RORγt, a multitask nuclear receptor at mucosal surfaces

Gérard Eberl

RORγt, a multitask nuclear receptor at mucosal surfaces

Gérard Eberl

Institut Pasteur, Microenvironment & Immunity Unit, 75724 Paris, France
INSERM U1224, 75724 Paris, France

Summary

RORγt is a nuclear hormone receptor that has followed an exponential success carrier. Its modest origins as an orphan receptor cloned from human pancreas blossomed within 15 years into a critical regulator of anti-microbial immunity and a major target in the fight against inflammatory pathologies. Here, I review its role as a transcription factor required for the generation of type 3 lymphoid cells, which induce the development of lymphoid tissues, provide resistance of epithelial stem cells to injury, maintain homeostasis with the symbiotic microbiota,orchestrate defense against extracellular microbes and regulate allergic responses. RORγt is also an intriguing molecule that is regulated by the circadian rhythm and includes cholesterol metabolites as ligands. RORγttherefore links anti-microbial immunity with circadian rhythms and steroids, the logic of which remains to be understood.

A brief history of RORγt

Two independent lines of research lead to the discovery ofRORγ and its shorter isoform RORγt. In the first line of research, PCR primers were designed to amplify cDNAs coding for conserved regions of the DNA binding domain of nuclear hormone receptors. This strategy aimed for the cloning of presumably all nuclear receptors expressed in a given tissue or cell line. Using this approach, novel orphan receptors named retinoid Z receptor (RZR)α and RZRβ were then identified in umbilical vain endothelial cells [1] and rat brain[2]. In addition, using hybridization cloning with the cDNA of the retinoic acid receptor (RAR)α, retinoic acid related orphan receptor (ROR)α was identified in rat brain and shown to be identical to RZRα[3]. This lead to the identification of the third member of the RZR/ROR family, RORγ, in a cDNA library from human pancreas,and later from mouse muscle, using the degenerate primer approach[4, 5]. Interestingly, melatonin was reported to activate the transcription factor activity of RORα and RORβin cultured cells[6-8], suggesting early on a role forthe ROR family of receptors in circadian rhythms.

In the second line of research, in a screen for proteins that confer resistance to TCR-mediated apoptosis,a shorter isoform of RORγ was identified by expression cloning of a thymocyte cDNA library in a T cell hybridoma[9]. While RORγ is expressed in a variety of organs, this new isoform was found to be primarily expressed in the thymus and was therefore named RORγt. Mice that lacked the expression of both RORγ and RORγt showed increased thymocyte apoptosis[9-11]. Such mice also had theremarkable phenotype of lacking lymph nodes and Peyer's patches. In knock-in mice in which the *Rorc(γt)* locus coded for GFP instead of RORγt, but still expressed RORγ, it was shown that RORγt is expressed by and is required for the development of fetal lymphoid tissue inducer (LTi) cells[12], a then enigmatic cell type that colonizes developing lymphoid tissues in the fetus before any other hematopoietic cell type[13-15].

It was nevertheless the association of RORγt with Th17 cells that shot RORγt to universal fame. In a landmark paper by Dan Cua and colleagues, it was demonstrated that IL-23, rather than IL-12,is the critical cytokine in experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis[16]. In a second paper, Cua reported that IL-23 promotes the generation of Th17 cells, the effector cells that driveEAE[17]. On the basis of transcriptome profiles, Th17 cells were

found to express high levels of RORγt as compared to Th1 cells, an observation that lead to the demonstration that Th17, like LTi cells, expressed RORγt and required RORγt for their generation[18].

Two years later, the world of RORγt$^+$ cells expanded again. A population of LTi-like cells was identified that express markers of NK cells, such as NKp46, as well as the signature cytokines IL-17 and IL-22, but do not cluster and induce the development of lymphoid tissues[19-22]. To keep controlof the expanding universe of LTi-likecells, the term "innate lymphoid cells" (ILCs) was coined[23, 24]. It was progressively realized that the ILC universe includes ILC1s, ILC2s and (RORγt$^+$) ILC3s, which mirror Th1, Th2 and Th17 cells in the expression of signature transcription factors, surface markers and effector cytokines[25].

