Published in final edited form as:

Curr Opin Toxicol. 2018 April ; 8: 66–80. doi:10.1016/j.cotox.2018.01.005.

Retinoic acid-related Orphan Receptor γ (ROR γ): connecting sterol metabolism to regulation of the immune system and autoimmune disease

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Abstract

Cholesterol and its metabolites are bioactive lipids that interact with and regulate the activity of various proteins and signaling pathways that are implicated in the control of a variety of physiological and pathological processes. Recent studies revealed that retinoic acid-related orphan receptors, ROR α and γ , members of the ligand-dependent nuclear receptor superfamily, exhibit quite a wide binding specificity for a number of sterols. Several cholesterol intermediates and metabolites function as natural ligands of ROR α and ROR γ and act as agonists or inverse agonists. Changes in cholesterol homeostasis that alter the level or type of sterol metabolites in cells, can either enhance or inhibit ROR transcriptional activity that subsequently result in changes in the physiological processes regulated by RORs, including various immune responses and metabolic pathways. Consequently, this might negatively or positively impact pathologies, in which RORs are implicated, such as autoimmune disease, inflammation, metabolic syndrome, cancer, and several neurological disorders. Best studied are the links between cholesterol metabolism, ROR γ t activity, and their regulation of Th17 differentiation and autoimmune disease. The discovery that Th17-dependent inflammation is significantly attenuated in ROR γ -deficient mice in several experimental autoimmune disease models, initiated a search for ROR modulators that led to the identification of a number of small molecular weight ROR γ inverse agonists. The inverse agonists suppress Th17 differentiation and IL-17 production and protect against autoimmunity. Together, these studies suggest that ROR γ t may provide an attractive therapeutic target in the management of several (inflammatory) diseases.

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Conflict of interest statement

Nothing declared

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Keywords

retinoic acid-related orphan receptor; ROR γ ; oxysterols; metabolism; (inverse) agonists; cholesterol biosynthesis; vitamin D3; transcription; Th17 cells; autoimmune disease; inflammation

1. Introduction

The retinoic acid-related orphan receptors alpha, beta, and gamma (ROR α - γ encoded by *RORA-C* or *NR1F1-3*) constitute a subfamily of nuclear receptors [1–4]. RORs exhibit a typical nuclear receptor domain structure consisting of an N-terminal domain, a highly-conserved DNA-binding domain (DBD) consisting of two C2-C2 zinc finger motifs, a ligand-binding domain (LBD), and a hinge domain spacing the DBD and LBD [1]. The DBD of RORs recognizes ROR response elements (ROREs) consisting of the RGGTCA consensus preceded by an A/T-rich sequence. RORs regulate transcription by binding as monomers to ROREs in the regulatory regions of target genes and the recruitment of co-activators or co-repressors [5,6].

Over the last ten years, it has become evident that RORs function as ligand-dependent transcription factors. ROR α / γ transcriptional activity can be modulated by various sterols and synthetic ligands that bind ROR and function either as agonists or inverse agonists [2,4,7–17]. The discovery that ROR transcriptional activity can be modulated by small (synthetic) molecules, opened the possibility that RORs may provide novel therapeutic targets in the management of various pathologies, in which RORs are implicated, such as autoimmune disease and type 2 diabetes. In addition, RORs may provide a target for various xenobiotics and thereby a mechanism by which environmental agents affect immunity and disease. This chapter provides an overview of the various links between cholesterol/sterol metabolism, RORs, and their regulation of immunity, particularly T helper 17 (Th17) cells, and their relationship to inflammatory disease.

2. ROR isoforms have different functions

The activity and function of RORs are controlled at many levels, including the generation of different isoforms. Via alternative splicing and/or alternative promoter usage the human *RORA* gene generates 4 isoforms (α 1–4) and human *RORC* two isoforms, ROR γ 1 and ROR γ t (ROR γ 2), while human *RORB* encodes one protein [1,18–21]. These ROR isoforms, which differ only in their N-terminus, exhibit different patterns of expression and regulate distinct genes and physiological processes. A clear example of this is ROR γ . ROR γ 1 is expressed in many peripheral tissues, including adipose, liver, kidney and muscle, where it regulates circadian rhythm, glucose and lipid metabolism [1,4,22–25]. In contrast, ROR γ t, to is selectively expressed in a variety of immune cells, including CD4⁺CD8⁺ (DP) thymocytes, T helper Th17 cells, type 3 innate lymphoid cells (ILC3), and lymphoid tissue inducer (LTi) cells, which constitute an ILC3 subtype [1,4,19,20,26–30]. ROR γ t regulates the survival and apoptosis of DP thymocytes and is essential for the development of ILC3 and Th17 cells, and the production of IL-17. ROR γ t is also required for the generation of LTi cells, which are essential for the development of peripheral lymph nodes and Peyer's patches. ROR γ -deficient mice lack Lti cells and peripheral lymph nodes [19,20]. ROR γ t is

also expressed in several other immune cells, including subtypes of CD8⁺Tc17, invariant natural killer and $\gamma\delta$ T-cells, and Foxp3⁺ T regulatory cells (T_{reg}) [31–36]. Foxp3⁺ROR γ ⁺ T_{regs} are preferentially expressed in colon and, as Th17 cells, their induction is regulated by the gut microbiota. However, in contrast to Th17 and ILC3 cells, ROR γ t appears not to be a significant regulator of IL-17 expression in Foxp3⁺ROR γ ⁺ T_{regs} indicating that the control of target gene transcription by ROR γ is cell context-dependent. Th17, ILC3, and Foxp3⁺ROR γ ⁺ T_{regs} all contribute to intestinal homeostasis and tolerance. Its expression in various immune cell populations with diverse functions indicates that ROR γ t has multiple roles in regulating immunologic homeostasis and inflammatory disease, including autoimmune disease. The complexity is further complicated by the plasticity of the various immune cell populations.

3. Regulation of ROR activity

The activity and function of RORs can be modulated by various posttranslational modifications (PTMs) [37]. Although the RORs are phosphorylated at multiple sites and can be acetylated, sumoylated, and ubiquitinated, the function of many of these PTMs has not yet been fully elucidated. Phosphorylation by protein kinase A (PKA) and sumoylation of ROR α has been reported to positively regulate its transcriptional activity [38,39]. Sumoylation of ROR α at Lys²⁴⁰ was enhanced by protein inhibitor of activated STAT (PIAS) proteins and shown to stimulate ROR α transcriptional activity. The histone acetylase, p300, acetylates ROR γ at several positions, while the deacetylase SIRT1 promotes deacetylation of ROR γ and increases its transcriptional activity, which consequently enhances the generation of Th17 cells and promotes autoimmunity [40]. The HECT E3 ubiquitin ligase, ITCH, was found to bind ROR γ and mediate its K48 poly-ubiquitination and subsequently its proteasomal degradation [41]. A recent study showed that a two-amino acid substitution in the hinge domain of ROR γ (Ser⁹²Ala-Leu⁹³Ala) decreased ubiquitination at Lys⁶⁹ within the ROR γ DBD; however, this ubiquitination did not affect ROR γ t protein stability [42]. Mice containing this mutation exhibited defective Th17 differentiation, while thymopoiesis and lymph node genesis were not affected, except for the development of Peyer's patches. ILC3 cells development was not disrupted in these mutant mice, but cells did not produce IL-17. The mechanism of this selectivity is not fully understood and might be due to differences in promoter context of target genes or differential expression and/or recruitment of transcriptional mediators in different ROR γ expressing cells.

4. Links between sterols and ROR signaling

Agonists and inverse agonists provide another mechanism to regulate ROR activity. A clear connection has been established between cholesterol intermediates/metabolites and their regulation of ROR activity. The first evidence for a link between RORs and sterols came from crystallography studies showing that cholesterol and cholesterol sulfate bind to the ligand-binding pocket of ROR α and function as ROR α agonists [7,43]. Subsequent studies demonstrated that several intermediates of the cholesterol biosynthesis pathway bind ROR γ and enhance ROR γ activity suggesting that they may function as endogenous ligands for ROR γ [8,11] (Figure 1). These studies identified zymosterone, zymosterol, 7-

dehydrocholesterol, and desmosterol among the most effective sterols activating ROR γ , while lanosterol, T-MAS, and cholesterol, were weak agonists. Cholesterol and other sterols can be metabolized by a number of cytochrome P450 enzymes, including CYP27A1, CYP46A1, CYP7A1 and CYP7B1, that lead to formation of a wide range of oxysterols [44–46]. CYP7A1 catalyzes the formation of 7-keto- and 7 α -hydroxysterols, while CYP27A1 catalyzes the formation of 25- and 27-hydroxysterols, and CYP46A1 the formation of hydroxysterols, such as 24(S)-hydroxycholesterol. The formation of 25-hydroxycholesterol can also be catalyzed by cholesterol 25-hydroxylase (Ch25h). A number of these oxysterols can act as ligands for ROR α and ROR γ [8–13,47–49]. These reports demonstrated that 7 α - and 7 β -hydroxycholesterol, 7-ketocholesterol, and 24S- and 24R-hydroxycholesterol, and 24,25-epoxycholesterol function as inverse agonists for ROR α and/or ROR γ , while 25-hydroxycholesterol, 27-hydroxycholesterol, 20 α -hydroxycholesterol, 22R-hydroxycholesterol, 7 α ,27- and 7 β ,27-dihydroxycholesterol act as agonists of ROR γ [2,10,45,50]. In addition to being hydroxylated, sterols can be sulfoconjugated by sulfotransferase SULT2B1 and generate sulfated sterols, such as cholesterol- and desmosterol sulfate, which function as ROR α / γ agonists [7,8].

