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REVIEW

The role of nuclear receptors in regulation of Th17/Treg biology and its implications for diseases

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Nuclear receptors in the cell play essential roles in environmental sensing, differentiation, development, homeostasis, and metabolism and are thus highly conserved across multiple species. The anti-inflammatory role of nuclear receptors in immune cells has recently gained recognition. Nuclear receptors play critical roles in both myeloid and lymphoid cells, particularly in helper CD4+ T-cell type 17 (Th17) and regulatory T cells (Treg). Th17 and Treg are closely related cell fates that are determined by orchestrated cytokine signaling. Recent studies have emphasized the interactions between nuclear receptors and the known cytokine signals and how such interaction affects Th17/Treg development and function. This review will focus on the most recent discoveries concerning the roles of nuclear receptors in the context of therapeutic applications in autoimmune diseases.
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The list of helper CD4+ T-cell subsets has grown extensively since the initial discovery of helper CD4+ T-cell type 1 (Th1) and type 2 (Th2).[1] Additions to this extending list include regulatory T cells (Treg), type 17 (Th17), follicular helper T cells (Tfh), and type 9 (Th9).[2–4] At the same time, unique master transcription factors have been discovered in each Th subset in association with their different immune functions.

The differentiation of Th17 and Treg subsets are dichotomous cell fates of CD4+ T cells similar to Th1 and Th2 subsets.[5] Different cytokines can have the potential to tip the subtle balance of between the Treg/Th17 lineages and alter by altering the fate of differentiating cells. This cell-fate decision is highly context-dependent, with transforming growth factor-β1 (TGF-β1) acting as a common cytokine for both Th17 and Treg differentiation.[6] Interleukin-6 (IL-6)- or IL-21-dependent signal transducer and activator of transcription-3 (STAT3) activation, together with TGF-β1, can induce differentiation of the Th17 subset that secretes the proinflammatory cytokines, IL-17A/F and IL-22.[7] The Th17 subset is critical for the immune response against bacterial and fungal infections.[8] Conversely, excessive levels of the Th17 subset in peripheral blood and lesions are associated with pathology in patients with multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, Crohn's disease, and ulcerative colitis.[9] Canonical transcription factors that confer a Th17 phenotype in CD4+ T cells are retinoic acid-related orphan receptors-γt (ROR-γt) and ROR-α, members of a nuclear receptor family of proteins.[7,10] ROR-γt and IL-17A/F expression levels can be further regulated by other transcription factors, including hypoxia-inducible factor-1α (HIF-1α), STAT1, STAT3, and STAT5.[11–14]

IL-2 and TGF-β1 induce and maintain the expression of forkhead box P3 (Foxp3), which is a master regulator of Tregs.[15] Tregs are critical for maintaining self-tolerance and homeostasis in a host organism. Increased numbers of Tregs hinder an effective immune response against tumors, while the loss of Tregs is associated with major autoimmune diseases.[16] The Treg subset is further divided into natural Tregs (nTregs) derived from the thymus and induced Tregs (iTregs) derived from the periphery.[17] Modes of Foxp3+ Treg-dependent immune suppression mediators include the (i) inhibition of proinflammatory cytokines such as interferon-γ (IFN-γ) and IL-2 and (ii) trans-suppression of T cells, dendritic cells, and macrophages through contact-dependent and contact-independent mechanisms.[18] Sustained Foxp3 expression is critical for the capacity of Tregs to negatively regulate the immune response, and its expression is tightly regulated by many transcription factors, including STAT5 and all-trans retinoic acid receptor (RAR).[19,20] More recently, it was found that Foxp3 expression can be further modified through HIF-1α, Stub1, and USP7 via posttranslational mechanisms.[14,21,22] Foxp3

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can also antagonize ROR-γt, thereby inhibiting Th17 differentiation in favor of Treg differentiation.[23,24]

Many recent studies have identified nuclear receptors as additional modulators of Th17/Treg development.[25,26] These nuclear receptors can either positively or negatively regulate cell differentiation and function. In this review, we will introduce the nuclear receptor superfamily and discuss how each subfamily differentially regulate Th17/Treg differentiation and function in relation to other transcription factors. In general, this review focuses on iTregs (denoted henceforth simply as "Treg"), and nTregs are specified as such when relevant.

Nuclear receptor signaling and mechanisms of gene regulation

Nuclear receptor-dependent signaling differs from cytokine-mediated signaling that is mediated by membrane-anchored receptors. Instead, a nuclear receptor signal is initiated by a lipid-derived molecule that penetrates the plasma membrane.[27] Thus, the signaling cascade begins in the cytosol, and subsequently, ligand-bound nuclear receptors translocate into the nucleus to regulate gene expression. A well-known subfamily of the nuclear receptor superfamily includes steroid receptors such as glucocorticoid and estrogen receptors, which were discovered initially in 1985.[28] Since then, our knowledge of nuclear receptors has significantly expanded.

