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Transgenic over-expression of interleukin-33 in osteoblasts results in decreased osteoclastogenesis

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ABSTRACT

Interleukin-33 (IL-33) is the most recently identified member of the IL-1 family of cytokines, which is primarily known for its proinflammatory functions. We have previously reported that IL-33 is expressed by bone-forming osteoblasts, and that administration of recombinant IL-33 to bone marrow cultures inhibits their differentiation into bone-resorbing osteoclasts. Likewise, while the inhibitory effect of IL-33 on osteoclast differentiation was fully abolished in cultures lacking the IL-33 receptor ST2, mice lacking ST2 displayed low bone mass caused by increased osteoclastogenesis. Although these data suggested a physiological role of IL-33 as an inhibitor of bone resorption, direct *in vivo* evidence supporting such a function was still missing. Here we describe the generation and bone histomorphometric analysis of a transgenic mouse model (*Col1a1-Il33*) over-expressing IL-33 specifically in osteoblasts. While we did not observe differences in osteoblast number and bone formation between wildtype and *Col1a1-Il33* mice, the number of osteoclasts was significantly reduced compared to wildtype littermates in two independent transgenic lines. Since we did not observe quantitative differences in the populations of eosinophils, neutrophils, basophils or M2-macrophages from the bone marrow of wildtype and *Col1a1-Il33* mice, our data demonstrate that an inhibition of osteoclastogenesis is one of the major physiological functions of IL-33, at least in mice.

1. Introduction

Bone is constantly remodeled by bone-forming osteoblasts and bone-resorbing osteoclasts, and a relative increase of bone resorption over bone formation can result in osteoporosis, one of the most prevalent diseases in the aged population [1,2]. Since excessive osteoclastogenesis is also detrimental in several other disorders, such as Morbus Paget, rheumatoid arthritis or metastatic bone disease, it is of central clinical importance to identify molecules specifically involved in the regulation of osteoclast differentiation and/or function [3–5]. Osteoclasts are multinucleated cells derived from mononuclear precursors of the monocyte/macrophage lineage [6]. In the differentiated state they are attached to the bone matrix, which is resorbed by simultaneous secretion of hydrochloric acid and matrix-degrading peptidases. Impaired osteoclast differentiation or function causes osteopetrosis, a high bone mass disorder accompanied by immunological defects due to bone marrow displacement [7]. Since combined efforts in human and mouse genetics have led to the identification of several genes, whose inactivation causes osteopetrosis, there is now a decent understanding of the molecular mechanisms regulating osteoclastogenesis [6]. Collectively, these findings provide the basis for an efficient anti-resorptive treatment of osteoporotic patients. In this regard, it is probably most important that specific cytokines and their receptors have been identified as putative target molecules.

One of the best characterized positive regulators of osteoclastogenesis is Rankl, a cytokine expressed by osteoblasts and activated T cells [8]. Rankl promotes osteoclast differentiation and function through binding to its receptor Rank, and mutations in either of the corresponding genes cause osteopetrosis in mice and humans [7]. The action of Rankl can be antagonized by its decoy receptor osteoprotegerin, whose inactivation in mice or humans results in excessive osteoclastogenesis [8,9]. This knowledge has already been translated into a novel anti-resorptive therapy, since a monoclonal antibody neutralizing Rankl has recently been approved for the treatment of postmenopausal osteoporosis [10]. Another cytokine stimulating osteoclastogenesis is interleukin-1 (IL-1), which is

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mostly known for its proinflammatory functions [11]. Based on cumulative evidence from various investigators, IL-1 is now considered to be a key molecule causing bone destruction in inflammatory disorders, such as rheumatoid arthritis or periodontal disease [12–15]. This is best underscored by the presence of multifocal osteolytic lesions in patients with inactivating mutations of the *ILRN* gene [16,17]. *ILRN* encodes an endogenous antagonist (IL-1Ra) of the IL-1 receptor, and a recombinant form of the protein has already been approved for the treatment of rheumatoid arthritis [18]. In this regard it is also important to state, that the initial discovery of IL-1 as an osteoclast-activating factor produced by mononuclear blood cells has opened a new area of research, now termed "osteoimmunology", as it provided one of the first examples for an interaction between bone and immune cells [19,20].