Triterpenes, which are produced by many plants, fruits, herbs, and fungi, are structurally closely related to sterols (Figure 2). Several triterpenes have been reported to bind ROR γ and function as natural ROR γ ligands. Among them, ursolic acid, which functions as an ROR γ inverse agonist [51] and ganoderone A, an antiviral triterpenoid produced by the fungus *Ganoderma pfeifferi*, which was recently identified as a very potent ROR γ agonist [16](Figure 2).

As was demonstrated for the binding of cholesterol sulfate to the ROR α (LBD) [7], X-ray crystal structure analysis confirmed the interaction of cholesterol sulfate and hydroxysterols with the ligand binding pocket of ROR γ [9,16,52,53]. Studies examining the crystal structures of apo (unbound) ROR γ (LBD) showed that, in the absence of ligand, ROR γ is in an active conformation that is capable of recruiting co-activators [9,16,52]. Study of X-ray structures of agonist-bound ROR γ (LBD) further revealed that binding of agonists stabilizes the active conformation of ROR γ and its interaction with coactivators, whereas most inverse agonists do the opposite. The hydrogen bond between H ϵ 2-His⁴⁷⁹ in helix 11 and OH-Tyr⁵⁰² in helix 12 (referred to as His-Tyr lock) plays a critical role in stabilizing the active conformation of ROR γ t. These studies further indicated that this stabilization involves the formation of different bonds with ROR γ [9,16,52]. For example, 25-hydroxycholesterol stabilizes H12 by interacting with Tyr⁵⁰² indirectly via a water-mediated hydrogen bond with its 25-OH group leaving the His-Tyr lock in place, whereas ganoderone A holds helix 12 (H12) in the agonist position through the formation of two strong hydrogen bonds with His⁴⁷⁹ and Tyr⁵⁰², thereby eliminating the His-Tyr lock. It is conceivable that such differences in agonist-ROR γ interaction might bring about different changes in ROR γ conformation that affect the specificity by which ROR γ recruits co-activator complexes and activates target gene transcription. Consequently, this might affect the regulation of ROR γ functions as well.

5. Vitamin D and lumisterol metabolites and RORs

Recently, several vitamin D3 and lumisterol metabolites were identified that function as weak ROR α/γ inverse agonists [15,53–55]. Previtamin D3, and subsequently vitamin D3, are formed in the skin from 7-dehydrocholesterol upon photochemical transformation by UVB. In the classical pathway, CYP2R1 and CYP27B1 convert vitamin D3 to 1,25(OH)D3, the active form that binds the vitamin D receptor (VDR), but also acts as a weak inverse agonist of ROR γ [11,15,53](Figure 3). In an alternative pathway in the skin, CYP11A1 catalyzes the formation of additional D3 metabolites, including 20(OH)D3, 22(OH)D3, 20,23(OH)D3, 20,22(OH)D3 and 17,20,23(OH)D3 [54,56]. Some of these metabolites are further hydroxylated by CYP27B1 at C1 α , by CYP24A1 and CYP27A1 at C24, C25, and C26 [54,56,57]. Several of the D3 metabolites, including 20(OH)D3, 20,23(OH)₂D3, 1,20,25(OH)₃D3, 17,20,23(OH)₃D3, 1,20(OH)₂D3, and 20,26(OH)₂D3 were able to inhibit RORE-mediated transactivation as well as the interaction between the ROR α/γ LBD with an LXXLL coactivator peptide [11,15,53]. Molecular modeling using established crystal structures of ROR α and ROR γ LBDs, further showed that these inverse agonists exhibit high docking scores supporting interaction of these vitamin D3 metabolites with the ligand binding pocket of ROR α/γ [15,53]. These findings suggest these noncalcemic D3 metabolites, which are produced endogenously, can act as ROR α/γ inverse agonists and modulate their activity and functions. These studies indicate that, in addition to VDR, RORs may provide an alternative pathway by which D3 and its metabolites regulate physiological processes. Previtamin D3 can also undergo photoisomerization to lumisterol (L3) under the influence of UVB [56], which then can undergo sequential hydroxylations by CYP11A1 producing 20(OH)L3, 22(OH)L3, and 20,22(OH)₂L3 [55](Figure 3). Cell-based and *in vitro* analyses demonstrated that several hydroxylumisterols exhibit inverse agonist activity, while molecular modeling supported the hypothesis that they act as ligands of ROR α and ROR γ [55].

6. Disruption of cholesterol biosynthesis and ROR α/γ activity

The discovery that various sterols function as ligands, predicted that alterations in sterol homeostasis that change the intracellular pool of ROR (inverse) agonists, would affect ROR transcriptional activity and as a consequence the physiological as well as pathological processes in which RORs are implicated. This hypothesis was strongly supported by studies examining the effect of disruption of cholesterol biosynthesis on ROR γ t-dependent regulation of Th17 differentiation and gene expression.

ROR γ t is required for the differentiation of naïve T cells into Th17 cells and for the transcriptional activation of *III17A/F*, *IL21*, and *IL23R* (Figure 4A). Interestingly, during Th17 differentiation, the expression of several cholesterol biosynthetic genes (e.g., *FDFT1*, *LSS*, and *DHCR24*) are enhanced, whereas that of genes involved in cholesterol metabolism (e.g., *CYP7A1*, *CYP27A1*) and efflux (e.g., *ABCA1*, *ABCG1*) are decreased [8]. In addition, Th17 differentiation is accompanied with an increase in the expression of the sulfotransferase *SULT2B1* and a decrease in sterol sulfatase (*STS*) expression [8]. A positive correlation between IL-17 levels and the expression of several cholesterol biosynthesis-related genes was also revealed by transcriptome analysis of RNA isolated from normal and

psoriatic skin biopsies [58]. Together, these findings are consistent with the concept that increased cholesterol biosynthetic gene expression would enhance the availability of ROR γ t agonists and subsequently stimulate ROR γ t activity, Th17 differentiation, and IL-17 production, and consequently promote inflammatory disease.

Several studies have demonstrated that disruption of the cholesterol biosynthesis pathway has a negative effect on ROR γ t activation and Th17 differentiation (Figure 4A and B). The cholesterol-lowering drugs, statins, bind to and inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGCR), which catalyzes the conversion of HMG-CoA to mevalonic acid, one of the first steps in cholesterol biosynthesis. Statins have been shown to inhibit Th17 differentiation and the expression of inflammatory cytokines [59]. This appears to involve several mechanisms including up-regulation of suppressor of cytokine secretion (SOCS3 and -7) and reduced activation of STAT3 and ROR γ t expression. In addition, the reduction in cholesterol biosynthesis by statins might diminish the generation of ROR γ t agonists, thereby reducing ROR γ t activation, ROR γ t-mediated Th17 differentiation, and activation of *Il17* (Figure 4A). The formation of squalene by squalene synthase (*Fdft1*) is another early step in the cholesterol biosynthetic pathway. A study examining the effect of disruption of squalene synthesis on ROR γ t activation demonstrated that the ROR γ -mediated transactivation of a RORE-driven reporter was greatly reduced in a squalene synthase (*Fdft1*)-deficient cell line, SXLT, while exogenous expression of *Fdft1* or the addition of squalene restored ROR γ t-mediated transactivation of the reporter [11] (Figure 4B).

Disruption of 14 α -demethylase cytochrome P450, *Cyp51a1* (lanosterol 14 α -demethylase), either in *Cyp51a1*-deficient mice or in cells treated with *Cyp51a1* inhibitors, such as azole-type fungicides, resulted in decreased synthesis of zymosterol and desmosterol and a significant reduction in ROR γ -mediated transactivation and consequently in Th17 differentiation and IL-17 expression [8,11,60] (Figure 4B). Consistent with this are similarities in the phenotype between *Cyp51a1*- and ROR γ -deficient mice. Branchial lymph node anlagen were absent in 75% of *Cyp51a1*^{-/-} mice and the number of IL17RA⁺ and CD4⁺ Lti cells was reduced. A recent study of a hepatocyte-specific knockout of *Cyp51a1* in mice, referred to as H^{Cyp51}^{-/-}, showed development of progressive liver injury and fibrosis [61]. Hepatic cholesterol biosynthesis was disrupted in these mice leading to lower levels of hepatic and blood cholesterol, as well as several cholesterol intermediates, including zymosterol and desmosterol, which act as ROR agonists. This reduced agonist levels are likely responsible for the observed reduction in ROR α and ROR γ transcriptional activity. In addition, in some of alterations in hepatic gene expression in H^{Cyp51}^{-/-} mice overlapped with ROR target genes consistent with the concept that some of these changes are due to reduced ROR α / γ activation.

7. Regulation of ROR γ activity by other sterol metabolic pathways

Cytochrome P450 enzymes play a key role in the regulation of various aspects of cholesterol homeostasis and many catalyze the formation of oxysterols, several of which act as (inverse) antagonists of ROR γ t. CYP27A1 catalyzes the formation of 27-hydroxysterols, including 27-hydroxycholesterol, 7 α , 27- and 7 β , 27-dihydroxycholesterol. These hydroxysterols,

which were reported to be preferentially produced by mouse Th17 cells, all act as ROR γ t agonists and stimulate Th17 differentiation [10]. The production of 27-hydroxycholesterols in Cyp27a1-deficient Th17 cells was greatly diminished as was the number of IL-17 producing cells in Cyp27a1-deficient mice suggesting that 27-hydroxysterols are an important source of ROR γ t agonists in these cells (Figure 4B). However, these findings appear at odds with another study showing that enzymes involved in oxysterol formation are expressed at low levels during Th17 differentiation [8].