Later, nonsteroidal receptors with different activation mechanisms were discovered. Steroid receptors form homodimers and interact with ligands,[29] whereas nonsteroidal receptors form heterodimers and have a common receptor, retinoid-X-receptor (RXR), which recognizes 9-*cis*-retinoic acid.[30] This subfamily of nuclear receptors consists of many members, including thyroid hormone receptors (T3R), all-trans retinoic acid receptors (RAR), peroxisome proliferator of activated receptors (PPAR), and liver-X-receptors (LXR). Each nonsteroidal receptor subfamily consists of multiple subtypes, which are expressed by different alleles and bind to a unique set of ligands[27] (Table1). However, the ligands for many nuclear receptors are still unknown, and receptors with unidentified ligands are often termed "orphan receptors".[31] Examples include ROR, nerve growth factor-induced clone B (NGFI-B), steroidogenic factor-1 (SF-1), and estrogen receptor-related (ERR) receptors.

The ligand-mediated activation of nuclear receptors can cause the induction or repression of gene expression in many different ways.[32] The regulatory mechanism is unique in that many nuclear receptors modulate gene expression by recruiting transcriptional co-activators or co-repressors in processes known as transactivation and transrepression, respectively. Transactivation involves the recruitment of histone acetylases such as P300/CBP and steroid receptor co-activators (SRCs), whereas transrepression involves the recruitment of histone deacetylase (HDAC) through the interaction between nuclear receptors and SMRT/NcoR (silencing mediator for retinoid and T3R/ nuclear co-repressor) complexes.

The underlying themes of ligand-mediated nuclear receptor activation can be summarized by the following principles. First, nuclear receptors require the presence of natural or synthetic ligands to direct Th17 or Treg differentiation. In this context, genetic deletion of the receptor of interest negates the effect of the ligand on the cells, indicating the ligand is specific for the receptor. Second, even in the absence of synthetic ligands, the deletion of a nuclear receptor can affect Th17 or Treg differentiation. This is true for many nuclear orphan receptors, suggesting the existence of endogenous ligands. In this review, we address the phenotypes of nuclear receptor knockout (KO) mice and the effects of agonist and antagonist treatments *in vitro* and/or *in vivo* models.

Retinoic acid-related orphan receptors (ROR)

ROR is a member of a nuclear receptor superfamily with unidentified ligands. This subfamily consists of the α, β, and γ subtypes, each of which have several isoforms generated by alternative splicing.[33] ROR-γt (one splicing isoform of RORγ, also known as RORγ2) was found to be a canonical transcription factor required for Th17 differentiation.[7] ROR-γt is uniquely expressed in IL-17A- and IL-17F-expressing Th17 cells differentiated by TGF-β1 and IL-6 or IL-21. Genetic deletion of murine ROR-γt in CD4+ T cells significantly decreased Th17 differentiation, as shown in both *in vitro* and *in vivo* studies. Nevertheless, the lack of ROR-γt does not completely abrogate Th17 differentiation, suggesting that an additional transcription factor promotes Th17 development. Another study found that the ROR-α subtype regulates Th17 differentiation as well, and that the combined genetic ablation of both ROR-γt and ROR-α leads to a complete loss of Th17 development.[10] In contrast, the overexpression of both ROR-γt and ROR-α synergistically enhances Th17 differentiation, suggesting that both receptor are critical for Th17 development. Both ROR-γt and ROR-α induce *Il17a/f* transcriptional activity, and ROR-γt interacts with HIF-1α and STAT3 in order to form a transcriptional complex.[14] Similar to murine Th17 cells, TGF-β1 together with IL-6, IL-1β and IL-23 induce ROR-γt in human naïe CD4+ T cells and ROR-γt expression is required for human Th17 differentiation.[34,35] However, ROR-α transcription levels were relatively low in differentiated human Th17 cells, and whether ROR-α plays a role in human Th17-cell development remains uncertain.[34]

Although endogenous ligands for ROR-γt and ROR-α have not yet been discovered, synthetic derivatives have been found to effectively antagonize both ROR subtypes. Digoxin and its derivatives inhibit ROR-γt activity; furthermore, the compounds inhibit both human and murine Th17 development *in vitro* and ameliorate the pathogenesis of experimental autoimmune encephalomyelitis (EAE) in mice.[36] SR1001, an inhibitor of both ROR-α and ROR-γt, similarly inhibits both human and murine Th17 development by promoting NcoR recruitment and blocking SRC2 recruitment to the murine *Il17a* promoter.[37]