A recent observation brings RORγt back full circle to its original description as a cousin of the melatonin receptorsRORα and RORβ [6-8]. RORγis a component of the transcriptional network of peripheral circadian clocks[26, 27], which regulates the transcription factor Nfil3, which in turn represses the expression of RORγt[28]. Deregulation of the circadian clock thus leads to a deregulation of Th17 cells and increased susceptibility to inflammatory pathology. Finally, and no less intriguing, cholesterol metabolites are natural ligands of RORγt[29, 30], possibly linking type 3 immunity to the endocrine system and metabolism, even though the biology of these ligands remains to be understood.

RORγt and lymphoid tissue development

LTi cells were first describedas CD3$^-$CD4$^+$or CD3$^-$IL-7Rα$^+$cells in the developing lymph nodes (LNs)[13, 14]and Peyer's patches (PPs)[15] (**Figure 1**). Mebius' and Nishikawa's labs characterizedLTi cells and their interactions with stroma cellswithin the lymphoid tissue anlagen[31, 32], but it is the discovery of RORγt that allowed to demonstrate formally that LTi cells are required for the development of lymphoid tissues[12]. In the fetus, LTi cells are the only cells expressing RORγt. In RORγt-deficient mice, LTi cells are absent and LNs and PPs fail to develop. These cells express several members of the TNF superfamily, such as soluble lymphotoxin (LT) $\alpha_3$and its membrane-bound variant LT$\alpha_1\beta_2$, TRANCE and TRANCE-L[14, 31, 33]. LT$\alpha_1\beta_2$ is essential for the development of lymphoid tissues[34-36], as it engages LTβR

on specialized stroma cells, which in turninduces the expression of adhesion molecules and chemokines that recruit lymphocytes and myeloid cells[37].

After birth, LTi cells cluster in hundreds of so-called cryptopatches (CPs)located between crypts of the intestinal lamina propria[38, 39]. CPs collect B cells todevelop into isolated lymphoid follicles (ILFs), which generate mostly T cell-independent IgA^+ B cells[40-42]. Surprisingly, the formation of ILFs from CPs requires bacterial microbiota[41, 43]. Proliferating bacteria release peptidoglycans from their cell wall, which is recognized by the innate receptor NOD-1 in epithelial cells[44]. This unleashes an activation cascade through the release of CCL-20, the activation of the $CCR6^+$ (the receptor for CCL-20) LTi cells and stroma cells in CPs and the recruitment of B cells[43, 45]. In RORγt-deficient mice, ILFs-like structures still develop[46]. However, these structures are termed tertiary lymphoid tissues (tLTs), which are induced by chronic inflammation in most organs independently of LTi cells, through the expression of $LT\alpha_1\beta_2$ by subsets of B cells, T cells or NK cells[47].

Intriguingly, LTi cells are retained in mature (adult) LNs and Peyer's patches, and locate in the cortex between B cell follicles[48]. Their functions in this region remains enigmatic, mainly because it has been so far impossible to investigate the consequence of a absence of LTi cells in adult lymphoid tissues, as RORγt-deficient mice lack both.

ILC3s

A population of intestinal lymphoid cells expressing the NK marker NKp46 in mouse and NKp44 in human, were found to co-express RORγt and IL-22[19-22]. These cells were originally named NK22 cells, but their requirement for RORγt rather than Eomesin development, as well as their cytokine profile, makes them more similar to LTi cells than to NK cells. However, in contrast to LTi cells, NK22 do not cluster in CPs, and therefore, are not involved in the development of lymph nodes, Peyer's patches and ILFs[49, 50]. Rather, NK22 are viewed as more "regular" effector cells that patrol the tissue and produce effector cytokines where it matters in terms of defense and injury[51].

