

# Adopted orphans as regulators of inflammation, immunity and skeletal homeostasis

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## Summary

Adopted orphan nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs), have emerged as key regulators of inflammation and immunity and likewise control skeletal homeostasis. These properties render them attractive targets for the therapy of various inflammatory and autoimmune diseases affecting the musculoskeletal system. This review summarises the current knowledge on the role of these families of receptors during innate and adaptive immunity as well as during the control of bone turnover and discuss the potential use of targeting these molecules during the treatment of chronic diseases such as osteoarthritis, rheumatoid arthritis and osteoporosis.

**Key words:** *inflammation; nuclear receptors; bone; macrophages; osteoblasts; osteoclasts; arthritis*

## Introduction

The musculoskeletal system is a prevalent target of chronic inflammatory diseases such as rheumatoid arthritis (RA) and osteoarthritis. Typically, such disorders exhibit a non-resolving inflammatory response that does not only lead to the local destruction of bone and cartilage, but likewise results in the impairment of systemic homeostatic processes such as fat, glucose and bone metabolism. Current approaches for the treatment of chronic inflammatory diseases are still largely based on pharmacologic compounds that primarily interfere with pro-inflammatory signalling cascades and hence suppress the inflammatory response. The most widely used anti-inflammatory substances are still glucocorticoids, which have revolutionised the therapy of inflammatory diseases since its discovery in the 1940's. The initial enthusiasm of clinicians using these compounds was soon compromised, when severe side effects of this therapeutic approach became evident. We now know that glucocorticoids exert their biological effects via the glucocorticoid receptor (GR), a member of the nuclear receptor (NR) super-family that shows a widespread expression throughout the body. In accordance with the ubiquitous expression profile of their receptor, glucocorticoids are not

only involved in the modulation of inflammation, but likewise regulate diverse processes such as glucose metabolism and bone homeostasis. This fact, in turn, explains many side effects observed after glucocorticoid therapy, which range from iatrogenic diabetes mellitus to osteoporosis [1]. These side effects constrain the long-term use of glucocorticoids during chronic inflammatory diseases. However, scientific progress during the past decades has resulted in the identification and characterisation of a large panel of additional NRs, some of which share the anti-inflammatory potential of the GR, but exert differential effects on fat, glucose, cartilage and bone homeostasis. Notably, the exact effects individual NRs exert on inflammation vary considerably between the different receptors, which is linked to their individual properties and the panel of genes regulated. Many of these NRs were initially referred to as “orphan receptors” since their specific endogenous ligands and their functions were often unknown. Meanwhile, the identification of various ligands of such orphan receptors resulted in the emergence of “adopted” orphan receptors, which include NRs such as peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs) and retinoic X receptors (RXRs) that bind fatty acids, oxysterols and retinoids, respectively [2].

Many of these NRs do not only exert potent anti-inflammatory effects, but likewise control homeostatic processes within the musculoskeletal system. However, their effects on bone, cartilage and muscle metabolism often oppose the effects exerted by glucocorticoids. Therefore, they have emerged as highly attractive targets for a therapeutic intervention during a variety of chronic inflammatory diseases. This review addresses the role of the PPAR, LXR and NR4a families of (adopted) orphan NRs during the regulation of inflammation and immunity and additionally highlights their impact on the musculoskeletal system in terms of bone, joint and cartilage metabolism.

## Orphan NRs as regulators of inflammation and immunity

Most NRs act as ligand-dependent transcription factors that share a conserved structure consisting of a carboxy-terminal ligand-binding, a central DNA-binding and as well as

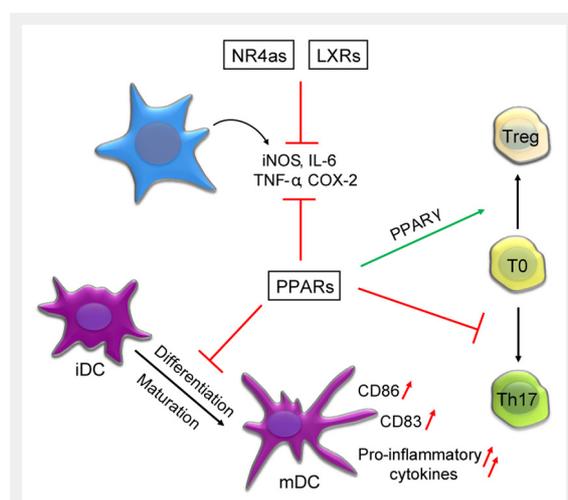
an amino-terminal trans-activation domain. By recruitment of co-activator and co-repressor complexes, NRs can both positively and negatively affect gene expression [3]. Mechanisms underlying the NR-mediated transcriptional regulation are complex and often differ between individual NR subgroups. In general, NRs can form monomers, homodimers or heterodimers, and bind to hormone responsive elements at the promoters of their target genes. Upon binding of their ligand, NRs attach to specific responsive elements and subsequently promote transcription of target genes [4, 5]. Thus NRs induce expression of a large set of genes. As this includes certain anti-inflammatory genes such as heme-oxygenase-1 and can fundamentally change the phenotype of the respective cells, these positive transcriptional effects can indirectly contribute to the reported anti-inflammatory effects of NRs [6, 7]. Some NRs such as the members of the PPAR family are also able to constitutively bind to a distinct subset of promoter elements, where they attract co-repressor complexes and block transcription in the absence of a ligand [3, 8]. Upon ligand binding, these co-repressors are exchanged by co-activators and transcription is initiated. Furthermore, many NRs seem to be capable of actively blocking the transcription of individual genes in a ligand-dependent manner. The underlying molecular events are still incompletely understood and a matter of debate. A current working model suggests that during this process (referred to as “trans-repression”), the NR does not directly bind, but rather tethers to the repressed promoter, where it triggers recruitment or interferes with the dissociation of co-repressors such as NCoR or SMRT [9, 10]. A typical example is the trans-repression of distinct inflammatory genes by PPAR $\gamma$  and LXRs [11–13]. Many