One might expect that hypercholesterolemic conditions lead to an increase in endogenous sterol levels and availability of ROR agonists. This would subsequently result in increased activation of ROR γ t and promote, for example, Th17 differentiation and the production of the inflammatory cytokine IL-17A. Low cholesterol diet might do the opposite. This hypothesis is supported by several reports. Apolipoprotein E (ApoE)-deficient mice have increased levels of total cholesterol and LDL-cholesterol. These mice spontaneously develop atherosclerosis and show an increased susceptibility to type II collagen-induced arthritis [62]. Since Th17 cells and the production of inflammatory cytokines, such as IL17A, play a critical role in the development of these pathologies, the increased susceptibility to these diseases might be in part related to the elevated blood levels of cholesterol that lead to an increase in ROR γ agonists and Th17 cells [62](Figure 4B). A different study reported that patients with chronic hepatitis C, when placed on a normocaloric, low cholesterol diet showed a significant reduction in Th17 cells and IL-17 levels, which might at least in part be related to reduced ROR γ t activation [63].

CD5L-like (CD5L/AIM), a member of the scavenger receptor cysteine-rich superfamily, is highly expressed in non-pathogenic Th17 cells, where it regulates lipid synthesis and modulates intracellular lipid homeostasis, but is down-regulated in pathogenic Th17 cells (Fig. 4A)[64]. CD5L reduces the content of polyunsaturated fatty acid (PUFA) in favor of saturated fatty acids (SFA) and limits cholesterol biosynthesis. The low expression of CD5L in pathogenic Th17 cells increases cholesterol biosynthesis and the generation of ROR γ t agonists, and promotes their pathogenicity. Similarly, deficiency in CD5L in mice leads to increased PUFA content and expression of several cholesterol biosynthetic enzymes, such as *Cyp51a1* and *Sc4mol*, in Th17 cells leading to increased availability of ROR γ t agonists (Figure 4B). Consequently, this leads to ROR γ t-dependent transactivation of *Il17* and *Il23r* promoters, thereby promoting a shift from non-pathogenic to pathogenic Th17 cells.

ROR α / γ activity might be influenced by a number of other cholesterol regulatory pathways, including sterol regulatory element binding protein 2 (SREBP2) and the LXR nuclear receptors [45,65–67]. The transcription factor SREBP2 regulates cholesterol biosynthesis in part by increasing the transcription of several genes involved in cholesterol biosynthesis, including *HMGCR* and *HMGCS*, thereby enhancing the availability of ROR γ agonists [66,67]. Activation of the T cell receptor (TCR) pathway results in activation of SREBP in favor of sterol-sulfate and cholesterol synthesis and might synergize with ROR γ in promoting Th17 differentiation and IL-17 synthesis [8,68]. LXRs play a critical role in cholesterol homeostasis by regulating the expression of *ABCA1* and *ABCG1*, which mediate cholesterol efflux [45,65]. Thus, LXR agonists reduce cholesterol levels in cells thereby reducing the availability of ROR α / γ agonists (Figure 4B).

8. Regulation of sterol metabolism by RORs

In addition to being regulated by sterols, RORs themselves regulate the expression of a number of genes involved in cholesterol transport and sterol metabolism. The hepatic expression of *ABCA1* and *ABCG1*, encoding reverse cholesterol transporters, is reduced in the liver of ROR α -deficient mice [69]. In HUVEC endothelial cells and the monocytic cell line, THP-1, *ABCA1* was found to be directly regulated by ROR α [70]. Treatment with an inverse ROR α agonist was shown to decrease plasma cholesterol levels and suppress atherosclerosis development in mice, which appeared in part to be related to increased *ABCG5/G8* expression and cholesterol excretion by the intestine [71]. The hepatic expression of sterol 12 α -hydroxylase, *CYP8B1*, and 7 α hydroxylase, *CYP7B1*, which are involved in the conversion of cholesterol into bile acids and the regulation of hepatic and serum cholesterol levels, have been reported to be directly regulated by ROR α and/or ROR γ [22,25,72]. ROR α regulates *CYP8B1* by directly binding to an RORE in its promoter and through the recruitment of the co-activator, CBP [72].

In addition, ROR α/γ have been implicated in the circadian regulation of several 3 β -hydroxysteroid dehydrogenases and sterol sulfotransferases [22,25]. ROR α/γ positively regulate the hepatic expression of the hydroxysteroid sulfotransferase, *Sult1B1*, and steroid/bile acid sulfotransferase, *Sult2A1* [22,73,74]. *Sult2A1* transcription was shown to be regulated directly by ROR α and ROR γ [73]. In macrophages, ROR α regulates cholesterol 25-hydroxylase (*Ch25h*), which converts cholesterol to 25-hydroxycholesterol, and promotes phagocytosis [50]. Regulation of sterol metabolic genes by RORs may enhance or reduce the synthesis of agonists or inverse agonist thereby modulating ROR γ activity, thereby creating a positive or negative feedback loop. Whether the regulation of sterol metabolic genes by RORs affects the availability of ROR (inverse) agonists and therefore ROR activation, needs further study.

9. Synthetic ROR γ ligands

Genetic deficiency of ROR γ t in mice greatly affects Th17 cell differentiation and *Il17* expression and protects mice against Th17-dependent inflammation in several experimental autoimmune disease models [75–78]. This, together with the discovery that RORs act as ligand-dependent transcription factors, prompted a search for synthetic ROR inverse agonists with therapeutic potential. Screens of chemical libraries for ROR modulators identified several series of synthetic small molecular weight molecules that function either as an agonist or inverse agonist [2,17,48,79–91]. Recently, a synthetic ROR γ agonist, SR0987, was also described [92]. Chemical structures of several synthetic ROR inverse agonists are shown in Figure 5. For a more comprehensive overview, we refer to several recent reviews [2,4,12–14].

X-ray crystallography of the ROR γ (LBD) in complex with various inverse agonists suggested the existence of distinct classes [16,93,94]. Most inverse agonists bind the canonical ligand binding pocket of ROR γ ; however, these interactions can be distinguished on basis of the formation of different bonds with ROR γ . In one class, binding of the inverse agonist causes repositioning of H12 that disrupts interaction with co-activators. Binding of a

second class of inverse agonists involves a “water trapping” mechanism, in which H12 remains in the active conformation and still allows interaction with co-activator peptides [16]. A different study revealed still another type of antagonism [93]. Indazole-type inverse agonists, such as MRL-871, do not interact with the canonical, orthosteric ligand binding pocket, but with a different, distal, allosteric binding site in ROR γ (LBD). MRL-871 reorients H12 and actively blocks binding of cofactors. Molecular studies revealed two additional modes of action by which inverse agonists can inhibit ROR γ transcriptional activity. The inverse agonists TMP778, TMP920, and GSK805 interact physically with the canonical ligand-binding pocket of ROR γ t and inhibit Th17 differentiation; however, interaction with TMP920 disrupts ROR γ t binding to DNA, whereas TMP778- and GSK805-bound ROR γ t are still associated with target genes and actually stabilized ROR γ t binding to a number of new genomic sites, suggesting that ROR γ binding to DNA remains largely intact [17]. Similarly, the inverse agonist Cpd1 did not affect the binding of ROR γ to ROREs (Guntermann, 2017 #1276). Binding of ROR γ t to the *Il17a* and *Il23r* promoter was found to be associated with a more open chromatin structure as indicated by H3AcK9/K14 acetylation and H3K4me3 methylation at these loci. This histone methylation and acetylation is downregulated in the presence of Cpd1 suggesting a less open chromatin structure.

10. ROR γ as therapeutic target

Treatment of cells and rodents with synthetic ROR γ inverse agonists induces many of the same effects as observed in ROR γ null mice, including inhibition of Th17 differentiation, RORE-mediated transactivation, activation of the *Il17* transcription, and IL-17 production [2,4,17,19,20,95–98]. Moreover, as observed in ROR γ null mice, ROR γ inverse agonists greatly alleviate Th17-dependent inflammation in several experimental autoimmune disease models in rodents [17,51,76,89,96,98–102]. The dual ROR α /ROR γ inverse agonist SR1001 and the ROR γ -selective inverse agonists, ursolic acid, digoxin, MRL-248, TMP778, TMP920, have been reported to ameliorate Th17-mediated experimental autoimmune encephalomyelitis (EAE) in mice and rats [17,51,103–106]. Treatment with inverse ROR γ agonists Bio-0554019, GSK2981278, TMP778, VTP-43742, JNJ-54271074 attenuated skin inflammation in imiquimod-induced psoriatic model as well as in other psoriatic models, the IL-23-injection model and K5.Stat3C transgenic mice [98–100,105,107–109]. Administration of the inverse ROR α /ROR γ inverse agonist SR1001 to NOD mice, a model of type 1 diabetes, significantly reduced diabetes incidence and insulinitis [101], while treatment of obese diabetic mice with SR1555 caused a significant reduction in fat mass and improved insulin sensitivity [110]. A different study showed that Cpd1 reduced hypersensitivity responses in a methylated bovine serum albumin-induced skin inflammation model in rats [102]. The inverse agonists, JNJ-54271074 and SR2211, have been reported to inhibit inflammation in a collagen-induced arthritis mouse model [89,99] and Cpd1 in antigen-induced arthritis in rats [102]. In an experimental murine model, treatment with TMP778 markedly alleviated chronic graft-versus-host disease (cGvHD), which represents a major complication of allogeneic stem cell transplantation, and may provide a new therapeutic approach in the management of cGvHD [111].