In addition to promoting Th17 differentiation, ROR-γt inhibits human Treg differentiation by binding and modulating Foxp3-promoter activity.[38] However, siRNA-mediated *Rorc*-γt knockdown in human Tregs did not alter Treg

Table 1. Nuclear receptors/agonists/antagonists and their effects on Th17/Treg differentiation

Nuclear receptors	subtypes	Cross-talk across nuclear receptors	Ligands	Antagonists	Ligand Effects on Th17	Ligand Effects on Treg	References
ROR	ROR-α, -β, -γ	Unknown	Unknown	Digoxin SR1001	Positive regulator of Th17 development; deletion of ROR-γt and ROR-α impairs Th17 differentiation. Ectopic expression of ROR-γt and ROR-α enhances Th17 development	No direct effects by ROR-γt and ROR-α on Treg development (Foxp3 expression)	(7), (10), (39), (14)
RAR/RXR	RAR-α, -β, -γ	RXR	Natural ligands: all-trans retinoic acid (RAR), 9-*cis*-retinoic acids (RXR) Synthetic ligands: AM580 (RAR), LG268 (RXR)	Ro 41-5253 LE135 LE540	Inhibit Th17 differentiation	Enhance Treg differentiation	(41), (19), (46), (44)
PPAR	PPAR-α	RXR LXR	Natural ligands : palmitic acid, oleic acid, and leukotriene B4 (LTB4) Synthetic ligands: WY14643, Gemfibrozil, Fenofibrate	GW6471	Deletion of PPAR-α in CD4+ T cells enhances Th1/Th17 differentiation	Deletion of PPAR-α in Tregs impairs suppressive capacity	(31), (32), (33), (34)
	PPAR-γ		Natural ligands: α-, γ-linoleic acid, arachidonic acid, 13-HODE (hydroxyoctadeca-9Z,11E-dienoic acid) and 15-deoxy-delta-prostaglandin J2 (15dPGJ2) Synthetic ligands: pioglitazone, thioglitazone, rosiglitazone	GW9662	Ligand treatment inhibits Th17 differentiation; deletion of PPAR-γ enhances Th17 differentiation	Impaired development of adipocyte tissue specific Treg development; intact development of peripheral Tregs	(26), (27), (28), (29), (30)
	PPAR-β/δ		Natural ligands: prostaglandin A1 (PGA1) and prostaglandin D2 (PGD2), Synthetic ligands: GW501516, L165041	GSK0660 GSK3787	Ligand treatment inhibits Th17 differentiation; Genetic knock-out of PPAR-β/δ in mice results in exacerbated EAE	Unknown	(35), (36)
AHR	AHR1 AHR2 (nonmammalian vertebrate species)	LXR	Synthetic ligands: tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene, 6-formylindolo(3,2-b) carbazol Natural ligands: heme metabolites, indigoids, kynurenine (Kyn), 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), other tryptophan metabolites	CH-233191	FICZ treatment enhances Th17 differentiation *in vitro* and exacerbates pathogenesis in EAE; the lack of AHR gene results in less severe EAE	TCDD treatment induces Treg differentiation and ameliorates EAE	(37), (38), (39), (40), (41), (42), (43), (44)

Table 1. (Continued)

Nuclear receptors	subtypes	Cross-talk across nuclear receptors	Ligands	Antagonists	Ligand Effects on Th17	Ligand Effects on Treg	References
NGFIB	Nr4a1 (Nur77), Nr4a2 (Nurr1), Nr4a3 (NOR-1)	Unknown	Unknown; orphan receptor	N/A	Deletion of all three isoforms results in complete abrogation of natural Treg(nTreg) development and the development of Th17	Deletion of Nr4a1 results in enhanced thymic Treg population; deletion of Nr4a2 inhibits iTreg development, while ectopic expression of Nr4a2 imparts functional iTreg	(45), (46), (47), (48), (49), (50)
VDR	VDR	Unknown	Natural ligands: 1,25-dihydroxyvitamin D3	N/A	Deletion of VDR leads to enhanced Th17 development	Deletion of VDR leads to impaired Treg development and loss of suppressive capacity by Tregs; addition of vitamin D enhances Treg development	(51), (52), (53)
LXR	LXR-α (NR1H3), LXR-β (NR1H2)	RXR AHR	Natural ligands: oxysterol Synthetic ligands: GW3965, T0901317	GSK2033	Deletion of LXR leads to enhanced Th17 development and severe pathogenesis during EAE; addition of LXR ligands impairs Th17 development	Addition of LXR enhances Treg development	(54), (55), (56)

suppressive capacity. In contrast, ROR-γt and ROR-α do not appear to interfere with murine Treg development because ROR-γt/ROR-α deficiency in murine CD4+ T cells minimally affects HIF-1α-mediated Foxp3 downregulation.[14,39] Human ROR-γt binds the human Foxp3 promoter, but not the murine Foxp3 promoter, suggesting that ROR family members may regulate Foxp3 in a species-specific manner.[38]