NRs do not only recruit co-activator and co-repressor complexes, but also directly interact with a variety of other proteins such as members of the NF- $\kappa$ B complex, different protein kinases and  $\beta$ -catenin [14–16]. Interactions can be direct or indirect sometimes using so-called co-regulators or even interfering with RNA elongation [17, 18]. Presumably larger complexes are involved. Thereby, NRs positively and negatively affect intracellular signalling and transcription at various levels. The effects distinct NR sub-families exert during inflammation and immunity are complex. Figure 1 summarises key findings on the role of the NR4a, LXR and PPAR families of NR during the innate and adaptive response.

### PPARs

Among the different adopted orphan NRs, members of the PPAR sub-family of NRs were shown to exert various potent anti-inflammatory effects rendering them promising candidates for the therapy of chronic inflammatory diseases. This NR sub-family includes three members (PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR $\gamma$ ) that bind regulatory DNA elements as heterodimers together with the retinoic X receptor. Although all PPAR members act via conserved responsive elements, they differ in their ligand-specificity and are differentially expressed throughout the body. Endogenous ligands include various fatty acids as well as locally produced eicosanoids. Notably, different commonly used drugs exert their effects via PPARs. Prominent examples include the insulin-sensitising class of thiazolidinediones that act via PPAR $\gamma$  and lipid-lowering fibrates that act via PPAR $\alpha$ . PPARs have been initially described as key regulators of fat and glucose metabolism, where the individual members of this NR family promote distinct metabolic programmes such as fatty acid oxidation (PPAR $\alpha$ ) and adipogenesis (PPAR $\gamma$ ), respectively [19]. Furthermore, all three members were shown to exert potent regulatory functions during the innate and adaptive immune response [20].

The immune-modulatory role of PPAR $\gamma$  has been most intensively studied in monocytes and macrophages. Various ligands for PPAR $\gamma$  were shown to block the inflammatory response in these cell types, where PPAR $\gamma$  is the predominant PPAR isoform [21–23]. Although some of the anti-inflammatory effects observed after the use of high doses of PPAR $\gamma$  ligands were subsequently shown to be PPAR $\gamma$ -independent [24], the role of PPAR $\gamma$  as key regulator of macrophage activation has meanwhile been settled [14]. A set of carefully executed experiments using PPAR-deficient cells as controls, revealed that ligand-induced activation of PPAR $\gamma$  does not generally block inflammatory signalling, but represses a specific subset of toll-like receptor (TLR)-induced genes in macrophages [25]. Interestingly, most of the affected pro-inflammatory genes do not contain PPAR responsive elements in their promoters. Instead, PPAR $\gamma$  blocks their expression by trans-repressing NF- $\kappa$ B- and AP1-dependent transcriptional activation. Ligand binding was shown to induce allosteric changes of PPAR $\gamma$  that enable its SUMOylation by SUMO1. Subsequently, SUMOylated PPAR $\gamma$  interacts with the NCoR co-repressor in complex with Histone deacetylase 3. NCoR, in turn, is known to constitutively silence a distinct subset of inflammatory genes in the steady state and usually dissoci-



**Figure 1**

Summary of the key findings on the role of the PPAR, LXR and NR4a family of NRs during inflammation and immune homeostasis. A common feature of these families of NRs are their anti-inflammatory effects that result in the inhibition of the expression of multiple pro-inflammatory cytokines such as inducible NO-Synthase (iNOS), TNF- $\alpha$ , Interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2). PPARs were additionally described to block the maturation of dendritic cells (DCs), which includes an attenuated expression of DC maturation markers such as CD86 and CD83 after activation of this NR sub-family. Also differentiation of T cells into distinct subsets was shown to be influenced by NRs, where PPARs interfere with the differentiation of Th17 cells and simultaneously promote the differentiation of regulatory T cells (Treg).

ates after arrival of an inflammatory signal. SUMOylated PPAR $\gamma$ , however, prevents the release of NCoR from these promoter regions thereby shutting down transcription of the respective genes [11]. This mechanism seems to mediate the ligand-induced repression of a number of inflammatory genes such as inducible NO-Synthase (iNOS) and TNF- $\alpha$  in macrophages [11, 13].

PPAR $\alpha$  and PPAR $\beta/\delta$  are also able to attenuate pro-inflammatory gene expression. However underlying molecular mechanisms are less well investigated than the trans-repressive capacity of PPAR $\gamma$  [26]. PPAR $\alpha$  is highly expressed in vascular smooth muscle and endothelial cells as well as in hepatocytes [27]. Here, this NR suppresses the expression of pro-inflammatory genes such as interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2) by interference with p65 and c-Jun as well as by the transcriptional induction of the NF- $\kappa$ B Inhibitor I $\kappa$ B [28–31]. PPAR $\beta/\delta$  is the most widely expressed PPAR family member. Accordingly, this member of the PPAR family has been implicated in the regulation of inflammatory signalling in diverse cell types such as endothelial cells, smooth muscle cells and keratinocytes, where it negatively regulates the expression of a large panel of chemokines and cytokines [32, 33]. On a molecular level, PPAR $\beta/\delta$  seems to control the activation status of macrophages at least partially via sequestration of the transcriptional repressor BCL-6, which is released after the binding of a ligand to PPAR $\beta/\delta$  [34].