It is to be expected that treatment with ROR γ inverse agonists would also affect the function of ROR γ in other ROR γ^+ immune cells, including DP thymocytes, ILC3s, ROR γ^+ T_{regs}, and ROR γ^+ δ . Treatment of mouse thymocytes with the inverse agonists was shown to down-regulate *Bcl2l1* expression and to increase apoptosis, while treatment of mice or rats with, respectively, the ROR γ inverse agonists MRL-248 or Cpd1, causes a reduction in the number of total thymocytes and perturbs DP thymocyte survival [97,105], as observed in ROR γ null mice [19,20]. Moreover, administration of MRL-248 in mice resulted in skewed cell receptor alpha (TCR α) gene rearrangements and limited T cell repertoire diversity [105]. In a myelin oligodendrocyte glycoprotein (MOG)-induced EAE model, MRL-248 reduced the number of MOG-specific CD4⁺ T cells and delayed autoimmune EAE progression. Although ILC3s can be pro-inflammatory, ROR γ^+ ILC3s play a critical role in intestinal immunity and protect against infection-mediated inflammation [112]. In response to infection with *Citrobacter rodentium*, Th17 cell responses and intestinal inflammation were significantly inhibited in mice treated with the inverse agonist GSK805, while cytokine expression in ILC3s, including IL-17, was largely maintained [113]. Thus ROR γ inverse agonists might provide an attractive strategy to treat inflammatory bowel disease.

The discovery that treatment with small molecular weight ROR γ inverse agonists protects against autoimmune disease in several experimental rodent models initiated a number of clinical trials [109,114,115]. Vitae Pharmaceuticals/Allergan successfully finished its Phase 1 trial with VTP-43742 (Figure 5) and a Phase 2a trial showed statistical significant efficacy in psoriatic patients [115]. These studies suggest that ROR γ inverse agonist might provide an attractive therapeutic alternative in the management of inflammatory autoimmune disease.

11. Lymphoma concerns

Ueda et al. [116] reported that ROR γ -deficient mice develop thymic lymphomas within three months of age that was confirmed in a later study [117]. Knockout of ROR γ in adult mice using conditional ROR γ knockout mice also leads to thymic lymphoma development; however, in mice, in which the *Rorc* locus was incompletely deleted, ROR γ -deficient thymocytes were rapidly replaced by wild type thymocytes [117]. These findings raised concerns whether therapeutic treatment with ROR γ antagonists increases the risk of developing T cell lymphoma. Chronic treatment of rats with the ROR γ inverse agonist Cpd1 was recently shown to cause progressive thymic changes that resemble those observed in ROR γ -deficient mice [97]. This study further showed that one rat developed preneoplastic, hypertrophic lesions in the cortical thymus. Although this may not occur in humans, these studies point at an apparent risk of T cell lymphoma with chronic ROR γ inverse agonist treatment at least in rodents.

Although these concerns may be real, the observed diversity in modes of interaction of inverse agonists with ROR γ (LBD) provides hope that future studies might be able to identify inverse agonists that target certain biological processes more specifically thereby minimizing possible side effects of therapeutic treatment [16,17,93]. A recent study [42] showing that a two-amino-acid substitution in ROR γ t disrupts Th17 differentiation, but not thymocyte development, is consistent with this prospect.

12. Further considerations

Multiple T helper cell lineages and immune cell types contribute to immunopathology. As mentioned above ROR γ t is expressed in a number of immune cell subtypes with pathogenic as well as protective functions, including Th17, RORt⁺T_{regs}, RORt⁺ γ δ , and ILC3s. For example, Th17 cells have a pro-inflammatory role in the development of autoimmune disease, but are also key players in host defense against extracellular pathogens [78,118]. In the gut, a delicate balance exists between anti- and pro-inflammatory mechanisms that maintain the epithelial barrier integrity and control intestinal homeostasis and inflammation. This involves complex, mutual interactions between the gut microbiota and different immune cells, such as Th17, RORt⁺T_{regs}, RORt⁺ γ δ , and ILC3s (reviewed in [31,35,112,119]). How ROR γ t inverse agonists affect these interactions is not yet fully understood.

Adding to this complexity is that microbiota, metabolism, and immunity, are also regulated by the circadian clock [120–122] and in several tissues ROR expression is under the control of the circadian clock and expressed in a rhythmic pattern [4,123]. In addition, RORs themselves participate in the regulation of circadian clock genes [4,123–125]. Together, these findings suggest that timing of administering inverse agonists to treat autoimmune disease could be important and that the greatest therapeutic benefits might be obtained at the circadian time of optimal ROR γ t expression.

One also needs to take into account that changes in sterol homeostasis influences the activity of many other signaling pathways [45,66]. Cholesterol is critical for membrane fluidity of the plasma membrane and can interact with and regulate the activity of many other proteins, including G protein-coupled receptors, such as GPR183 [126] and Smoothened (SMO), a component of Hedgehog signaling [127], and several other nuclear receptors, such as LXRs [45,128]. Several (inverse) agonists have been reported to interact with both LXRs and RORs. It is therefore likely that the regulation of physiological processes by RORs intersects with many of the other cholesterol/sterol mediated regulatory signaling pathways.

13. Concluding remarks

An increasing number of studies have demonstrated that ROR γ t has multiple functions in immunity and immunopathology. Autoimmune disease is a complex disease in which metabolism, the microbiome, circadian clock, and multiple cell immune cells play mutually dependent roles. Further studies are needed to understand the precise molecular mechanisms underlying these interrelationships and how loss of ROR γ t function affects these interactions and immunopathologies. The discovery that loss of ROR γ t function protects against autoimmune disease and the identification of synthetic ROR γ inverse agonists that protect against autoimmune disease in several experimental rodent models has made ROR γ a conceivable therapeutic target. Successful phase 1 and phase 2 clinical trials suggest that ROR γ inverse agonists may offer an attractive alternative therapy for autoimmune disease. To what extent the risk of developing lymphoma is a concern needs further study. Discovery of inverse agonists that target ROR γ t-regulated physiological processes more specifically may get around certain side effects. Like ROR γ t, ROR α is also expressed in a number of

immune cells, including Th17 and ILC2s; however, its role in immunity and immunopathology is less well understood. Identification of high affinity ROR α (inverse) agonists will not only increase our understanding of the role of ROR α in immunity and immune disease, but may also uncover its therapeutic potential.

Acknowledgments

The author would like to thank Drs. David Scoville and Caleb Sutherland for their comments on the manuscript. AMJ research was supported by the Intramural Research Program of the NIEHS, NIH (Z01-ES-101585) and partially by NIH grants R21AR066505 and 1R01AR071189-01A1 to AS.

References

1. Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl Recept Signal.* 2009; 7:e003. [PubMed: 19381306]
2. Solt LA, Burris TP. Action of RORs and their ligands in (patho)physiology. *Trends Endocrinol Metab.* 2012; 23:619–627. [PubMed: 22789990]
3. Jetten AM, Kang HS, Takeda Y. Retinoic acid-related orphan receptors alpha and gamma: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity. *Front Endocrinol (Lausanne).* 2013; 4:1. [PubMed: 23355833]
4. Cook DN, Kang HS, Jetten AM. Retinoic Acid-Related Orphan Receptors (RORs): Regulatory Functions in Immunity, Development, Circadian Rhythm, and Metabolism. *Nucl Receptor Res.* 2015; 2 pii: 101185.
5. Giguere V, McBroom LD, Flock G. Determinants of target gene specificity for ROR alpha 1: monomeric DNA binding by an orphan nuclear receptor. *Mol Cell Biol.* 1995; 15:2517–2526. [PubMed: 7739535]
6. Medvedev A, Yan ZH, Hirose T, Giguere V, Jetten AM. Cloning of a cDNA encoding the murine orphan receptor RZR/ROR gamma and characterization of its response element. *Gene.* 1996; 181:199–206. This study, together with a previous report by the same laboratory, describe the discovery of the mouse and human homologs of the third member of the retinoic acid-related orphan receptor subfamily, ROR γ . The amino acid sequence of mouse ROR γ exhibits an 88% overall identity to that of human ROR γ . Analysis of the ROR gamma-response element (RORE) revealed that it binds as a monomer to response elements composed of a single core motif GGTC A preceded by a 6 bp AT-rich sequence. [PubMed: 8973331]
7. Kallen J, Schlaeppi JM, Bitsch F, Delhon I, Fournier B. Crystal structure of the human RORalpha Ligand binding domain in complex with cholesterol sulfate at 2.2 Å. *J Biol Chem.* 2004; 279:14033–14038. In this study, the X-ray crystal structures of eight different classes natural and synthetic (inverse) agonists are analyzed. Several new molecular mechanisms for ROR γ t agonism and inverse agonism were proposed. One class of inverse agonists acts via steric clash that prevents co-activator binding and the other involving “water trapping”. These new mechanistic insights should prove useful for the design and optimization of further ROR γ t modulators. [PubMed: 14722075]
8. Hu X, Wang Y, Hao L-Y, Liu X, Lesch CA, et al. Sterol metabolism controls T(H)17 differentiation by generating endogenous ROR γ agonists. *Nature Chem Biol.* 2015; 11:141–147. This report shows that during Th17 differentiation, cholesterol biosynthesis and uptake programs are induced, whereas their metabolism and efflux programs are suppressed, which result in increased accumulation of the cholesterol precursor, desmosterol, a potent endogenous ROR γ agonist. Blocking cholesterol biosynthesis with inhibitors inhibits Th17 differentiation. The study shows that sterol synthesis, mobilization and metabolism play a critical role in the regulation of Th17 differentiation by regulating ROR γ activation. [PubMed: 25558972]
9. Jin L, Martynowski D, Zheng S, Wada T, Xie W, et al. Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORgamma. *Mol Endocrinol.* 2010; 24:923–929. [PubMed: 20203100]