Besides osteoprotegerin and IL-1Ra, there are other cytokines negatively affecting osteoclastogenesis, such as IFNγ, IL-4 and IL-18, the latter belonging to the IL-1 family of proteins [21,22]. We have previously reported that the third member of this family, IL-33, is expressed by bone-forming osteoblasts, which led us to analyze the potential role of IL-33 in bone remodeling [23]. While we did not observe a major influence of recombinant IL-33 on osteoblast differentiation and matrix mineralization, we found that osteoclast formation from bone marrow precursor cells was inhibited by IL-33, and that this influence was dependent on the presence of the IL-33 receptor ST2 [23]. Likewise, we observed that ST2-deficient mice displayed a low bone mass phenotype explained by increased osteoclastogenesis with normal bone formation. Taken together, these findings suggested that the negative influence of IL-33 on osteoclastogenesis is also relevant *in vivo*, but they failed to provide direct evidence, as it is virtually impossible to rule out the existence of additional ligands for ST2. Here we describe the generation of a mouse model with increased *Il33* expression in osteoblasts. Since our bone histomorphometric analysis revealed decreased osteoclastogenesis compared to wildtype littermates, it is now possible to conclude that IL-33 acts as an inhibitor of osteoclast differentiation, not only *in vitro*, but also *in vivo*. Taken together, these data expand our current knowledge in the field of osteoimmunology and raise the possibility that a stimulation of the IL-33/ST2 interaction may be an alternative approach for blocking excessive bone resorption not only in osteoporosis, but also in other diseases associated with excessive osteoclastogenesis.

2. Materials and methods

2.1. Animals

For the generation of *Col1a1-Il33* transgenic mice the ORF encoding IL-33 was placed under the control of an osteoblast-specific 2.3 kb *Col1a1* promoter fragment (kindly provided by Dr. B. de Crombrugghe, Houston, USA). Pronucleus injection into fertilized oocytes was performed according to standard protocols. Genotyping of the offspring was performed by Southern Blotting following digestion with KpnI using the 3′-UTR of the *hGH* gene as a probe, or by PCR using primers located within the β-*globin* intron (5′-ACT ACA TCC TGG TCA TCA TCC TGC-3′) and the *Il33* cDNA (5′-TGC ACC ATG AGA TCC CAT TCT A-3′). The two transgenic founders were mated with wildtype mice (C57Bl/6) to establish independent transgenic lines. The skeletal phenotype from the two founder animals and their offspring was analyzed at 24 weeks of age. All mice received two injections of calcein before sacrifice, which enabled us to perform dynamic histomorphometry. The animal experiments were approved by the animal facility of the University Medical Center Hamburg-Eppendorf and by the "Amt für Gesundheit und Verbraucherschutz" (95/08, Org139).

2.2. Skeletal analysis

After their initial analysis by contact X-ray (Faxitron X-ray Corp.), the vertebral bodies L2 to L5 and one tibia from each animal were dehydrated and embedded non-decalcified into methyl methacrylate for sectioning. Sections were either stained with toluidine blue or by the von Kossa/van Gieson procedure as described [23]. Static and cellular histomorphometry was carried out on toluidine blue-stained sections using the OsteoMeasure system (Osteometrics, Decatur, USA) following the guidelines of the American Society of Bone and Mineral Research [24]. Dynamic histomorphometry for determination of the bone formation rate was performed on two consecutive non-stained 12 μm-sections. TRAP activity staining was performed on decalcified sections using naphthol AS-MX phosphate (Sigma) and Fast Red Violet LB salt (Sigma) in 40 mM acetate buffer (pH 5).

2.3. Primary osteoblasts

Primary osteoblasts were isolated from calvariae of newborn mice as described [25]. At 80% confluency, cells were differentiated by adding β-glycerophosphate and ascorbic acid to a final concentration of 10 mM and 50 μg/ml, respectively. For alizarin red staining cells were fixed with 90% ethanol, washed twice with water and incubated with 40 mM alizarin red staining solution (pH 4.2) for 10 min at room temperature, followed by five subsequent washes with water. To quantify matrix mineralization, the stained cultures were incubated with 10% acetic acid for 30 min, before heating for 10 min at 85 °C. After removing the cellular remnants by centrifugation, 400 μl of supernatant was neutralized with 150 μl 10% ammonium hydroxide, and 150 μl of each sample were used to determine absorption at 405 nm. To determine the IL-33 concentration in osteoblast medium and in serum, we used a commercially available ELISA (R&D Systems, #M3300).