These potent anti-inflammatory effects of the PPAR family of NRs explain the fact that genetic deletion of PPAR $\gamma$  was shown to result in the exacerbation of chronic inflammatory diseases, where conditional deletion of PPAR family members in individual cell types revealed anti-inflammatory roles not only in monocytes and macrophages, but also in parenchymal cell types such as intestinal epithelial cells [35, 36]. Deletion of PPAR $\alpha$  or PPAR $\beta/\delta$  also results in the exacerbation of the inflammatory response in murine models of contact dermatitis [37, 38], airway inflammation [39] or steatohepatitis [40]. Accordingly, treatment with PPAR $\gamma$  as well as with PPAR $\alpha$  ligands attenuated disease severity in such models of inflammation. Amongst others, activation of these NRs were shown to exert protective effects during animal models of colitis [35, 36, 41–43] and allergic airway disease [44, 45].

Likewise, PPARs were shown to critically impact on the progression of atherosclerosis as one of the most frequent chronic inflammatory diseases and leading cause of death in modern societies. Within the vascular wall, PPAR $\alpha$  and PPAR $\beta/\delta$  are mainly expressed in vascular smooth muscle cells and endothelial cells, whereas PPAR $\gamma$  is the predominant PPAR isoform in monocyte-derived macrophages and foam cells within the atherosclerotic plaque. Within cells of the vascular wall, ligand-induced activation of all three PPAR members was shown to exert anti-inflammatory effects thereby reducing the expression of adhesion molecules, chemokines and pro-inflammatory cytokines [28, 46–48]. *In vivo* studies determining the effects of PPAR ligands showed clear anti-atherogenic effect of PPAR $\alpha$  and PPAR $\gamma$  ligands, which reduced the size of atherosclerotic lesions and foam cell formation within the plaque. In contrast, ligands for PPAR $\beta/\delta$  were not effective [49]. Studies using PPAR-deficient mice confirmed a protective role of

PPAR $\gamma$  in murine models of atherosclerosis [49, 50], whereas studies using PPAR $\alpha$  and PPAR $\beta/\delta$ -deficient mice revealed conflicting data on the exact role of these NR during atherogenesis [34, 51, 52]. In addition to the direct anti-inflammatory properties of PPAR agonists, the induction of anti-inflammatory and vascular-protective genes, such as heme oxygenase-1 [6], as well as changes in metabolic parameters [52] seems to contribute to their beneficial effects within the vascular wall.

In addition to their anti-inflammatory actions, PPARs were shown to directly and indirectly influence the adaptive immune response and to contribute to the maintenance of immunological tolerance. Notably, uptake of apoptotic cells by macrophages was shown to exert immune-modulatory effects via activation of PPAR $\gamma$  and PPAR $\beta/\delta$  within the phagocyte ensuring the non-inflammatory clearance of apoptotic cell-derived auto-antigens [53, 54]. On a molecular level, apoptotic cells seem to induce sumoylation of PPAR $\gamma$  and thereby prevent the dissociation of the NCoR co-repressor complex from promoters of inflammatory genes [53]. PPAR  $\beta/\delta$ , in turn, promotes the expression of distinct opsonins such as complement component Iq $\beta$  that facilitate the uptake of the apoptotic cell [54]. Furthermore, PPARs were shown to modulate the differentiation, maturation and function of professional antigen-presenting cells such as dendritic cells (DCs). Activation of all three PPAR subtypes results in an altered DC phenotype with an attenuated expression of co-stimulatory molecules and a reduced expression of pro-inflammatory cytokines as well as an impaired migratory capacity of the DC [55–63]. Accordingly, activation of PPAR $\gamma$  and PPAR $\alpha$  were shown to reduce the antigen-presenting capacity of DCs [59, 62, 64]. Mechanistically, the effects PPARs exert on DCs seem to involve not only trans-repression mechanisms and interactions with other transcription factors such as STAT6 [58], but also a PPAR-induced shift in the metabolic programming of these cells [7, 65–67]. Apart from their regulatory role in antigen-presenting cells, PPARs also directly influence cells of the adaptive immune system. An example is the PPAR $\gamma$ - and PPAR $\alpha$ -mediated blockade of the activation of T and B cells, where these NRs also exert pro-apoptotic effects in certain lymphocyte subsets [68–76]. PPARs also orchestrate the differentiation of naïve T cells into distinct T effector cell subsets such as Th1, Th17 or regulatory T cells. An example is the block of Th17 differentiation by PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR $\gamma$  [77–79]. Th17 cells and regulatory T cells represent distinct T cell types that fulfil seemingly opposite tasks. Whereas Th17 cells have been both implicated in the defence against extracellular pathogens and the pathogenesis of distinct autoimmune diseases such as rheumatoid arthritis and multiple sclerosis [80], regulatory T cells provide protection against such autoimmune disorders [81]. Accordingly, T-cell-specific deletion of PPAR $\gamma$  results in an increased differentiation of Th17 cells as well as in an exacerbation of models for Th17-mediated autoimmune disease including experimental autoimmune encephalomyelitis [79], a murine model for multiple sclerosis. Mice that received PPAR $\gamma$  or PPAR $\beta/\delta$  ligands, in turn, showed an attenuated disease course [78, 79]. In addition to its negative effects on Th1 and Th17 differentiation, PPAR $\gamma$  seems to promote the ac-

cumulation of immune-modulatory regulatory T cells [82, 83].