All-trans retinoic acid receptor (RAR)

RAR consists of the α, β, and γ subtypes, and its preferential ligand is the naturally produced all-trans retinoic acid (atRA), an active metabolite of vitamin A. In addition, a RA synthetic derivative AM580 acts as a RAR agonist.[40] Once bound to its ligand, RAR forms a heterodimer with RXR and binds to retinoic acid response elements (RAREs) to regulate gene expression.[41] AtRA is known to enhance Treg and inhibit Th17 differentiation. The addition of atRA to murine CD4+ T cells enhances Foxp3 expression *in vitro* under Treg-polarizing conditions and inhibits IL-17A expression under Th17-polarizing conditions.[19] The administration of atRA *in vivo* significantly inhibits Th17 and Th1 responses in an EAE model, although Foxp3 expression levels were unaffected in CD4+ T cells. This result suggests that additional signals may counteract atRA activity *in vivo*. There appears to be little redundancy among RAR subtypes because RAR-α deficiency in murine CD4+ T cells is sufficient to abrogate atRA-mediated Treg enhancement. It is not clear whether atRA has similar effects in human Tregs, although atRA may stabilize Foxp3 expression and suppress Th17 differentiation in both murine and human nTregs.[42,43]

Consistent with RAR-RXR heterodimer formation, treatment with an RXR agonist augments RAR-dependent enhancement of Foxp3 expression.[44] Furthermore, this effect was abrogated in the presence of an RXR antagonist. Several mutual and nonexclusive mechanisms have been proposed for atRA-dependent Foxp3 enhancement in T cells. Some results have shown that atRA directly regulates Foxp3 expression by enhancing the phosphorylation of Smad3 and inhibiting the expression of the IL-6 receptor in murine naïve CD4+ T cells.[45] In contrast, other researchers have shown that Smad3 is dispensable for atRA-mediated regulation, and atRA functions indirectly by inhibiting AP-1/NFATc1-mediated Foxp3 regulation.[46] Additionally, atRA is reported to indirectly enhance Foxp3 expression by inhibiting cytokines such as IFN-γ, IL-4, and IL-21 from $CD44^{hi}$ memory CD4+ T cells.[47]

Identified RAR-α antagonists include Ro 41-5253 and LE-135. Ro 41-5253 effectively inhibits Foxp3 induction in murine CD4+ T cells under Treg-promoting conditions *in vitro*.[48] LE-135 appears to effectively mitigate atRA-dependent Foxp3 expression in colonic biopsies of ulcerative colitis patients and in colonic tissue from 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced murine colitis.[49] Furthermore, MS patients who received vitamin A supplements showed decreased T-cell proliferation in response to peptide stimulation *in vitro* compared to placebo-treated patients.[50,51] Collectively, these studies

present strong evidence that RAR represents an important immunomodulatory agent in human autoimmune diseases.[52]

Peroxisome proliferator of activated receptor (PPAR)

PPARs consist of the subtypes α, β, and γ similar to the case for ROR and RAR. PPAR was discovered for its ability to induce rapid proliferation of peroxisomes in hepatocytes treated with anti-diabetic drugs such as fibrate and glitazone, which are synthetic ligands of PPAR.[53] Subsequently, it was found that PPAR induces acyl-CoA oxidase (ACO) and mediates peroxisomal β-oxidation.[54] PPAR is one of nuclear receptors with extensively identified natural and synthetic ligands, as shown in Table 1. Additionally, each subtype of PPAR appears to play a nonredundant role in Treg/Th17 development.