2.4. FACS analysis

Analyses of surface marker expression via flow cytometry were conducted after blocking unspecific binding sites with COHN fraction II (Sigma), using the following fluorescently labeled primary antibodies: CD11b (CD11b-fluorescein, BD Biosciences, orCD11b-Alexa647, eBiosciences) CCR3/CD193 (CCR3-phycoerythrin, R&D Systems), Ly-6G (Ly-6G-phycoerythrin, BD Biosciences), CD23 (CD23-fluorescein, BD Biosciences), and CD206/MMR (CD206-Alexa647, BioLegend). Additionally, appropriately matched and fluorescently conjugated isotype controls were used. Flow cytometry was performed with a FACSCalibur flow cytometer (BD Biosciences). Data were processed with CellQuest-Pro software (BD Biosciences).

2.5. Statistical analysis

All data are presented as means ± standard deviation. Statistical analysis was performed using unpaired, two-tailed Student's *t*-test. Asterisks indicate statistically significant differences ($*p < 0.05$).

3. Results

To address the role of IL-33 in bone remodeling *in vivo*, we generated a construct (Fig. 1A) containing the ORF encoding IL-33 under the control of an osteoblast-specific 2.3 kb promoter fragment from the *Col1a1* gene [26,27]. This construct was injected into fertilized mouse oocytes, and the offspring was analyzed for integration of the transgene. Using this approach we obtained two transgenic founder animals (Fig. 1B) that were bred with

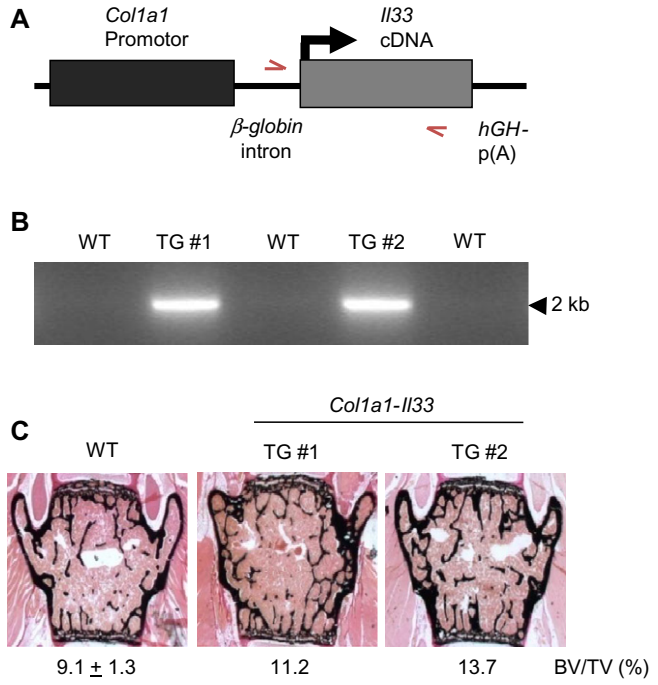

Fig. 1. Generation of *Col1a1-Il33* mice. (A) Schematic representation of the construct used for osteoblast-specific over-expression of *Il33*. The primers used for genotyping are indicated by red arrows. (B) Identification of two transgenic founder animals (TG#1, TG#2) by genomic PCR. (C) von Kossa/van Gieson staining of non-decalcified vertebral body sections from 24 week old female wildtype mice (WT) and the two founder animals. The trabecular bone volume (BV/TV, bone volume per tissue volume) is given below. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

wildtype mice to generate two independent transgenic lines. To get a first impression on their skeletal phenotype both founder animals were analyzed by non-decalcified histology at 24 weeks of age, where we found a higher trabecular bone volume compared to wildtype controls in both cases (Fig. 1C).

To obtain statistically relevant information, we next analyzed the transgenic offspring of the two founders compared to wildtype littermates (Fig. 2). By analyzing six female mice per group at 24 weeks of age, we found that the mean trabecular bone volume was higher in transgenic offspring from both founder animals, albeit the observed difference was statistically significant only for the second transgenic line. The same was the case for the increase in trabecular number, and the reduction in trabecular separation, whereas trabecular thickness was not different from the wildtype littermates.

We next performed cellular and dynamic histomorphometry to quantify the osteoblast surface and the bone formation rate. In line with our previous *in vitro* findings, where we did not observe an influence of IL-33 on matrix mineralization of cultured osteoblasts [23], we found no significant differences between wildtype and transgenic mice from both founder lines (Fig. 3A). In contrast, when we performed TRAP activity staining to visualize bone-resorbing osteoclasts, we observed that their number was reduced in transgenic mice compared to the wildtype controls (Fig. 3B). This was confirmed by histomorphometric quantification, where we found a significant decrease of the osteoclast surface per bone surface in transgenic offspring from both founders, when compared to wildtype littermates.