10. Soroosh P, Wu J, Xue X, Song J, Sutton SW, et al. Oxysterols are agonist ligands of ROR γ and drive Th17 cell differentiation. *Proc Natl Acad Sci U S A*. 2014; 111:12163–12168. Oxysterols elicit profound effects on immune and inflammatory responses. This study demonstrates that several naturally occurring oxysterols act as ROR γ agonists by binding directly to recombinant ROR γ ligand binding domain and promoting recruitment of a coactivators. The most potent and selective activator for ROR γ , 7 β , 27-dihydroxycholesterol, enhances the Th17 differentiation of murine and IL-17 production. Mice deficient in CYP27A1, a key enzyme in generating these oxysterols, showed significant reduction of IL-17-producing cells. CYP27A1 is shown to be important for the generation of these ROR γ agonists and adaptive IL-17-dependent immune responses. [PubMed: 25092323]
11. Santori FR, Huang P, van de Pavert SA, Douglass EF Jr, Leaver DJ, et al. Identification of Natural ROR γ Ligands that Regulate the Development of Lymphoid Cells. *Cell Metab*. 2015; 21:286–297. ROR γ -deficient mice exhibit defective development of thymocytes, lymphoid organs, Th17 cells, and type 3 innate lymphoid cells. This study shows that several intermediates of the cholesterol biosynthesis, such as desmosterol and zymosterol, enhance ROR γ activation and ROR γ -dependent transcription. The results suggest that they function as ROR γ ligands. They stabilized the ROR γ ligand-binding domain and induced coactivator recruitment. The report suggests that these intermediates play a role in regulating lymphocyte development by binding ROR γ . [PubMed: 25651181]
12. Fauber BP, Magnuson S. Modulators of the nuclear receptor retinoic acid receptor-related orphan receptor-gamma (ROR γ or RORc). *J Med Chem*. 2014; 57:5871–5892. [PubMed: 24502334]
13. Kojetin DJ, Burris TP. REV-ERB and ROR nuclear receptors as drug targets. *Nat Rev Drug Discov*. 2014; 13:197–216. [PubMed: 24577401]
14. Cyr P, Bronner SM, Crawford JJ. Recent progress on nuclear receptor ROR γ modulators. *Bioorg Med Chem Lett*. 2016; 26:4387–4393. [PubMed: 27542308]
15. Slominski AT, Kim TK, Takeda Y, Janjetovic Z, Brozyna AA, et al. ROR α and ROR γ are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. *FASEB J*. 2014; 28:2775–2789. [PubMed: 24668754]
16. Kallen J, Izaac A, Be C, Arista L, Orain D, et al. Structural States of ROR γ : X-ray Elucidation of Molecular Mechanisms and Binding Interactions for Natural and Synthetic Compounds. *ChemMedChem*. 2017; 12:1014–1021. [PubMed: 28590087]
17. Xiao S, Yosef N, Yang J, Wang Y, Zhou L, et al. Small-molecule ROR γ antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. *Immunity*. 2014; 40:477–489. [PubMed: 24745332]
18. Giguere V, Tini M, Flock G, Ong E, Evans RM, et al. Isoform-specific amino-terminal domains dictate DNA-binding properties of ROR α , a novel family of orphan hormone nuclear receptors. *Genes & Dev*. 1994; 8:538–553. [PubMed: 7926749]
19. Sun Z, Unutmaz D, Zou YR, Sunshine MJ, Pierani A, et al. Requirement for ROR γ in thymocyte survival and lymphoid organ development. *Science*. 2000; 288:2369–2373. [PubMed: 10875923]
20. Kurebayashi S, Ueda E, Sakaue M, Patel DD, Medvedev A, et al. Retinoid-related orphan receptor gamma (ROR γ) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. *Proc Natl Acad Sci USA*. 2000; 97:10132–10137. This study, together with that of Sun, Z., et al. (PNAS, 2000), identified for the time several immune functions of ROR γ by generating and characterizing mice deficient in ROR γ . ROR γ -deficient mice lack peripheral and mesenteric lymph nodes and Peyer's patches, indicating that ROR γ expression is indispensable for lymph node organogenesis. Thymopoiesis is greatly impaired in ROR γ -deficient mice due to accelerated apoptosis of the CD4(+)CD8(+) subpopulation. This was at least in part related to the greatly reduced level of expression of the anti-apoptotic gene Bcl-X(L). These two studies showed that ROR γ is an critical regulator of several immune cells. [PubMed: 10963675]
21. Hirose T, Smith RJ, Jetten AM. ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. *Biochem Biophys Res Commun*. 1994; 205:1976–1983. This is the first study describing the discovery of the ROR γ gene. [PubMed: 7811290]

22. Kang HS, Angers M, Beak JY, Wu X, Gimble JM, et al. Gene expression profiling reveals a regulatory role for ROR alpha and ROR gamma in phase I and phase II metabolism. *Physiol Genomics*. 2007; 31:281–294. [PubMed: 17666523]
23. Schmidt SF, Madsen JG, Frafjord KO, Poulsen L, Salo S, et al. Integrative Genomics Outlines a Biphasic Glucose Response and a ChREBP-RORgamma Axis Regulating Proliferation in beta Cells. *Cell Rep*. 2016; 16:2359–2372. [PubMed: 27545881]
24. Takeda Y, Kang HS, Freudenberg J, DeGraff LM, Jothi R, et al. Retinoic acid-related orphan receptor gamma (RORgamma): a novel participant in the diurnal regulation of hepatic gluconeogenesis and insulin sensitivity. *PLoS Genet*. 2014; 10:e1004331. [PubMed: 24831725]
25. Takeda Y, Kang HS, Lih FB, Jiang H, Blaner WS, et al. Retinoid acid-related orphan receptor gamma, RORgamma, participates in diurnal transcriptional regulation of lipid metabolic genes. *Nucleic Acids Res*. 2014; 42:10448–10459. [PubMed: 25143535]
26. Montaldo E, Juelke K, Romagnani C. Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice. *Eur J Immunol*. 2015; 45:2171–2182. [PubMed: 26031799]
27. Eberl G, Littman DR. The role of the nuclear hormone receptor RORgamma in the development of lymph nodes and Peyer's patches. *Immunol Rev*. 2003; 195:81–90. [PubMed: 12969312]
28. Ivanov II, Zhou L, Littman DR. Transcriptional regulation of Th17 cell differentiation. *Semin Immunol*. 2007; 19:409–417. [PubMed: 18053739]
29. Bar-Ephraim YE, Mebius RE. Innate lymphoid cells in secondary lymphoid organs. *Immunol Rev*. 2016; 271:185–199. [PubMed: 27088915]
30. Melo-Gonzalez F, Hepworth MR. Functional and phenotypic heterogeneity of group 3 innate lymphoid cells. *Immunology*. 2017; 150:265–275. [PubMed: 27935637]
31. Ohnmacht C. Tolerance to the Intestinal Microbiota Mediated by ROR(gamma)(+) Cells. *Trends Immunol*. 2016; 37:477–486. [PubMed: 27255270]
32. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, et al. The microbiota regulates type 2 immunity through RORgamma(+) T cells. *Science*. 2015; 349:989–993. Together with Sefik et al. (Ref. 33) showed the complex interrelationships that exist between microbiota homeostasis, different RORγ-positive immune cell populations in the gut, host defence, and inflammatory responses. [PubMed: 26160380]
33. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, et al. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science*. 2015; 349:993–997. [PubMed: 26272906]
34. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol*. 2010; 10:479–489. [PubMed: 20559326]
35. Burkett PR, Meyer zu Horste G, Kuchroo VK. Pouring fuel on the fire: Th17 cells, the environment, and autoimmunity. *J Clin Invest*. 2018 In Press.
36. Kim BS, Lu H, Ichiyama K, Chen X, Zhang YB, et al. Generation of RORgamma(+) Antigen-Specific T Regulatory 17 Cells from Foxp3(+) Precursors in Autoimmunity. *Cell Rep*. 2017; 21:195–207. [PubMed: 28978473]
37. Rutz S, Eidenschenk C, Kiefer JR, Ouyang W. Post-translational regulation of RORgamma-A therapeutic target for the modulation of interleukin-17-mediated responses in autoimmune diseases. *Cytokine Growth Factor Rev*. 2016; 30:1–17. [PubMed: 27481185]
38. Ermisch M, Firla B, Steinhilber D. Protein kinase A activates and phosphorylates RORalpha4 in vitro and takes part in RORalpha activation by CaMK-IV. *Biochem Biophys Res Commun*. 2011; 408:442–446. [PubMed: 21514275]
39. Hwang EJ, Lee JM, Jeong J, Park JH, Yang Y, et al. SUMOylation of RORalpha potentiates transcriptional activation function. *Biochem Biophys Res Commun*. 2009; 378:513–517. [PubMed: 19041634]
40. Lim HW, Kang SG, Ryu JK, Schilling B, Fei M, et al. SIRT1 deacetylates RORgamma and enhances Th17 cell generation. *J Exp Med*. 2015; 212:607–617. [PubMed: 25918343]
41. Kathania M, Khare P, Zeng M, Cantarel B, Zhang H, et al. Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-gamma ubiquitination. *Nat Immunol*. 2016; 17:997–1004. [PubMed: 27322655]