PPAR-γ was originally discovered as a critical mediator of adipocyte differentiation.[55] Recently, it was found that PPAR-γ is highly expressed in CD4+ T cells and regulates cellular differentiation. Treatment with pioglitazone, a PPAR-γ ligand, significantly impaired Th17 development from murine naïve CD4+ T cells under Th17-polarizing conditions *in vitro*.[56] In addition, *in vivo* experiments showed that pioglitazone-fed mice developed less severe paralysis in an EAE model, whereas *Pparg-γ* cKO mice exhibited exacerbated paralysis. *In vitro*, pioglitazone treatment specifically inhibited human Th17 development (i.e., showed expression of IL-17A/F, IL-21, IL-22, and IL-23 receptors) in peripheral T cells from both healthy and MS patients.[56] PPAR-γ has been shown to inhibit *Il17a* expression by recruiting NcoR and SMRT to the *Rorc* promoter and blocking transcription.[56] Alternatively, PPAR-γ can inhibit the DNA binding activity of STAT3, a critical downstream mediator of IL-6.[57,58]

At the same time, PPAR-γ is essential for the development of Tregs highly enriched in the murine adipose tissue.[55] Mice that are deficient in PPAR-γ in Foxp3+ cells have significantly reduced Tregs in visceral adipose tissue in comparison to WT littermates. On the other hand, the development of Tregs in the spleen and lymph nodes does not change in these mice, suggesting that PPAR-γ may not directly regulate peripheral nTreg development *in vivo*. Another study found that ciglitazone, which is another PPAR-γ agonist, can enhance murine Treg development and Treg-suppressive capacity in a murine graft-versus-host disease model.[59] In contrast, PPAR-γ deficiency in Tregs in a murine colitis model increased the susceptibility of the mice to pathogenesis.[60]

Murine CD4+ T cells deficient in PPAR-α or PPAR-β/δ showed enhanced expression of IFN-γ (Th1) and IL-17A (Th17) in an EAE model.[61] It was found that these PPAR-α effects are gender-dependent, with male *Ppara-α* KO mice showing more severe paralysis and higher levels of IFN-γ and IL-17A secretion but with female *PPAR-α* KO mice showing pathogenic responses that were comparable to their WT littermates.[62] Interestingly, in humans, the gender dependence of Th1 and Th17 development in EAE applies to PPAR-α as well as PPAR-γ.[63] Mice treated with a synthetic PPAR-α or PPAR-β/δ agonist are also less susceptible to paralysis and show enhancement of the Th2 subset.[64,65]

It is not clear whether PPAR-α directly regulates Treg development in mice, although we found that WT and *Ppara-α* KO CD4+ T cells are equally capable of expressing Foxp3 under Treg-polarizing conditions *in vitro* (unpublished data). In human T cells, PPAR-α agonists bezafibrate and GW7647 can stabilize Foxp3 expression through epigenetic modification of the *Foxp3* promoter.[66] Therefore, PPAR-α is likely critical for maintaining the suppressive functions of both murine and human Tregs *in vitro*.[66,67] The molecular mechanism behind PPAR-α- and PPAR-β/δ-dependent Th17/Treg development remains to be elucidated, although one possible mechanism involves the interaction among PPAR subtypes.[68] Similar to other nuclear receptors, PPARs represent important therapeutic targets in major autoimmune diseases. For example, treatment with a PPAR-γ agonist, pioglitazone, was effective in treating patients with MS.[69] Additionally, PPAR-β/δ is highly expressed in plaques of psoriasis patients, and PPAR-β/δ antagonists GSK3787 and GSK0660 effectively ameliorated disease severity in a murine psoriasis model.[70]

Aryl hydrocarbon receptor (AHR)

The aryl hydrocarbon receptor (AHR) is activated by environmental toxins and is required to metabolize toxins in the liver.[71] Some of these toxins include halogenated aromatic hydrocarbons such as tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic aromatic hydrocarbons such as 3-methylcholanthrene.[72] In addition to these environmental ligands, endogenous ligands include dietary supplements and tryptophan metabolites such as 6-formylindolo[3,2-b]carbazole (FICZ).[73,74] Due to its critical role in protecting hosts from environmental toxins, AHR is highly conserved in vertebrates and only one subtype exists in mammals, although some nonmammalian organisms express two subtypes.[75]

Similar to ROR function, AHR is critical for Th17 development. AHR is highly expressed in the murine/human Th17 subset, and FICZ treatment enhances IL-17A/F and IL-22 expression in murine/human CD4+ T cells *in vitro* without altering ROR-γt expression.[76] Furthermore, the genetic KO of AHR in mice ameliorated paralysis in an EAE model, even though Th17 differentiation in the *AHR*-KO CD4+ T cells *in vitro* remains relatively intact. In a subsequent study by the same group, it was found that CH-223191, an AHR antagonist, can inhibit IL-17A expression in murine Th17-polarized CD4+ T cells *in vitro*.[77] At a molecular level, AHR appears to directly interact and inhibit STAT1 activity, a negative regulator of Th17 development.[78]