Its multiple anti-inflammatory and immune-modulatory effects explain the reported beneficial effects that PPARs exert during chronic inflammatory diseases of the musculo-skeletal system. In animal models of autoimmune arthritis, genetic deletion of PPAR $\gamma$  causes an exacerbation, whereas PPAR $\gamma$  ligand-treatment ameliorates disease severity [68, 84–86]. Furthermore, ligand-induced activation of PPAR $\gamma$  and PPAR $\alpha$  was shown to beneficially influence inflammatory disease activity in patients suffering from rheumatoid arthritis [87–89] and psoriatic arthritis [90], respectively. Treatment with PPAR $\gamma$  and PPAR $\alpha$  agonists, likewise, protects from degenerative joint disease in animal models of osteoarthritis, where activation of these NRs blocks expression of inflammatory cytokines and matrix metalloproteinases and thereby ameliorates proteoglycan degradation [91–95]. Cartilage-specific deletion of PPAR $\gamma$ , in turn, results in a spontaneous osteoarthritis phenotype [96, 97].

### LXRs

In analogy to PPARs, the Liver X receptors (LXR $\alpha$  and LXR $\beta$ ) are a second group of adopted orphan NRs that exert potent anti-inflammatory effects [98]. Like PPARs, they form heterodimers with RXR, whereas their natural ligands have been identified as oxysterols. While LXRs have been initially implicated in the regulation of cholesterol metabolism, these NRs have meanwhile emerged as crucial regulators of both innate and adaptive immunity. In response to the binding of a ligand, LXRs also become SUMOylated and prevent the dissociation of co-repressors from the promoter regions of inflammatory genes that partially overlap with genes that are suppressed by activation of the GR or PPAR $\gamma$  [13, 25, 98]. Thereby LXRs attenuate the expression of a distinct set of TLR-induced genes such as iNOS, COX-2 or IL-6 in macrophages [99]. Interestingly, there seems to be a reciprocal inhibition between LXR- and TLR-induced signalling, as pathogen-induced TLR activation blocks the LXR-mediated efflux of cholesterol in macrophages [100]. LXR $\alpha\beta^{-/-}$  mice show an impaired macrophage response to pathogens as well as an increased susceptibility to infection indicating that expression of LXRs is crucial for a proper function of macrophages [101, 102]. The uptake of apoptotic cells also results in the activation of LXRs ingesting macrophages, where these NRs contribute to the non-inflammatory removal of apoptotic cell-derived antigens and the maintenance of self-tolerance [103]. Furthermore, LXRs were shown to control the differentiation of distinct macrophage subsets such as marginal zone macrophages in the spleen and to contribute to neutrophil homeostasis in the steady state [104, 105]. Recent evidence also points towards a key role of LXR-mediated regulation of cholesterol homeostasis during the regulation of T cell proliferation implying a direct function of this NR during the adaptive immune response [106]. In accordance with an anti-inflammatory role of LXRs, synthetic LXR ligands were reported to exert anti-inflammatory effects *in vivo* and ameliorate the disease course in murine models of atherosclerosis and contact dermatitis [107–109]. The consequences of LXR activation during allergic airway dis-

ease and autoimmune arthritis are controversial, and both beneficial and detrimental effects of an LXR agonist treatment in murine asthma models and during collagen-induced arthritis have been described [110–114]. Reduced LXR signalling seems to contribute to catabolic metabolism in osteoarthritic cartilage [115], whereas LXR ligands block matrix degradation and alleviate pain during animal models of osteoarthritis [116].

### NR4a1–3

Another group of NRs that were shown to crucially modulate the innate and adaptive immune response is the NR4a family of NRs [117, 118]. The three members (NR4a1–3 or alternatively termed as Nur77, Nurr1 and NOR-1) are differentially expressed throughout the body. Notably, they lack a classical ligand-binding domain and endogenous ligands for this NR subgroup have not been described so far. These findings render them “true orphans”, although recently a compound that was isolated from an endophytic fungus was identified to be able to act as an agonist for Nur77 [119]. Stimulation of cells with various pro-inflammatory, activation signals such as LPS or oxidised LDL results in the rapid transcriptional induction of NR4a expression within 1 hour [117]. By recruiting the CoREST co-repressor complex and blocking p65-mediated transcriptional activation of NF- $\kappa$ B target genes, Nurr1 was shown to prevent an overwhelming inflammatory response by microglia and astrocytes thereby providing protection from an inflammation-associated loss of dopaminergic neurons in the CNS [120]. Its family member, Nur77, was shown to interfere with the LPS-induced activation of macrophages by blocking the phosphorylation of the NF- $\kappa$ B subunit p65 [121]. In mouse models of atherosclerosis, deletion of Nur77 resulted in an increased expression of inflammatory genes in macrophages and an accelerated development of atherosclerotic plaques [121, 122]. Recent data from our lab suggests that the expression of Nur77 is rapidly induced in macrophages that recognise apoptotic cells. Subsequently this NR interferes with NF- $\kappa$ B-signalling and dampens pro-inflammatory signalling pathways within the phagocyte ensuring the non-inflammatory clearance of dying cells [123]. These findings suggest that the NR4a subgroup of NRs is not only involved in regulatory feedback loops, but also integrates pro- and anti-inflammatory signalling pathways to fine-tune the immune response. Furthermore, Nur77 was shown to act as an essential factor for the differentiation of Ly6C<sup>low</sup> resident monocytes, a distinct subset of blood monocytes that, in contrast to Ly6C<sup>high</sup> monocytes, migrate into the tissue during the steady state and seem to fulfil homeostatic functions. Absence of Nur77 resulted in significantly reduced numbers of these “patrolling” monocytes in the bone marrow and blood of NR4a1<sup>-/-</sup> mice [124]. Notably, the three NR4a family members also control the development of thymic regulatory T cells and mice lacking all three NR4a members do not only lack this T cell subset, but also suffer from a fatal systemic autoimmune disorder [125]. As mice that are deficient for only one of the three NR4a members do not exhibit an obvious T cell phenotype, these findings point towards a certain redundancy in the function of members this NR sub-family. Little is known about a role of