We thereafter focussed our analysis on the second founder line and isolated primary calvarial osteoblasts to analyze IL-33 production. Surprisingly, the cultures from transgenic mice displayed a moderate increase in matrix mineralization, when compared to the corresponding wildtype cultures at day 15 of differentiation (Fig. 4A). Most importantly, however, while the IL-33 concentration in the medium of these cells was more than threefold increased in transgenic cultures, there was no significant difference in the IL-33 serum concentration between wildtype and transgenic mice (Fig. 4B). Since this suggested that *Col1a1-Il33* mice only display a local increase of IL-33 levels, it was interesting to address the question of whether this would specifically affect osteoclastogenesis or hematopoietic differentiation in general. This was addressed by FACS analysis of the bone marrow using the same antibodies that we have previously applied to monitor the effects of IL-33 *in vitro* [23]. Here we did not detect any statistically significant changes in the population of eosinophils, neutrophils,

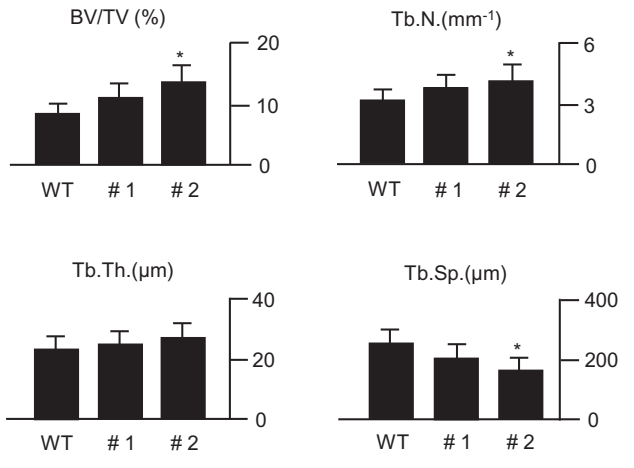

Fig. 2. Increased bone mass in *Col1a1-Il33* mice. Histomorphometric quantification of the trabecular bone volume (BV/TV, bone volume per tissue volume), trabecular number (Tb.N.), trabecular thickness (Tb.Th.) and trabecular separation (Tb.Sp.) of both founder lines (#1, #2) compared to wildtype littermates. All bars represent mean ± SD ($n = 6$ mice per group). Asterisks indicate statistically significant differences ($p < 0.05$).

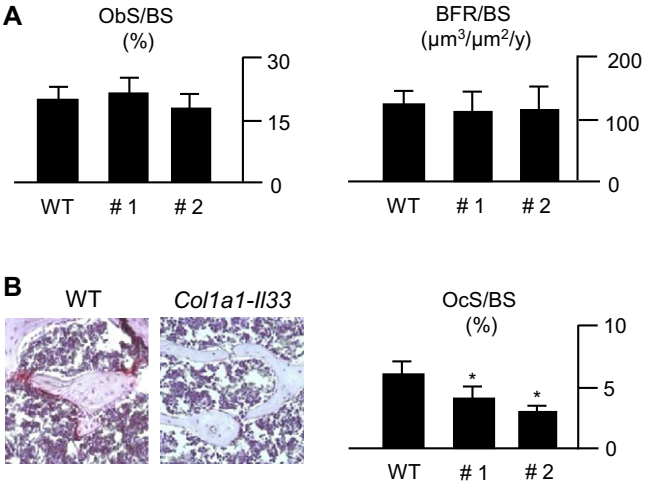

Fig. 3. Decreased osteoclastogenesis in *Col1a1-Il33* mice. (A) Histomorphometric quantification of the osteoblast surface per bone surface (ObS/BS) and the bone formation rate per bone surface (BFR/BS). (B) TRAP activity staining for osteoclasts in decalcified spine sections from 24 week old female wildtype and *Col1a1-Il33* transgenic mice. The reduction in bone resorption was confirmed by the histomorphometric quantification of osteoclast surface per bone surface (OcS/BS) in transgenic mice. All values represent mean ± SD ($n = 6$ mice per group). Asterisks indicate statistically significant differences compared to wildtype littermates ($p < 0.05$).