The role of AHR in Treg development remains unclear, as FICZ treatment reportedly had no effect on murine Treg development (i.e., Foxp3 expression) either *in vitro* and *in vivo*, suggesting the FICZ treatment effect is specific to Th17.[76] In contrast, another group found that murine naïve CD4+ T cells from *Ahr*-KO mice are less capable of Treg development compared to those of WT littermates.[78] Furthermore, the AHR ligand TCDD can reportedly substitute for TGF-β1 in murine Treg differentiation *in vitro*.[79] In addition, TCDD treatment can generate functional human Tregs *in vitro* through Smad1

and Aiolos.[80] Consistent with this *in vitro* data, administration of TCDD to WT mice can suppress paralysis in EAE.

It is interesting that TCDD and FICZ, which are ligands of AHR, exert different effects on Th17/Treg development. Nevertheless, AHR transcript levels are much lower in the Treg subset than in that of the Th17 subset,[76] and TCDD-dependent Treg enhancement can result from a survival advantage of Tregs against TCDD toxicity.[81] However, more recent studies with endogenous ligands such as 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) and kynurenine (Kyn) have shown the critical roles of AHR in murine Treg development *in vivo* and *in vitro*.[82,83] Although AHR ligand-mediated Th17/Treg regulation clearly requires further examination, AHR is a promising therapeutic target for the treatment of human autoimmune diseases.

Nerve growth factor-induced clone B (NGFIB)

NGFIB is an immediate early gene induced by a variety of external signals, including T-cell receptors (TCRs) on T cells.[84] NGFIB is involved in diverse cellular processes such as metabolism, apoptosis, and DNA repair. The three subtypes of NGFIB include Nr4a1 (Nurr1), Nr4a2 (Nurr77), and Nr4a3 (Nor1), which play nonredundant roles. Ligands for Nr4a1, Nr4a2, and Nr4a3 are not yet known; thus, these receptors are classified as orphan receptors.[85] The roles of NGFIB in T cells have been recently recognized in the context of Th17 and Treg subsets, similar to many other nuclear receptors. Nr4a1 is essential for the clonal deletion of thymocytes and the development of Tregs in mice.[86] *Nr4a1* KO mice have increased percentages of CD4+ and CD8+ T cells in the thymus compared with WT littermates, a phenotype that has been attributed to diminished levels of the pro-apoptotic molecule, Bim. Concurrently, these mice express a higher percentage of nTregs in the thymus, suggesting a critical role of Nr4a1 in early T-cell development.

Nr4a2, another subtype of the NGFIB subfamily, is critical for murine Th17 development.[87] The genetic ablation of Nr4a2 in CD4+ T cells resulted in a failure to induce Th17 differentiation *in vitro*. Similarly, siRNA-mediated gene silencing of *Nr4a2* in T cells ameliorated pathogenesis in murine EAE. The mechanism of Nr4a2 is interesting, because the impaired Th17 response is not accompanied by decreased ROR-γt expression. Instead, Nr4a2 seems to be a modulator of IL-23R and thus expression of IL-17A/F and IL-21. In addition, Nr4a2 is required for murine Treg development and function.[88] Deletion of *Nr4a2* in CD4+ T cells impairs Treg development, whereas the exogenous expression of Nr4a2 can lead to functional Tregs *in vitro*. This effect seems to apply to Nr4a2 but not to Nr4a1 and Nr4a3, suggesting nonredundant roles of the three NGFIB subtypes in Treg development.

In contrast, NGFIB subtypes appear to play redundant roles in the development of nTregs in mice.[89] Single KO of these genes is not sufficient to impair thymic Foxp3+ T cells, suggesting some degree of redundancy in function. In contrast, KO of all three subtypes results in severe autoimmunity in mice due to a loss of both thymic and peripheral nTregs and enhanced Th1 and Th17 responses. It is not clear why NGFIB subtypes appear to have redundant roles in nTregs and not iTregs. It can be speculated that the loss of nTreg development is more detrimental to hosts in evolutionary terms, and thus organisms develop redundant mechanisms to compensate for the potential loss of one subtype. Alternatively, this variance in redundancy of NGFIB subtypes may reflect a more complex difference in the molecular nature of nTregs and iTregs.