this NR sub-family during inflammatory joint disease. However, constitutive expression of Nur77 in T cells results in the amelioration of collagen-induced arthritis [126] and its family member Nurr1 was shown to repress the expression of matrix metalloproteinases in osteoarthritic cartilage [127].

## Orphan nuclear receptors as regulators of skeletal homeostasis

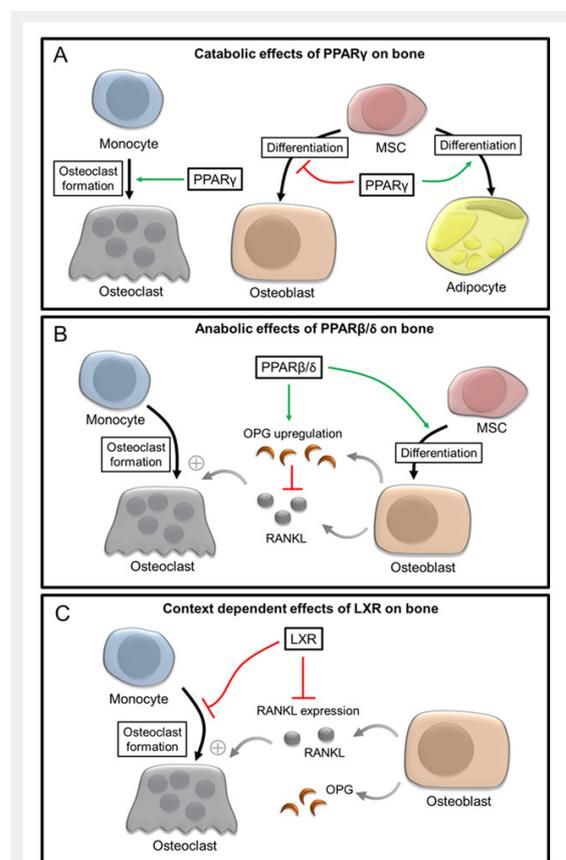
Given the potential of NRs to serve as targets for the treatment of chronic inflammatory musculoskeletal diseases such as rheumatoid arthritis and osteoarthritis, it is important to consider their impact on skeletal homeostasis, where many of them control multiple aspects of bone biology including the differentiation and function of bone-forming osteoblasts and of bone-resorbing osteoclasts. Furthermore, NRs are involved in the coordination of systemic calcium and phosphate metabolism as well as in the differentiation of mesenchymal stem cells (MSCs). The role of classic-

al steroid receptors such as oestrogen receptors, the glucocorticoid receptor as well as the vitamin D receptor during bone homeostasis is well studied and reviewed in detail elsewhere [128]. The beneficial effects of vitamin D on bone are exploited during the treatment of skeletal diseases such as osteoporosis, where the administration of vitamin D is a first line therapy [129]. The catabolic effects glucocorticoids exert on bone homeostasis, on the other hand, represent a major problem during the glucocorticoid-based treatment of chronic inflammatory diseases [130]. Recent data widened our understanding of the pivotal role adopted orphan receptors such as PPARs and LXRs play in bone biology. While the anti-inflammatory and immune modulatory effects of PPARs, LXRs and members of the NR4a family often overlap, these NRs differentially affect bone turnover. The various reported effects of PPARs and LXRs on bone homeostasis are summarised in figure 2.