42. He Z, Ma J, Wang R, Zhang J, Huang Z, et al. A two-amino-acid substitution in the transcription factor ROR γ disrupts its function in TH17 differentiation but not in thymocyte development. *Nat Immunol.* 2017; 18:1128–1138. [PubMed: 28846085]
43. Kallen JA, Schlaeppi JM, Bitsch F, Geisse S, Geiser M, et al. X-ray structure of the hROR α LBD at 1.63 Å: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of ROR α . *Structure.* 2002; 10:1697–1707. In this study, the X-ray crystal structures of eight different classes natural and synthetic (inverse) agonists are analyzed. Several new molecular mechanisms for ROR γ agonism and inverse agonism were proposed. One class of inverse agonists acts via steric clash that prevents co-activator binding and the other involving “water trapping”. These new mechanistic insights should prove useful for the design and optimization of further ROR γ modulators. [PubMed: 12467577]
44. Guillemot-Legris O, Mutemberezi V, Muccioli GG. Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids. *Trends Mol Med.* 2016; 22:594–614. [PubMed: 27286741]
45. Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: From cholesterol metabolites to key mediators. *Prog Lipid Res.* 2016; 64:152–169. [PubMed: 27687912]
46. Acimovic J, Goyal S, Kosir R, Golicnik M, Perse M, et al. Cytochrome P450 metabolism of the post-lanosterol intermediates explains enigmas of cholesterol synthesis. *Sci Rep.* 2016; 6:28462. [PubMed: 27334049]
47. Wang Y, Kumar N, Solt LA, Richardson TI, Helvering LM, et al. Modulation of retinoic acid receptor-related orphan receptor α and γ activity by 7-oxygenated sterol ligands. *J Biol Chem.* 2010; 285:5013–5025. [PubMed: 19965867]
48. Fauber BP, Rene O, Deng Y, DeVoss J, Eidenschenk C, et al. Discovery of 1-{4-[3-fluoro-4-((3s, 6r)-3-methyl-1,1-dioxo-6-phenyl-[1,2]thiazinan-2-ylmethyl)-phenyl]-piperazin-1-yl}-ethanone (GNE-3500): a potent, selective, and orally bioavailable retinoic acid receptor-related orphan receptor C (ROR γ or ROR γ) inverse agonist. *J Med Chem.* 2015; 58:5308–5322. [PubMed: 26061388]
49. Wang Y, Kumar N, Crumbley C, Griffin PR, Burris TP. A second class of nuclear receptors for oxysterols: Regulation of ROR α and ROR γ activity by 24S-hydroxycholesterol (cerebrosterol). *Biochim Biophys Acta.* 2010; 1801:917–923. [PubMed: 20211758]
50. Tuong ZK, Lau P, Du X, Condon ND, Goode JM, et al. ROR α and 25-Hydroxycholesterol Crosstalk Regulates Lipid Droplet Homeostasis in Macrophages. *PLoS One.* 2016; 11:e0147179. [PubMed: 26812621]
51. Xu T, Wang X, Zhong B, Nurieva RI, Ding S, et al. Ursolic acid suppresses interleukin-17 (IL-17) production by selectively antagonizing the function of ROR γ t protein. *J Biol Chem.* 2011; 286:22707–22710. [PubMed: 21566134]
52. Li X, Anderson M, Collin D, Muegge I, Wan J, et al. Structural studies unravel the active conformation of apo ROR γ nuclear receptor and a common inverse agonism of two diverse classes of ROR γ inhibitors. *J Biol Chem.* 2017; 292:11618–11630. [PubMed: 28546429]
53. Slominski AT, Kim TK, Hobrath JV, Oak ASW, Tang EKY, et al. Endogenously produced nonclassical vitamin D hydroxy-metabolites act as “biased” agonists on VDR and inverse agonists on ROR α and ROR γ . *J Steroid Biochem Mol Biol.* 2017; 173:42–56. [PubMed: 27693422]
54. Slominski AT, Li W, Kim T, Semak I, Wang J, Zjawiony J, et al. Novel activities of CYP11A1 and their potential physiological significance. *J Steroid Biochem Mol Biol.* 2015; 151:25–37. [PubMed: 25448732]
55. Slominski AT, Kim TK, Hobrath JV, Janjetovic Z, Oak ASW, et al. Characterization of a new pathway that activates lumisterol in vivo to biologically active hydroxylumisterols. *Sci Rep.* 2017; 7:11434. [PubMed: 28900196]
56. Slominski AT, Kim TK, Shehabi HZ, Semak I, Tang EKY, et al. In vivo evidence for a novel pathway of vitamin D3 metabolism initiated by P450 scc and modified by CYP27B1. *FASEB J.* 2012; 26:3901–3915. [PubMed: 22683847]
57. Slominski AT, Kim TK, Li W, Postlethwaite A, Thieu EW, et al. Detection of novel CYP11A1-derived secosteroids in the human epidermis and serum and pig adrenal gland. *Sci Rep.* 2015; 5:14875.doi: 10.1038/srep14875 [PubMed: 26445902]

58. Varshney P, Narasimhan A, Mittal S, Malik G, Sardana K, et al. Transcriptome profiling unveils the role of cholesterol in IL-17A signaling in psoriasis. *Sci Rep*. 2016; 6:19295. [PubMed: 26781963]
59. Ulivieri C, Baldari CT. Statins: from cholesterol-lowering drugs to novel immunomodulators for the treatment of Th17-mediated autoimmune diseases. *Pharmacol Res*. 2014; 88:41–52. [PubMed: 24657239]
60. Kojima H, Muromoto R, Takahashi M, Takeuchi S, Takeda Y, et al. Inhibitory effects of azole-type fungicides on interleukin-17 gene expression via retinoic acid receptor-related orphan receptors alpha and gamma. *Toxicol Appl Pharmacol*. 2012; 259:338–345. [PubMed: 22289359]
61. Urlep Z, Lorbek G, Perse M, Jeruc J, Juvan P, et al. Disrupting Hepatocyte Cyp51 from Cholesterol Synthesis Leads to Progressive Liver Injury in the Developing Mouse and Decreases RORC Signalling. *Sci Rep*. 2017; 7:40775. [PubMed: 28098217]
62. Postigo J, Genre F, Iglesias M, Fernandez-Rey M, Buelta L, et al. Exacerbation of type II collagen-induced arthritis in apolipoprotein E-deficient mice in association with the expansion of Th1 and Th17 cells. *Arthritis Rheum*. 2011; 63:971–980. [PubMed: 21225684]
63. Maggio R, Viscomi C, Andreozzi P, D'Ettore G, Viscogliosi G, et al. Normocaloric low cholesterol diet modulates Th17/Treg balance in patients with chronic hepatitis C virus infection. *PLoS One*. 2014; 9:e112346. [PubMed: 25532016]
64. Wang C, Yosef N, Gaublumme J, Wu C, Lee Y, et al. CD5L/AIM Regulates Lipid Biosynthesis and Restrains Th17 Cell Pathogenicity. *Cell*. 2015; 163:1413–1427. [PubMed: 26607793]
65. Bovenga F, Sabba C, Moschetta A. Uncoupling nuclear receptor LXR and cholesterol metabolism in cancer. *Cell Metab*. 2015; 21:517–526. [PubMed: 25863245]
66. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell*. 2006; 124:35–46. [PubMed: 16413480]
67. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. *Nat Rev Endocrinol*. 2017
68. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol*. 2013; 14:489–499. [PubMed: 23563690]
69. Lau P, Fitzsimmons RL, Raichur S, Wang SC, Lechtken A, et al. The orphan nuclear receptor, RORalpha, regulates gene expression that controls lipid metabolism: staggerer (SG/SG) mice are resistant to diet-induced obesity. *J Biol Chem*. 2008; 283:18411–18421. [PubMed: 18441015]
70. Coban N, Gulec C, Ozsait-Selcuk B, Erginel-Unaltuna N. CYP19A1, MIF and ABCA1 genes are targets of the RORalpha in monocyte and endothelial cells. *Cell Biol Int*. 2017; 41:163–176. [PubMed: 27925372]
71. Billon C, Sitaula S, Burris TP. Inhibition of RORalpha/gamma suppresses atherosclerosis via inhibition of both cholesterol absorption and inflammation. *Mol Metab*. 2016; 5:997–1005. [PubMed: 27689012]
72. Pathak P, Li T, Chiang JY. Retinoic acid-related orphan receptor alpha regulates diurnal rhythm and fasting induction of sterol 12alpha-hydroxylase in bile acid synthesis. *J Biol Chem*. 2013; 288:37154–37165. [PubMed: 24226095]
73. Ou Z, Shi X, Gilroy RK, Kirisci L, Romkes M, et al. Regulation of the human hydroxysteroid sulfotransferase (SULT2A1) by RORalpha and RORgamma and its potential relevance to human liver diseases. *Mol Endocrinol*. 2013; 27:106–115. [PubMed: 23211525]
74. Kang HS, Okamoto K, Takeda Y, Beak JY, Gerrish K, et al. Transcriptional profiling reveals a role for RORalpha in regulating gene expression in obesity-associated inflammation and hepatic steatosis. *Physiol Genomics*. 2011; 43:818–828. [PubMed: 21540300]
75. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity*. 2008; 29:138–149. [PubMed: 18599325]
76. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity*. 2008; 28:29–39. In this study, the investigators report that Th17 cells also highly express another related nuclear receptor, RORα, that is induced by transforming growth factor-beta and interleukin-6 (IL-6), which is dependent on signal transducer and activator of

transcription 3. Overexpression of ROR α promotes Th17 differentiation. ROR α -deficiency resulted in reduced IL-17 expression in vitro and in vivo. It was further shown that ROR α and ROR γ coexpression synergistically enhance Th17 differentiation, while ROR α /ROR γ double knockout mice exhibit a greater impaired Th17 generation and completely protected mice against experimental autoimmune encephalomyelitis. The study concludes that Th17 differentiation is directed by both nuclear receptors, ROR α and ROR γ . [PubMed: 18164222]

77. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, et al. The Orphan Nuclear Receptor ROR γ Directs the Differentiation Program of Proinflammatory IL-17(+) T Helper Cells. *Cell*. 2006; 126:1121–1133. IL-17-producing Th17 lymphocytes are major contributors to autoimmune disease. This report identifies ROR γ as a key transcription factor of Th17 lineage differentiation. ROR γ regulates the transcription of IL-17 and the related cytokine IL-17F in naïve T helper cells. ROR γ -deficient mice T cells lack tissue-infiltration of Th17 cells and show an attenuated autoimmune disease. This study indicated for the first time that ROR γ is a key regulator of immune homeostasis and might have potential as a therapeutic target in inflammatory diseases. [PubMed: 16990136]
78. Jetten AM. Immunology: A helping hand against autoimmunity. *Nature*. 2011; 472:421–422. [PubMed: 21525918]
79. Fauber BP, Rene O, Burton B, Everett C, Gobbi A, et al. Identification of tertiary sulfonamides as ROR γ inverse agonists. *Bioorg Med Chem Lett*. 2014; 24:2182–2187. [PubMed: 24685544]
80. Kumar N, Kojetin DJ, Solt LA, Kumar KG, Nuhant P, et al. Identification of SR3335 (ML-176): a synthetic ROR α selective inverse agonist. *ACS Chem Biol*. 2011; 6:218–222. [PubMed: 21090593]
81. Kinzel, O., Gege, C., Steeneck, C., Kleymann, G., Hoffmann, T. Seven-membered sulfonamides as modulators of RAR-related orphan receptor-gamma (ROR γ , NR1F3). WO 2013/064231. 2013.
82. Steeneck, C., Kinzel, O., Gege, C., Kleymann, G., Hoffmann, T. Pyrrolo carboxamides as modulators of orphan nuclear receptor RAR-related orphan receptor-gamma (ROR γ , NR1F3) activity and for the treatment of chronic inflammatory and autoimmune diseases. WO 2013/079223. 2013.
83. Glick, GD., Toogood, PL., Romero, AG., Vanhuis, CA., Aicher, TD., et al. Tetrahydroquinoline and related bicyclic compounds for inhibition of ROR γ activity and treatment of disease. WO 2012/064744. 2012.
84. Birault, V., Campbell, AJ., Harrison, S., Le, J. Sulfonamide compounds and their use in the modulation of retinoid-related orphan receptor. WO 2013/045431. 2013.
85. Wang Y, Cai W, Zhang G, Yang T, Liu Q, et al. Discovery of novel N-(5-(arylcarbonyl)thiazol-2-yl)amides and N-(5-(arylcarbonyl)thiophen-2-yl)amides as potent ROR γ inhibitors. *Bioorg Med Chem*. 2014; 22:692–702. [PubMed: 24388993]
86. van Niel MB, Fauber BP, Cartwright M, Gaines S, Killen JC, et al. A reversed sulfonamide series of selective ROR γ inverse agonists. *Bioorg Med Chem Lett*. 2014; 24:5769–5776. [PubMed: 25453817]
87. Doebelin C, Patouret R, Garcia-Ordóñez RD, Chang MR, Dharmarajan V, et al. N-Arylsulfonyl Indolines as Retinoic Acid Receptor-Related Orphan Receptor gamma (ROR γ) Agonists. *ChemMedChem*. 2016; 11:2607–2620. [PubMed: 27879053]
88. Olsson RI, Xue Y, von Berg S, Aagaard A, McPheat J, et al. Benzoxazepines Achieve Potent Suppression of IL-17 Release in Human T-Helper 17 (TH 17) Cells through an Induced-Fit Binding Mode to the Nuclear Receptor ROR γ . *ChemMedChem*. 2016; 11:207–216. [PubMed: 26553345]
89. Chang MR, Lyda B, Kamenecka TM, Griffin PR. Pharmacologic repression of retinoic acid receptor-related orphan nuclear receptor gamma is therapeutic in the collagen-induced arthritis experimental model. *Arthritis Rheumatol*. 2014; 66:579–588. [PubMed: 24574218]
90. Huang Z, Xie H, Wang R, Sun Z. Retinoid-related orphan receptor gamma is a potential therapeutic target for controlling inflammatory autoimmunity. *Expert Opin Ther Targets*. 2007; 11:737–743. [PubMed: 17504012]
91. Hintermann S, Guntermann C, Mattes H, Carcache DA, Wagner J, et al. Synthesis and Biological Evaluation of New Triazolo- and Imidazopyridine ROR γ Inverse Agonists. *ChemMedChem*. 2016; 11:2640–2648. [PubMed: 27902884]

92. Chang MR, Dharmarajan V, Doebelin C, Garcia-Ordóñez RD, Novick SJ, et al. Synthetic RORγ agonists enhance protective immunity. *ACS Chem Biol*. 2016; 11:1012–1018. [PubMed: 26785144]
93. Scheepstra M, Leysen S, van Almen GC, Miller JR, Piesvaux J, et al. Identification of an allosteric binding site for RORγ inhibition. *Nat Commun*. 2015; 6:8833. [PubMed: 26640126]
94. Fujita-Sato S, Ito S, Isobe T, Ohyama T, Wakabayashi K, et al. Structural basis of digoxin that antagonizes RORγ receptor activity and suppresses Th17 cell differentiation and interleukin (IL)-17 production. *J Biol Chem*. 2011; 286:31409–31417. [PubMed: 21733845]
95. de Wit J, Al-Mossawi MH, Huhn MH, Arancibia-Carcamo CV, Doig K, et al. RORγ inhibitors suppress T(H)17 responses in inflammatory arthritis and inflammatory bowel disease. *J Allergy Clin Immunol*. 2016; 137:960–963. [PubMed: 26611672]
96. Huh JR, Leung MW, Huang P, Ryan DA, Krout MR, et al. Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORγ activity. *Nature*. 2011; 472:486–490. [PubMed: 21441909]
97. Guntermann C, Piaia A, Hamel ML, Theil D, Rubic-Schneider T, et al. Retinoic-acid-orphan-receptor-C inhibition suppresses Th17 cells and induces thymic aberrations. *JCI Insight*. 2017; 2:e91127. [PubMed: 28289717]
98. Smith SH, Peredo CE, Takeda Y, Bui T, Neil J, et al. Development of a Topical Treatment for Psoriasis Targeting RORγ: From Bench to Skin. *PLoS One*. 2016; 11:e0147979. [PubMed: 26870941]
99. Xue X, Soroosh P, De Leon-Tabaldo A, Luna-Roman R, Sablad M, et al. Pharmacologic modulation of RORγ translates to efficacy in preclinical and translational models of psoriasis and inflammatory arthritis. *Sci Rep*. 2016; 6:37977. [PubMed: 27905482]
100. Skepner J, Ramesh R, Trocha M, Schmidt D, Baloglu E, et al. Pharmacologic inhibition of RORγ regulates Th17 signature gene expression and suppresses cutaneous inflammation in vivo. *J Immunol*. 2014; 192:2564–2575. [PubMed: 24516202]
101. Solt LA, Banerjee S, Campbell S, Kamenecka TM, Burris TP. ROR Inverse Agonist Suppresses Insulinitis and Prevents Hyperglycemia in a Mouse Model of Type 1 Diabetes. *Endocrinology*. 2015;en20141677.
102. Guendisch U, Weiss J, Ecoeur F, Riker JC, Kaupmann K, et al. Pharmacological inhibition of RORγ suppresses the Th17 pathway and alleviates arthritis in vivo. *PLoS One*. 2017; 12:e0188391. [PubMed: 29155882]
103. Solt LA, Kumar N, Nuhant P, Wang Y, Lauer JL, et al. Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature*. 2011; 472:491–494. [PubMed: 21499262]
104. Wang T, Niwa S, Bouda K, Matsuura S, Homma T, et al. The effect of Am-80, one of retinoids derivatives on experimental allergic encephalomyelitis in rats. *Life Sci*. 2000; 67:1869–1879. [PubMed: 11043609]
105. Guo Y, MacIsaac KD, Chen Y, Miller RJ, Jain R, et al. Inhibition of RORγT Skews TCRα Gene Rearrangement and Limits T Cell Repertoire Diversity. *Cell Rep*. 2016; 17:3206–3218. [PubMed: 28009290]
106. Wang Y, Cai W, Cheng Y, Yang T, Liu Q, et al. Discovery of Biaryl Amides as Potent, Orally Bioavailable, and CNS Penetrant RORγ Inhibitors. *ACS Med Chem Lett*. 2015; 6:787–792. [PubMed: 26191367]
107. Takaishi M, Ishizaki M, Suzuki K, Isobe T, Shimozato T, et al. Oral administration of a novel RORγ antagonist attenuates psoriasis-like skin lesion of two independent mouse models through neutralization of IL-17. *J Dermatol Sci*. 2017; 85:12–19. [PubMed: 27726924]
108. Banerjee D, Zhao L, Wu L, Palanichamy A, Ergun A, et al. Small molecule mediated inhibition of RORγ-dependent gene expression and autoimmune disease pathology in vivo. *Immunology*. 2016; 147:399–413. [PubMed: 26694902]
109. Gege C. Retinoid-related orphan receptor gamma t (RORγ) inhibitors from Vitae Pharmaceuticals (WO2015116904) and structure proposal for their Phase I candidate VTP-43742. *Expert Opin Ther Pat*. 2016; 26:737–744. [PubMed: 26895086]