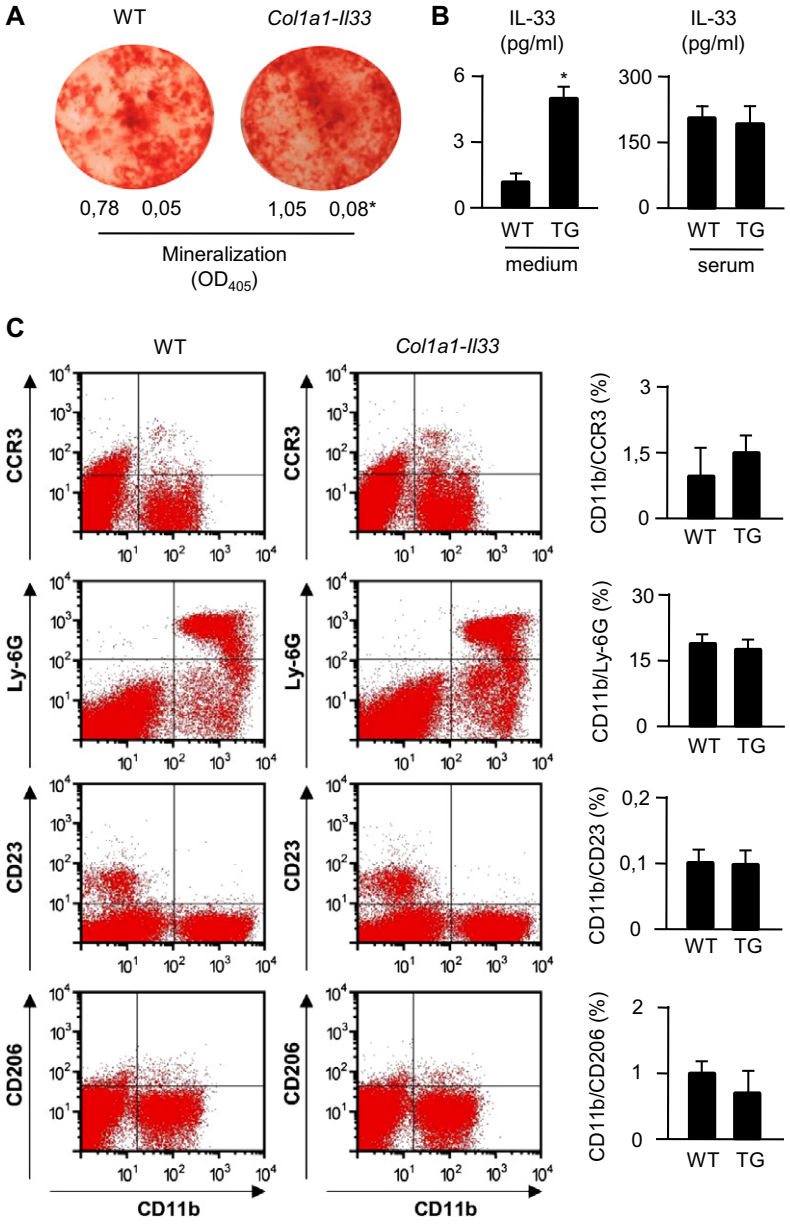

Fig. 4. Overexpression of *Il-33* in osteoblasts is not associated with changes in bone marrow composition. (A) Alizarin red staining of mineralized matrix in primary osteoblast cultures at day 15 of differentiation. Quantification of mineralization is indicated below. (B) IL-33 levels in medium of cultured osteoblasts ($n = 3$ cultures per group) and in serum of wildtype and *Col1a1-Il33* transgenic mice ($n = 6$ mice per group). (C) FACS analysis of bone marrow using the indicated antigens for the quantification of eosinophils (CD11b/CCR3), neutrophils (CD11b/Ly-6G), basophils (CD11b/CD23), or M2-macrophages (CD11b/CD206). Bars represent mean ± SD of three independent experiments. Asterisks indicate statistically significant differences ($p < 0.05$).

basophils or M2-macrophages between wildtype and transgenic littermates (Fig. 4C), thereby demonstrating that the primary role of osteoblast-derived IL-33 is to inhibit osteoclast differentiation.

4. Discussion

IL-33 is the most recently identified member of the IL-1 family of cytokines and serves as a ligand for the receptor ST2 [28]. It has been shown to act on a variety of immune cells, including Th2 lymphocytes, mast cells, granulocytes and macrophages [29–33]. Mostly based on experiments with ST2-deficient mice, several investigators have provided evidence for a critical role of IL-33 in different pathologies, such as asthma, allergy, arthritis and atherosclerosis [34–38]. Interestingly, however, the recent development and analysis of a mouse model lacking IL-33 only confirmed a role of IL-33 as an amplifier of innate immunity, while it was apparently dispensable for acquired immune responses [39]. These findings not only suggested the existence of additional ST2 ligands with pathophysiological relevance, but they also demonstrated that it is virtually impossible to draw definite conclusions regarding the functions of IL-33 from the analysis of ST2-deficient mice. This is exactly the reason why it was important to address the possible role of IL-33 in bone remodeling by the generation of transgenic mice over-expressing *Il33* in osteoblasts.