### PPARs

First clues for a key role of PPAR $\gamma$  during skeletal homeostasis came from the observation that patients suffering from type 2 diabetes, who received PPAR $\gamma$ -activating thiazolidinediones as insulin sensitisers, showed an increased risk of osteoporosis and osteoporosis-related fractures [131, 132]. This severe side effect of a therapy with these PPAR $\gamma$ -activating compounds is attributed to the fact that PPAR $\gamma$  serves as master regulator of adipogenesis and promotes the differentiation of mesenchymal stem cells (MSCs) into adipocytes resulting in a simultaneous PPAR $\gamma$ -mediated block of the differentiation of MSCs into chondrocytes, muscle cells and osteoblasts [133, 134] thereby explaining the detrimental effects this NR exerts on bone formation. Support for such a scenario comes from studies on mice carrying a haplo-insufficiency of PPAR $\gamma$  and that show an increased bone mass. Moreover, PPAR $\gamma^{+/-}$  MSCs have an enhanced potential to differentiate into osteoblasts [135]. Treatment of mice with PPAR $\gamma$  agonists, on the other hand, resulted in a decreased bone mass, which was paralleled by an increased number of adipocytes in the bone marrow [136, 137]. On a molecular level, this PPAR $\gamma$ -mediated block of osteoblast differentiation seems to be based on a reciprocal inhibition between PPAR $\gamma$  and the Wnt-signalling pathway in MSCs and osteoblast precursors. Wnt signalling, in turn, acts as a key driver of osteoblast differentiation and bone formation [15, 138–141]. Whereas agonists of the Wnt pathway induce the expression of key transcription factors of osteoblastogenesis such as Runx2 and osterix, their expression is blocked by PPAR $\gamma$  [136]. Recent data suggest an additional role of PPAR $\gamma$  during the differentiation of bone-resorbing osteoclasts, which might contribute to the catabolic effects this NR exerts on bone homeostasis. Conditional deletion of PPAR $\gamma$  in osteoclast precursors resulted in increased bone mass and a reduced number of osteoclasts, whereas the PPAR $\gamma$  agonist rosiglitazone fostered the differentiation of osteoclasts, indicating an important pro-osteoclastogenic role of this NR [142].

In contrast to PPAR $\gamma$ , its family member PPAR $\beta/\delta$  exerts potent anabolic effects on bone turnover and homeostasis. Ligand-induced activation of this NR fosters the development of mature osteoblasts [16, 143, 144]. Mechanistic-



**Figure 2**

Role of PPAR $\gamma$ , PPAR $\beta/\delta$  and LXRs during the control of osteoblast and osteoclast differentiation. (A) Catabolic effects of PPAR $\gamma$  on bone homeostasis are attributed to the PPAR $\gamma$ -mediated promotion of osteoclast differentiation and its inhibitory role during the differentiation of mesenchymal stem cells (MSC) into osteoblasts. (B) PPAR $\beta/\delta$  exert its anabolic effects on bone homeostasis via promotion of the osteoblast differentiation and regulation of the RANKL/OPG ratio thereby indirectly influencing osteoclastogenesis. (C) During states of increased osteoclastogenesis and high bone turnover such as inflammation and osteoporosis, activation of LXRs is able to both directly and indirectly interfere with osteoclast differentiation via blockade of osteoclastogenesis and influencing the RANKL/OPG ratio.

ally, activation of PPAR $\beta/\delta$  promotes the transcription of the Wnt co-receptor LRP5 and additionally interacts with  $\beta$ -catenin thereby amplifying Wnt signalling activity in osteoblast precursors [16]. Likewise, PPAR $\beta/\delta$  regulates the ratio between the pro-osteoclastogenic cytokine RANKL and its natural decoy receptor OPG. Both proteins are produced by osteoblasts and osteocytes and ligand-induced activation of PPAR $\beta/\delta$  results in a decreased RANKL/OPG ratio and an attenuation of osteoblast-mediated osteoclastogenesis [16]. Accordingly, PPAR $\beta/\delta$ -deficient mice exhibit a decreased bone mass, an increased RANKL/OPG ratio and a consecutive increase in the differentiation of osteoclasts. Pharmacological activation of PPAR $\beta/\delta$ , in turn, results in the protection from ovariectomy-induced bone loss highlighting the potential of PPAR $\beta/\delta$  as a novel target for the treatment of osteoporosis and related diseases in humans [16]. Importantly, the effects PPAR $\beta/\delta$  exerted on osteoclastogenesis were indirect and related to the regulation of the RANKL/OPG ratio provided by osteoblasts. Neither deletion of PPAR $\beta/\delta$ , nor treatment with specific concentrations of PPAR $\beta/\delta$  ligands altered the intrinsic potential of osteoclast precursors to form mature osteoclasts, although previous studies using high (and probably non-specific) concentrations of different PPAR agonists observed such inhibitory effects of various PPAR ligands on osteoclastogenesis [145]. Notably, treatment of ovariectomised rats with a PPAR $\beta/\delta$ -specific ligand resulted in a decreased bone density [146]. This, however, might be linked to the previously reported opposing consequences of a PPAR $\beta/\delta$ -activation on the metabolism of mice and humans on the one hand and of rats on the other hand [147].

*In vitro* data shows that the ligand-induced activation of PPAR $\alpha$  mice also stimulates osteoblast differentiation [143]. Likewise, treatment of mice with the PPAR $\alpha$  agonist bezafibrate resulted in increased periosteal bone formation [143]. However, PPAR $\alpha$ -deficient mice do not exhibit any alterations in their bone structure [148] and patients receiving lipid-lowering fibrates did not show a reduced risk of bone fractures [149].

### LXRs

The role of LXRs during bone metabolism is less clear. Activation of LXRs was shown to directly interfere with osteoclastogenesis and to additionally attenuate the RANKL/OPG ratio provided by osteoblasts [150, 151]. However, LXR-deficient mice show only mild alteration in their bone structure suggesting a rather minor role of LXRs during the regulation of physiological bone turnover [152]. Likewise, long-term treatment with LXR agonists did not significantly alter the structure and density of bones of non-challenged mice [153]. In contrast, treatment with LXR ligands efficiently blocked the increased osteoclastogenesis after ovariectomy or during inflammation, thereby protecting mice both from ovariectomy-induced osteoporosis and local inflammatory bone loss [151, 154]. These context-dependent effects LXR ligands exert on bone turnover render them highly attractive tools for future therapeutic approaches in the treatment of related human diseases.