Other families of nuclear receptors

Other nuclear receptors that regulate Treg/Th17 development and function include the vitamin D receptor (VDR) and liver-X-receptor (LXR). The active metabolite of vitamin D is 1,25-dihydroxyvitamin D3 ($1,25(OH)_2VD_3$), which binds to VDR. VDR also forms a heterodimer with RXR and binds to vitamin D response elements (VDREs). The human Foxp3 promoter contains VDREs, and the addition of vitamin D can enhance Foxp3 expression in human T cells under Treg-polarizing conditions and enhance Treg suppressive capacity through cell–cell contacts.[90] Another study found that $1,25(OH)_2VD_3$ inhibits human Treg proliferation but has no effects on its suppression.[91] In a murine model, deletion of VDR in CD4+ T cells leads to enhanced Th17 development with increased IL-17A and IL-21 expression.[92] At the the same time, *Vdr*-KO CD4+ T cells have significantly decreased expression of Foxp3 in CD4+ T cells under Treg-polarizing conditions. Thus, VDR determines Treg/Th17 cell fates through the reciprocal inhibition of alternative fates.

Vitamin D has been implicated in major autoimmune diseases. Serum levels of $1,25(OH)_2VD_3$ were significantly correlated with the suppressive capacity of peripheral Tregs in patients with MS.[93] Additionally, low vitamin D levels in serum are associated with an increased severity of inflammatory diseases, such as MS, RA, and inflammatory bowel disease.[94] Because the oral administration of vitamin D3 to patients with Crohn's disease was found to ameliorate disease severity,[95] vitamin D may be an important regimen for autoimmune diseases.[96]

Finally, LXR is another important nuclear receptor, and it consists of two subtypes, LXR-α and LXR-β. LXR is a crucial sensor of cholesterol in cells and regulates its metabolism through transcriptional regulation.[97] For example, LXR activation by ligands can antagonize the sterol regulatory element binding protein (SREBP) pathway for cholesterol synthesis.[98] Such changes can negatively affect T-cell activation and proliferation. Treatment with the LXR agonist T0901317 inhibits differentiation of murine CD4+ T cells, while treatment with the LXR antagonist GSK2033 enhances cellular proliferation and Th1/Th2/Th17 differentiation. Consistent with the antagonist-treatment results, murine CD4+ T cells deficient in LXR show increased Th17 development under polarizing conditions compared with WT CD4+ T cells *in vitro*.[99] In contrast, the administration of LXR ligands in mice inhibits Th17 development *in vitro* and suppresses EAE *in vivo*. Interestingly, SREBP-1, which is induced by LXR activation, suppresses IL-17A expression through physical interaction with AHR. Although LXR agonists have been effective in decreasing inflammation in atherosclerosis

