANKL to generate multinuclear osteoclasts. The expression of TREM2 (*Trem2*), Sirpβ1 (*Sirpb1*), Clec5a (*Clec5a*) and Cyclin D1 (*Ccnd1*) at the mRNA level was assessed by Q-PCR. Results are representative of three different experiments.
Figure S2. β-catenin deficiency does not affect M-CSF- and RANKL-induced signaling pathways in OcP. (A and B) OcP generated from control LysM-Cre+/+ β-catenin+/+ (βcat+/+) and LysM-Cre+/+ β-cateninfl/fl (βcatΔ/Δ) mice were starved from M-CSF for 4 h and then exposed to 50 ng/ml M-CSF (A) or were starved from M-CSF and serum for 4 h and then exposed to 100 ng/ml RANKL (B). After the indicated times total cell lysates were prepared and subjected to immunoblotting analysis using antibodies to the indicated proteins. Actin served as loading control. Results are representative of at least three different experiments.