Here we found, through the analysis of two independent transgenic lines, that an increased IL-33 release by osteoblasts causes reduced osteoclastogenesis *in vivo*. This confirmed our previous data, where we demonstrated the same function of IL-33 *in vitro*, together with increased osteoclastogenesis in ST2-deficient mice [23]. In addition, we were able to show that the over-expression

of IL-33 in osteoblasts does not affect bone formation *in vivo*, which is again in line with our previous findings in ST2-deficient mice [23]. Taken together, these data demonstrate that IL-33 is a physiologically relevant inhibitor of osteoclast formation, at least in mice, which is in full agreement with findings recently reported by others, where the administration of recombinant IL-33 inhibited osteoclast formation and cartilage destruction in a TNF-transgenic mouse model [40].

Since we previously found that the negative effect of IL-33 on osteoclastogenesis from bone marrow precursor cells is accompanied by a shift within different cell populations of the myeloid lineage [23], we further used FACS analysis to determine the number of eosinophils, neutrophils, basophils or M2-macrophages in the bone marrow of the *Col1a1-Il33* mice. Here we failed to detect significant differences compared to wildtype littermates, thus suggesting a direct negative influence of IL-33 on terminal osteoclast differentiation. These findings are not only in line with our previous observation showing that IL-33 also inhibits osteoclastogenic differentiation of the macrophage cell line RAW264.7, but they underscore that this effect represents the most relevant function of IL-33 *in vivo*, at least in terms of regulating bone remodeling. Based on all of these arguments, it is fair to conclude that IL-33 has a bone-protective function, and it is reasonable to speculate that an activation of the IL-33/ST2 axis might be an alternative approach to treat disorders with excessive bone resorption, such as osteoporosis, Morbus Paget, metastatic bone disease or arthritis.