110. Chang MR, He Y, Khan TM, Kuruvilla DS, Garcia-Ordenez R, et al. Antiobesity Effect of a Small Molecule Repressor of RORgamma. *Mol Pharmacol.* 2015; 88:48–56. [PubMed: 25904554]
111. Forcade E, Paz K, Flynn R, Griesenauer B, Amet T, et al. An activated Th17-prone T cell subset involved in chronic graft-versus-host disease sensitive to pharmacological inhibition. *JCI Insight.* 2017;2.
112. Withers DR, Hepworth MR. Group 3 Innate Lymphoid Cells: Communications Hubs of the Intestinal Immune System. *Front Immunol.* 2017; 8:1298. [PubMed: 29085366]
113. Withers DR, Hepworth MR, Wang X, Mackley EC, Halford EE, et al. Transient inhibition of ROR-gammat therapeutically limits intestinal inflammation by reducing TH17 cells and preserving group 3 innate lymphoid cells. *Nat Med.* 2016; 22:319–323. [PubMed: 26878233]
114. Bronner SM, Zbieg JR, Crawford JJ. RORgamma antagonists and inverse agonists: a patent review. *Expert Opin Ther Pat.* 2017; 27:101–112. [PubMed: 27629281]
115. Gege C. RORgammat inhibitors as potential back-ups for the phase II candidate VTP-43742 from Vitae Pharmaceuticals: patent evaluation of WO2016061160 and US20160122345. *Expert Opin Ther Pat.* 2017; 27:1–8. [PubMed: 27852111]
116. Ueda E, Kurebayashi S, Sakaue M, Backlund M, Koller B, et al. High incidence of T-cell lymphomas in mice deficient in the retinoid- related orphan receptor RORgamma. *Cancer Res.* 2002; 62:901–909. In this study, the investigators show that changes in homeostasis in the thymus of ROR γ -deficient mice are associated with a high incidence of T-cell lymphomas that metastasized frequently to spleen and liver. No other tumor types were detected in any of ROR γ -deficient mice. Lymphoma formation was associated with increased cellular proliferation and an increase in the number of apoptotic cells. Analysis of the immunophenotype of the lymphoblastic cells showed that the CD4 and CD8 subpopulations varied substantially among different lymphomas. The established cell lines consisted mostly of CD44-CD25+CD4-CD8- cells. Thus, the loss of ROR γ disturbs homeostasis in the thymus by enhancing apoptosis and cellular proliferation. The latter lead via an unknown mechanism to increased susceptibility to the development of T-cell lymphoma. [PubMed: 11830550]
117. Liljevald M, Rehnberg M, Soderberg M, Ramnegard M, Borjesson J, et al. Retinoid-related orphan receptor gamma (RORgamma) adult induced knockout mice develop lymphoblastic lymphoma. *Autoimmun Rev.* 2016; 15:1062–1070. [PubMed: 27491564]
118. Lee Y, Kuchroo V. Defining the functional states of Th17 cells. *F1000Res.* 2015; 4:132. [PubMed: 27006754]
119. Thaïss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature.* 2016; 535:65–74. [PubMed: 27383981]
120. Wang Y, Kuang Z, Yu X, Ruhn KA, Kubo M, et al. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science.* 2017; 357:912–916. [PubMed: 28860383]
121. Yu X, Rollins D, Ruhn KA, Stubblefield JJ, Green CB, et al. TH17 cell differentiation is regulated by the circadian clock. *Science.* 2013; 342:727–730. [PubMed: 24202171]
122. Man K, Loudon A, Chawla A. Immunity around the clock. *Science.* 2016; 354:999–1003. [PubMed: 27885005]
123. Takeda Y, Jothi R, Birault V, Jetten AM. ROR γ directly regulates the circadian expression of clock genes and downstream targets in vivo. *Nucleic Acids Res.* 2012; 40:8519–8535. [PubMed: 22753030]
124. Solt LA, Kojetin DJ, Burris TP. The REV-ERBs and RORs: molecular links between circadian rhythms and lipid homeostasis. *Future Med Chem.* 2011; 3:623–638. [PubMed: 21526899]
125. Takeda Y, Kang HS, Angers M, Jetten AM. Retinoic acid-related orphan receptor gamma directly regulates neuronal PAS domain protein 2 transcription in vivo. *Nucleic Acids Res.* 2011; 39:4769–4782. [PubMed: 21317191]
126. Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, et al. Oxysterols direct immune cell migration via EBI2. *Nature.* 2011; 475:524–527. [PubMed: 21796212]

127. Nachtergaele S, Mydock LK, Krishnan K, Rammohan J, Schlesinger PH, et al. Oxysterols are allosteric activators of the oncoprotein Smoothed. Nat Chem Biol. 2012; 8:211–220. [PubMed: 22231273]
128. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, et al. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci U S A. 1999; 96:266–271. [PubMed: 9874807]

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Figure 1. Intermediates of the cholesterol biosynthetic pathway function as ROR γ agonists

Shown is a schematic view of the Bloch cholesterol biosynthetic pathway. In peroxisomes, acetyl CoA is converted to mevalonate by HMGCR, a step that is inhibited by statins, and then via multiple steps into farnesyl pyrophosphate, which is then converted into squalene in the endoplasmic reticulum. This is subsequently converted into cholesterol via the steps indicated. Several cholesterol intermediates can act as ROR γ agonists, but zymosterol and desmosterol exhibit the highest activity. Deficiency in Fdft1 or Cyp51A1, enzymes acting upstream in the cholesterol biosynthetic pathway, inhibit the synthesis of cholesterol and the availability of ROR γ agonists. DHCR24 can convert 7-dehydrodesmosterol to 7-dehydrocholesterol, which acts as an agonist of ROR γ . ACAT1, Acetyl-CoA Acetyltransferase 1; HMGCS1, 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1; HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; FDT1, Farnesyl-Diphosphate Farnesyltransferase 1; SQLE, Squalene Epoxidase; LSS, Lanosterol Synthase; TM7SF2, Transmembrane 7 Superfamily Member 2; FAXDC2/SC4MOL, Fatty Acid Hydroxylase Domain Containing 2; NSDHL, NAD(P) Dependent Steroid Dehydrogenase-Like; HSD17B7, Hydroxysteroid 17-Beta Dehydrogenase 7; EBP, Emopamil Binding Protein; SC5D, Sterol-C5-Desaturase; DHCR7, 7-Dehydrocholesterol Reductase; DHCR24, 24-Dehydrocholesterol Reductase.

Figure 2.
Chemical structure of two sterol ROR γ agonists, cholesterol sulfate, 25-hydroxycholesterol, and two triterpenes, ganoderone A and ursolic acid, which act as an ROR γ agonist and ROR γ inverse agonist, respectively.

Figure 3. Hydroxylated derivatives of vitamin D function as weak ROR α /ROR γ inverse agonists
 After absorption of UVB the ROR γ agonist, 7-dehydrocholesterol, is phototransformed to vitamin D (D3) and lumisterol (L3). Vitamin D is activated through sequential hydroxylation at C25 and C1 α to produce 1,25 (OH)₂D3 that, in addition of acting on vitamin D receptor (VDR), also serves as an inverse agonist of RORs. Vitamin D3 and lumisterol can also be hydroxylated by CYP11A1 to produce hydroxylumisterols or hydroxyderivatives of vitamin D that can further be hydroxylated by other CYPs to produce hydroxyderivatives that can also act as inverse agonists of RORs. Weak inverse agonists shown in red; 7DHC, ROR γ agonist 7-dehydrocholesterol.

Figure 4A

Figure 4B

Figure 4. Changes in cholesterol/sterol homeostasis alter ROR γ transcriptional activity and subsequently the regulation of gene expression, physiological functions and disease, in which ROR γ is involved

A. The formation of ROR γ t agonists by the cholesterol biosynthetic pathway leads to activation of ROR γ t, which is required for Th17 differentiation and the expression of target genes, such as *Il17*, *Il21*, and *Il23R*. The HMG-CoA reductase inhibitors, statins, inhibit the generation of ROR γ t agonists resulting in reduced ROR γ t activation and Th17 differentiation. Statins also reduce ROR γ t expression and Th17 differentiation by inhibiting STAT3, which is required for ROR γ t transcription. The scavenger receptor CD5L is expressed at high levels in nonpathogenic Th17 cells, where it suppresses cholesterol biosynthesis and the formation of ROR γ t agonists. It becomes expressed at low levels in

pathogenic Th17 cells leading to increased cholesterol biosynthesis, generation of ROR γ t agonists, and pathogenicity of Th17 cells. **B.** Inhibition of CYP51A1 by azoles, deficiency in CYP51A1 or FDFT1 inhibit cholesterol biosynthesis, thereby reducing the availability of ROR γ agonists and ROR γ t activation. Statins and CD5L have a negative effect on cholesterol biosynthesis and ROR γ t activation. This subsequently reduces Th17 differentiation and *Il17* expression, and suppresses the development of inflammatory disease. Other mechanisms that affects the generation of ROR γ t agonists are CYP27A1 deficiency, which reduces the generation of 27-hydroxycholesterol. Induction of *ABCG1/A1* expression by LXRs increase cholesterol efflux and potentially reduce the availability of ROR γ t agonists and *Il17* expression, whereas ApoE-deficiency and high fat/cholesterol diet (HFD) have the opposite effect by increasing cholesterol levels.

Figure 5. Chemical structures of several ROR γ inverse agonists

SR1001 (Scripps Institute) is a dual ROR α /ROR γ inverse agonist, while all others are ROR γ -selective inverse agonists.