### NR4a1–3

Members of the NR4a family are also expressed in osteoblasts [155, 156], where they regulate genes involved in bone formation such as osteopontin, osteocalcin, alkaline phosphatase and collagen type I alpha I [155–157]. Interestingly, expression of NR4a receptors is rapidly induced following stimulation of osteoblasts or MSCs with parathyroid hormone. However the exact function of the NR4a sub-family of NRs during bone metabolism remains to be elucidated.

## Implications and future therapeutic strategies

The identification of the PPAR, LXR and NR4a families of NRs as potent modulators of inflammation and immunity has rendered them attractive targets for the treatment of inflammatory diseases affecting the musculoskeletal system such as rheumatoid arthritis and osteoarthritis. Their role as master regulators of fat and glucose homeostasis as well as during skeletal development might be a simultaneous advantage and an obstacle for their clinical use. On the one hand, these pleiotropic functions increase the risk of side effects like osteoporosis as observed during a therapy with PPAR $\gamma$ -activating thiazolidinediones or glucocorticoids. On the other hand, agonists for PPAR $\alpha$  or PPAR $\beta/\delta$  might evolve as anti-inflammatory compounds that allow the simultaneous treatment of alterations of fat, glucose and bone metabolism such as insulin resistance and osteoporosis as disorders that accompanied chronic inflammatory diseases. Another strategy would be the combination of different NR agonists that display a partial agonistic and antagonistic action profile such as ligands for PPAR $\gamma$  and PPAR $\beta/\delta$  in order to increase the anti-inflammatory potential of this treatment and minimise side effects (e.g. on bone). However, the prerequisite for a successful treatment strategy that targets NRs is a profound knowledge of their pleiotropic function and here we are just beginning to understand the complexity of the NR network that silently orchestrates our daily life in health and disease.

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Figures (large format)