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References

[1] M. Zaidi, Skeletal remodeling in health and disease, Nat. Med. 13 (2007) 791–801.
[2] P. Sambrook, C. Cooper, Osteoporosis, Lancet 367 (2006) 2010–2018.
[3] S. Seitz, M. Priemel, J. Zustin, F.T. Beil, J. Semler, H. Minne, T. Schinke, M. Amling, Paget's disease of bone: histologic analysis of 754 patients, J. Bone Miner. Res. 24 (2009) 62–69.
[4] K. Okamoto, H. Takayanagi, Osteoclasts in arthritis and Th17 cell development, Int. Immunopharmacol. 11 (2011) 543–548.
[5] R.E. Coleman, Clinical features of metastatic bone disease and risk of skeletal morbidity, Clin. Cancer Res. 12 (2006) 6243s–6249s.
[6] J.R. Edwards, G.R. Mundy, Advances in osteoclast biology: old findings and new insights from mouse models, Nat. Rev. Rheumatol. 7 (2011) 235–243.
[7] A. Villa, M.M. Guerrini, B. Cassani, A. Pangrazio, C. Sobacchi, Infantile malignant, autosomal recessive osteopetrosis: the rich and the poor, Calcif. Tissue Int. 84 (2009) 1–12.
[8] B.F. Boyce, L. Xing, Functions of RANKL/RANK/OPG in bone modeling and remodeling, Arch. Biochem. Biophys. 473 (2008) 139–146.
[9] M.P. Whyte, Paget's disease of bone and genetic disorders of RANKL/OPG/RANK/NF-kappaB signaling, Ann. NY Acad. Sci. 1068 (2006) 143–164.
[10] R. Baron, S. Ferrari, R.G. Russell, Denosumab and bisphosphonates: different mechanisms of action and effects, Bone 48 (2011) 677–692.
[11] C.A. Dinarello, IL-1: discoveries, controversies and future directions, Eur. J. Immunol. 40 (2010) 599–606.
[12] M. Sabatini, B. Boyce, T. Aufdemorte, L. Bonewald, G.R. Mundy, Infusions of recombinant human interleukins 1 alpha and 1 beta cause hypercalcemia in normal mice, Proc. Natl. Acad. Sci. USA 85 (1988) 5235–5239.
[13] J. Zwerina, K. Redlich, K. Polzer, L. Joosten, G. Krönke, J. Distler, A. Hess, N. Pundt, T. Pap, O. Hoffmann, J. Gasser, C. Scheinecker, J.S. Smolen, W. van den Berg, G. Schett, TNF-induced structural joint damage is mediated by IL-1, Proc. Natl. Acad. Sci. USA 104 (2007) 11742–11747.
[14] K. Polzer, L. Joosten, J. Gasser, J.H. Distler, G. Ruiz, W. Baum, K. Redlich, K. Bobacz, J.S. Smolen, W. van den Berg, G. Schett, J. Zwerina, Interleukin-1 is essential for systemic inflammatory bone loss, Ann. Rheum. Dis. 69 (2010) 284–290.
[15] K.S. Kornman, A. Crane, H.Y. Wang, F.S. di Giovine, M.G. Newman, F.W. Pirk, T.G. Wilson Jr., F.L. Higginbottom, G.W. Duff, The interleukin-1 genotype as a severity factor in adult periodontal disease, J. Clin. Periodontol. 24 (1997) 72–77.
[16] I. Aksentijevich, S.L. Masters, P.J. Ferguson, P. Dancey, J. Frenkel, A. van Royen-Kerkhoff, R. Laxer, U. Tedgård, E.W. Cowen, T.H. Pham, M. Booty, J.D. Estes, N.G. Sandler, N. Plass, D.L. Stone, M.L. Turner, S. Hill, J.A. Butman, R. Schneider, P. Babyn, H.I. El-Shanti, E. Pope, K. Barron, X. Bing, A. Laurence, C.C. Lee, D. Chapelle, G.I. Clarke, K. Ohson, M. Nicholson, M. Gadina, B. Yang, B.D. Korman, P.K. Gregersen, P.M. van Hagen, A.E. Hak, M. Huizing, P. Rahman, D.C. Douek, E.F. Remmers, D.L. Kastner, R. Goldbach-Mansky, An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist, N. Engl. J. Med. 360 (2009) 2426–2437.
[17] S. Reddy, S. Jia, R. Geoffrey, R. Lorier, M. Suchi, U. Broeckel, M.J. Hessner, J. Verbsky, An autoinflammatory disease due to homozygous deletion of the IL1RN locus, N. Engl. J. Med. 360 (2009) 2438–2444.
[18] G.D. Kalliolias, S.N. Liossis, The future of the IL-1 receptor antagonist anakinra: from rheumatoid arthritis to adult-onset Still's disease and systemic-onset juvenile idiopathic arthritis, Expert Opin. Investig. Drugs 17 (2008) 349–359.
[19] F.E. Dewhirst, P.P. Stashenko, J.E. Mole, T. Tsurumachi, Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 beta, J. Immunol. 135 (1985) 2562–2568.
[20] H. Takayanagi, Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems, Nat. Rev. Immunol. 7 (2007) 292–304.
[21] I.B. McInnes, G. Schett, Cytokines in the pathogenesis of rheumatoid arthritis, Nat. Rev. Immunol. 7 (2007) 429–442.
[22] N.J. Horwood, N. Udagawa, J. Elliott, D. Grail, H. Okamura, M. Kurimoto, A.R. Dunn, T. Martin, M.T. Gillespie, Interleukin 18 inhibits osteoclast formation via T cell production of granulocyte macrophage colony-stimulating factor, J. Clin. Invest. 101 (1998) 595–603.