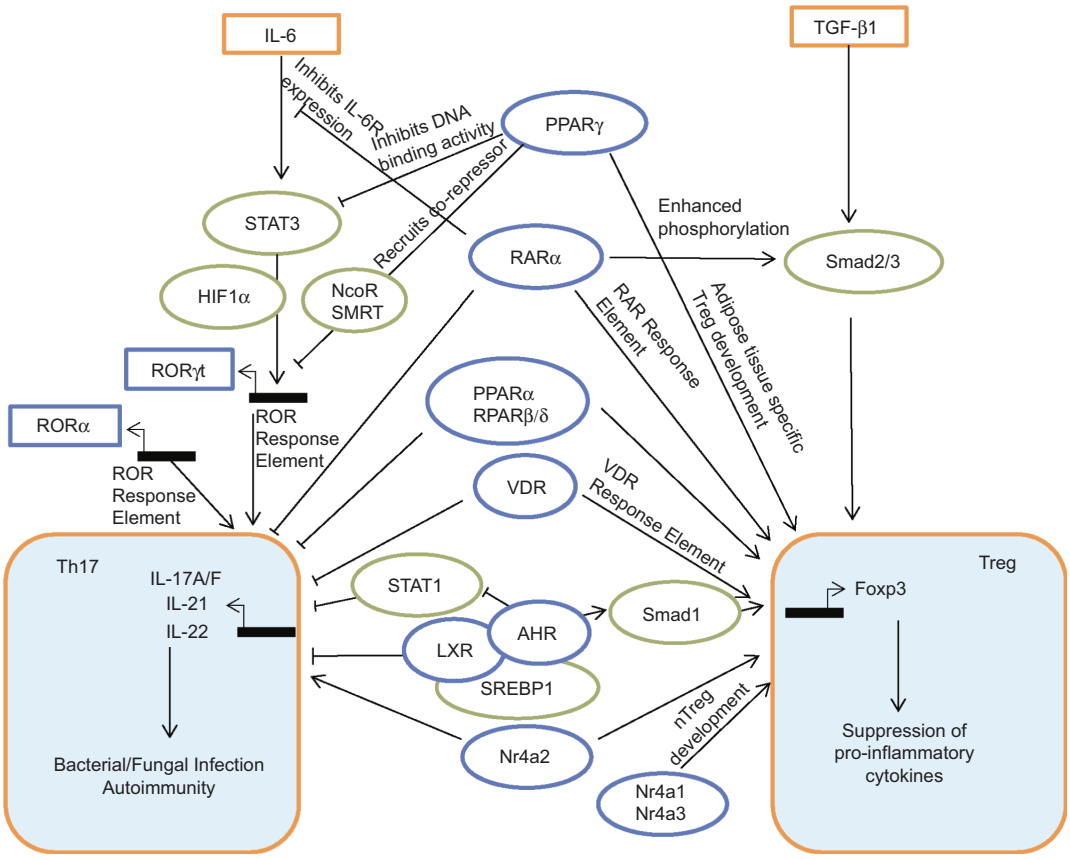

Figure 1. Schematic illustration of nuclear receptor-mediated Treg/Th17 differentiation: Pointed arrows represent positive regulation and blunt arrows represent negative regulation of the target. If known, specific mechanisms are indicated next to the arrows. Nuclear receptors are indicated in blue circles or boxes. Other transcription factors (non-nuclear receptors) are indicated by green circles. Overlapping nuclear receptors indicate direct physical interactions. Abbreviations: sterol regulatory element binding protein-1 (SREBP-1), liver-X-receptors (LXR), vitamin D receptor (VDR), aryl hydrocarbon receptor (AHR), peroxisome proliferator of activated receptor (PPAR), all-trans retinoic acid receptor (RAR), retinoic acid receptor-related orphan receptor (ROR).

and some neurodegenerative diseases,[100,101] these compounds need to be examined for therapeutic efficacy in autoimmune diseases.

Summary

Although our understanding of the critical roles of nuclear receptor families has been growing, it is yet to be investigated how nuclear receptors interact with each other and such interaction affects Th17/Treg development. For example, PPAR-β/δ activation can antagonize PPAR-γ activity. Thus, it warrants more comprehensive analysis of other nuclear receptor activation by agonist treatment or genetic deletion of a particular receptor. This type of analysis may reveal physical interactions among nuclear receptors such as AHR and LXR. Finally, although many studies have focused on the downstream effects of nuclear receptor signaling, yet upstream modulators of nuclear receptors need to be further investigated (Figure 1).

One of many challenges involved in studying nuclear receptors is their unique transrepression and transactivation mechanisms of gene regulation. These modes of regulation require additional co-activators and co-repressors. Additionally, each subfamily of nuclear receptors consists of different subtypes that may play redundant or nonredundant roles. For many subfamilies, the KO of a single subtype in mice results in the manifestation of a clear Th17/Treg phenotype (e.g., ROR, PPAR), but many other subfamilies do not follow this pattern, suggesting that certain genes are redundantly regulated by different subtypes (e.g., NGFIB). Finally, the context-dependent activation of nuclear receptors makes its regulation more complex. For example, although PPAR-γ expression is highly induced in both Th2 and Th17 subsets, ligand treatment only affects Th17 differentiation. This result suggests that individual cytokines affect the responses of nuclear receptors and cause differential activation by natural and synthetic ligands.

Another emerging point of interest is the connection between nuclear receptors and cellular metabolism.[102] It has been found that Tregs utilize fatty acid oxidation as a major source of their energy, while Th17 cells use glycolysis.[103] Additionally, it appears that the mammalian target of rapamycin (mTOR) and estrogen-related receptor-α (ERRa), both of which are sensors of environmental nutrients, can differentially affect T-cell lineage decisions.[104–106] Nevertheless, it is not clear how these metabolic

regulators modulate IL-17A and Foxp3 expression at the molecular level, and the interactions between nuclear receptors and these metabolic regulators remain to be explored.

Finally, nuclear receptors serve as important therapeutic targets. Alteration of the Treg/Th17 ratio in patients is correlated with many autoimmune diseases.[107,108] Nevertheless, many agonists and antagonists are nonspecific to a single subtype of nuclear receptor, and result in "off-target" effects (Table 1). Thus, the development of highly specific agonists or antagonists is critical for successful treatment of autoimmune diseases. At the same time, their use in cancer must be examined as altering the Th17/Treg balance may prove beneficial for anti-tumor immunity. It is known that deletion of Tregs and loss of their suppressive functions can enhance anti-tumor immune responses.[109] One study found that PPAR-α-KO mice exhibit a better control of murine B16-melanoma because of decreased Treg function.[110] In contrast, the use of agonists that favor Treg development may impose a risk of attenuated anti-tumor immune responses. These questions warrant future investigation into the effects of nuclear receptors in cancer.

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