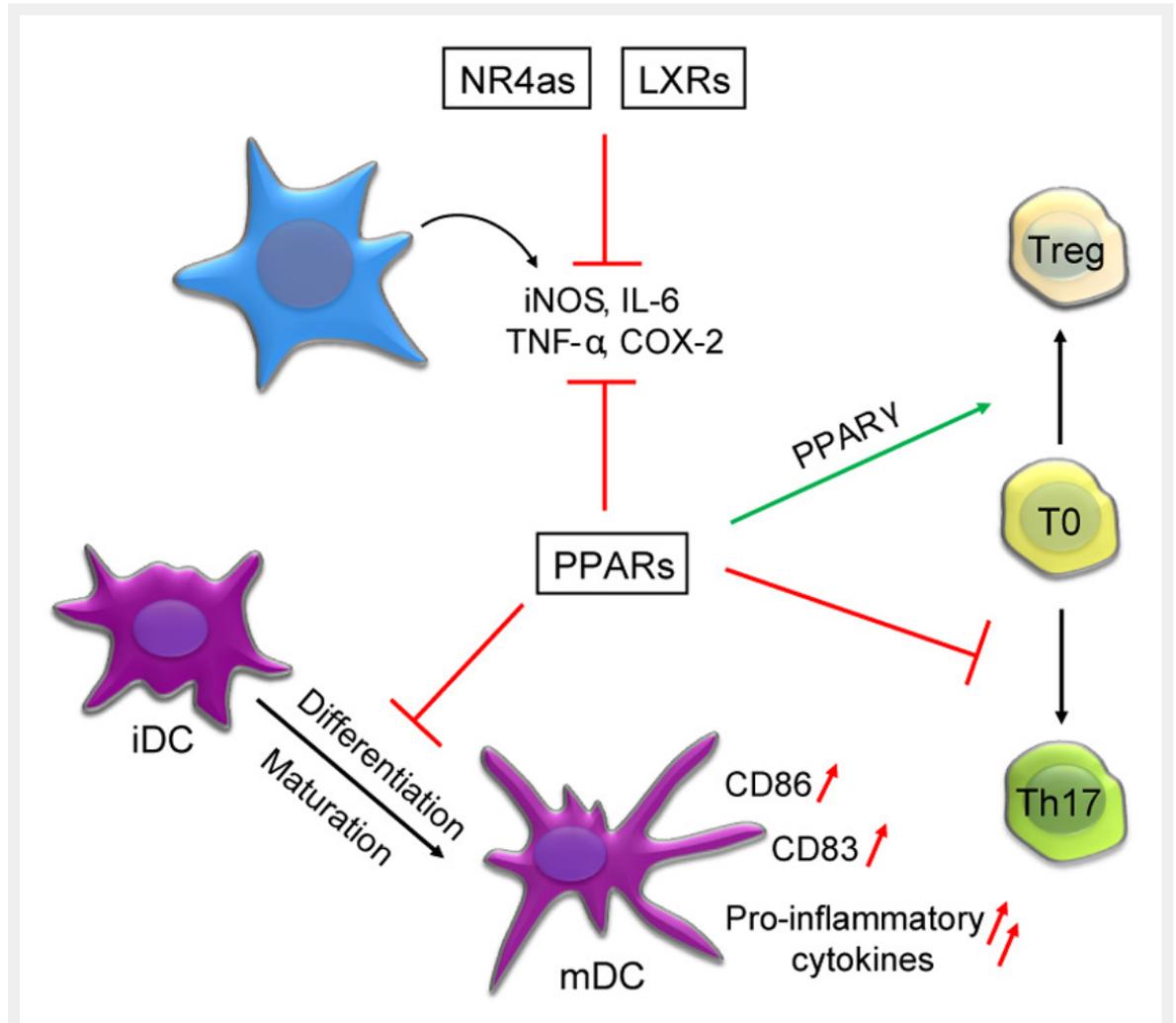
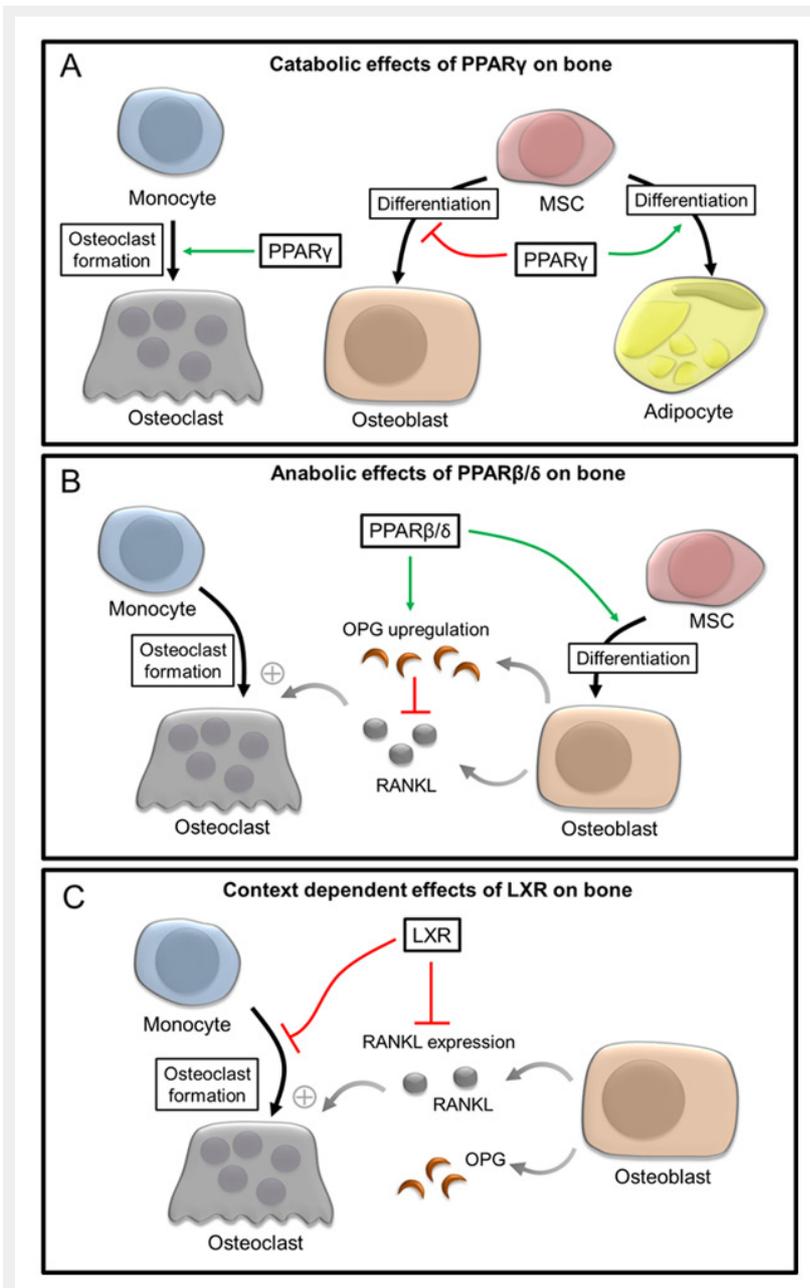


Figure 1

Summary of the key findings on the role of the PPAR, LXR and NR4a family of NRs during inflammation and immune homeostasis. A common feature of these families of NRs are their anti-inflammatory effects that result in the inhibition of the expression of multiple pro-inflammatory cytokines such as inducible NO-Synthase (iNOS), TNF- $\alpha$ , Interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2). PPARs were additionally described to block the maturation of dendritic cells (DCs), which includes an attenuated expression of DC maturation markers such as CD 86 and CD83 after activation of this NR sub-family. Also differentiation of T cells into distinct subsets was shown to be influenced by NRs, where PPARs interfere with the differentiation of Th17 cells and simultaneously promote the differentiation of regulatory T cells (Treg).



**Figure 2**

Role of PPAR $\gamma$ , PPAR $\beta/\delta$  and LXRs during the control of osteoblast and osteoclast differentiation. (A) Catabolic effects of PPAR $\gamma$  on bone homeostasis are attributed to the PPAR $\gamma$ -mediated promotion of osteoclast differentiation and its inhibitory role during the differentiation of mesenchymal stem cells (MSC) into osteoblasts. (B) PPAR $\beta/\delta$  exert its anabolic effects on bone homeostasis via promotion of the osteoblast differentiation and regulation of the RANKL/OPG ratio thereby indirectly influencing osteoclastogenesis. (C) During states of increased osteoclastogenesis and high bone turnover such as inflammation and osteoporosis, activation of LXRs is able to both directly and indirectly interfere with osteoclast differentiation via blockade of osteoclastogenesis and influencing the RANKL/OPG ratio.