[23] J. Schulze, T. Bickert, F.T. Beil, M.M. Zaiss, J. Albers, K. Wintges, T. Streichert, K. Klaetschke, J. Keller, T.N. Hissnauer, A.S. Spiro, A. Gessner, G. Schett, M. Amling, A.N. McKenzie, A.K. Horst, T. Schinke, Interleukin-33 is expressed in differentiated osteoblasts and blocks osteoclast formation from bone marrow precursor cells, J. Bone Miner. Res. 26 (2011) 704–717.
[24] A.M. Parfitt, M.K. Drezner, F.H. Glorieux, J.A. Kanis, H. Malluche, P.J. Meunier, S.M. Ott, R.R. Recker, Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee, J. Bone Miner. Res. 2 (1987) 595–610.
[25] J. Albers, J. Schulze, F.T. Beil, M. Gebauer, A. Baranowsky, J. Keller, R.P. Marshall, K. Wintges, F.W. Friedrich, M. Priemel, A.F. Schilling, J.M. Rueger, K. Cornils, B. Fehse, T. Streichert, G. Sauter, F. Jakob, K.L. Insogna, B. Pober, K.P. Knobeloch, U. Francke, M. Amling, T. Schinke, Control of bone formation by the serpentine receptor Frizzled-9, J. Cell Biol. 192 (2011) 1057–1072.
[26] J. Schulze, S. Seitz, H. Saito, M. Schneebauer, R.P. Marshall, A. Baranowsky, B. Busse, A.F. Schilling, F.W. Friedrich, J. Albers, A.S. Spiro, J. Zustin, T. Streichert, K. Ellwanger, C. Niehrs, M. Amling, R. Baron, T. Schinke, Negative regulation of bone formation by the transmembrane Wnt antagonist Kremen-2, PLoS One 5 (2010) e10309.
[27] R. Dacquin, M. Starbuck, T. Schinke, G. Karsenty, Mouse alpha1(I)-collagen promoter is the best known promoter to drive efficient Cre recombinase expression in osteoblast, Dev. Dyn. 224 (2002) 245–251.
[28] J. Schmitz, A. Owyang, E. Oldham, Y. Song, E. Murphy, T.K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, D.M. Gorman, J.F. Bazan, R.A. Kastelein, IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines, Immunity 23 (2005) 479–490.
[29] M. Komai-Koma, D. Xu, Y. Li, A.N. McKenzie, I.B. McInnes, F.Y. Liew, IL-33 is a chemoattractant for human Th2 cells, Eur. J. Immunol. 37 (2007) 2779–2786.
[30] E. Schneider, A.F. Petit-Bertron, R. Bricard, M. Levasseur, A. Ramadan, J.P. Girard, A. Herbelin, M. Dy, IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production, J. Immunol. 183 (2009) 3591–3597.
[31] P.N. Pushparaj, H.K. Tay, S.C. H'ng, N. Pitman, D. Xu, A. McKenzie, F.Y. Liew, A.J. Melendez, The cytokine interleukin-33 mediates anaphylactic shock, Proc. Natl. Acad. Sci. USA 106 (2009) 9773–9778.
[32] B. Stolarski, M. Kurowska-Stolarska, P. Kewin, D. Xu, F.Y. Liew, IL-33 exacerbates eosinophil-mediated airway inflammation, J. Immunol. 185 (2010) 3472–3480.
[33] M. Kurowska-Stolarska, B. Stolarski, P. Kewin, G. Murphy, C.J. Corrigan, S. Ying, N. Pitman, A. Mirchandani, B. Rana, N. van Rooijen, M. Shepherd, C. McSharry, I.B. McInnes, D. Xu, F.Y. Liew, IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation, J. Immunol. 183 (2009) 6469–6477.
[34] A.M. Miller, Role of IL-33 in inflammation and disease, J. Inflamm. (Lond.) 8 (2011) 22.
[35] A.M. Miller, D. Xu, D.L. Asquith, L. Denby, Y. Li, N. Sattar, A.H. Baker, I.B. McInnes, F.Y. Liew, IL-33 reduces the development of atherosclerosis, J. Exp. Med. 205 (2008) 339–346.
[36] D.E. Smith, IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma, Clin. Exp. Allergy 40 (2010) 200–208.
[37] F.Y. Liew, N.I. Pitman, I.B. McInnes, Disease-associated functions of IL-33: the new kid in the IL-1 family, Nat. Rev. Immunol. 10 (2010) 103–110.
[38] D. Xu, H.R. Jiang, P. Kewin, Y. Li, R. Mu, A.R. Fraser, N. Pitman, M. Kurowska-Stolarska, A.N. McKenzie, I.B. McInnes, F.Y. Liew, IL-33 exacerbates antigen-

induced arthritis by activating mast cells, Proc. Natl. Acad. Sci. USA 105 (2008) 10913–10918.
[39] K. Oboki, T. Ohno, N. Kajiwara, K. Arae, H. Morita, A. Ishii, A. Nambu, T. Abe, H. Kiyonari, K. Matsumoto, K. Sudo, K. Okumura, H. Saito, S. Nakae, IL-33 is a crucial amplifier of innate rather than acquired immunity, Proc. Natl. Acad. Sci. USA 107 (2010) 18581–18586.
[40] M.M. Zaiss, M. Kurowska-Stolarska, C. Böhm, R. Gary, C. Scholtysek, B. Stolarski, J. Reilly, S. Kerr, N.L. Millar, T. Kamradt, I.B. McInnes, P.G. Fallon, J.P. David, F.Y. Liew, G. Schett, IL-33 shifts the balance from osteoclast to alternatively activated macrophage differentiation and protects from TNF-alpha-mediated bone loss, J. Immunol. 186 (2011) 6097–6105.