Given their common dependence on RORγt and their similar cytokine profiles, LTi cells and NK22 cells were grouped together as ILC3s, while lymphoid cells that produce the type 2 cytokines IL-4, IL-5 and IL-13 were grouped as ILC2s, and those

that produce IFNγ as ILC1s[23, 24].A difficulty arose when it was found that ILC3s can downregulate RORγt and upregulate Tbet to produce IFNγin a context of intense inflammation[52]. These cells were termed ex-ILC3s.Another form of ILC3 plasticity was found *in vitro*using human ILC lines. When stimulated through TLR2, such lines expressed IL-5 and IL-13 in addition to IL-22[53]. It remains to be assessed to what extent such plasticity is operational *in vivo*, underlying for example the rapid response of ILCs to diverse types of tissue perturbations.

The prompt responsiveness of ILCsto infection and injuryplaces them upstream in the immune response. ILC3s are activated by the inducer cytokines IL-1β and IL-23 produced by macrophages and DCs, typically in response to infection by extracellular bacteria and fungi. Activated ILC3s produce the effectors cytokines IL-17, IL-22, GM-CSF, LT$\alpha_3$and LT$\alpha_1\beta_2$, which in turn induce the production of anti-microbial peptides (AMPs) by epithelial cells, of neutrophil-recruiting chemokines by stromal cells and epithelial cells[25, 54], and the activation of B cells [55, 56]. This antigen-independent mode of activation of ILCs is similar to that of memory and "innate" T cells, at least in terms of kinetics[57]. It does not require selection and expansion of antigen-specific clones, but rather relies on the expansion of populations. It remains to be understood whether sub-subsets of ILCs exist and specifically expand in reaction to particular infections and injuries, thereby providing a form of immunological memory to challenges, as described for NK cells[58].

ILC3s play a critical role early in the protection to enteropathogens, such as the proteobacteria *Citrobacter rodentium*, a homologue of the human *E. coli*intestinal pathogens[19], to *Salmonella enterica*[52]and to rotaviruses[59], through their production of IL-22. They also mediate containment of bacterial symbionts that live within Peyer's patches[60], and more generally protect from dextran sodium sulfate (DSS)-induced inflammation through the containment of symbiotic bacteria[46, 61]. The secretion by ILC3s of IL-22 also plays a role in the protection of epithelial cells from apoptosisinduced by chemotherapy or irradiationin the intestine and the thymus[62-64], as well as in the protection of hepatocytes from inflammation[65]. Intriguingly, ILC3s are relatively resistant to irradiation and chemotherapy, possibly as a consequence of their low turnover[12, 49, 66].

On the other hand, ILC3s and IL-22 are involved in the progression of colon cancer[67], presumablybecause they protect epithelial cells from apoptosis through the

activation of the transcription factor STAT-3[62-64]. In addition, ILC3s contribute to inflammatory pathology through their capacity to co-expressIFNγduring *Salmonella* infection in mouse[52] and in animals and patients suffering from inflammatory bowel disease (IBD)[68, 69]. The expression of IL-17 by ILC3s is involved in obesity-associated asthma induced by high fat diet[70], possibly as a consequence of a loss of containment of the intestinal microbiota and an induction of ILC3s in adipose tissue by incoming bacteria and bacterial compounds[71].

Another surprising feature of ILC3s is their expression of MHC class II[12, 14], as well as of several components of the class II antigen-processing pathway. This feature allows the ILC3s to present antigens and repress the activity of specific T cells in the intestine during homeostasis[72], and to induce T cells activation in the spleen[73]. The relative role of ILC3s, DCs and macrophages in the MHC class II-restricted activation and regulation of T cells[74], at least in the intestine and the lymphoid tissues, remains to be measured.

So, ILC3s are pivotal to many processes in mucosal immunity, from the development of lymphoid tissues and the containment of the microbiota, the early immunity to pathogens and the protection of epithelial cells, to the exacerbation of inflammatory pathology and the progression of cancer. Even though ILC3s depend on RORγt for their development, it remains to be determined whether RORγt is also required for their maintenance, an important consideration when targeting RORγt with agonists or antagonists to regulate type 3 immune responses[75, 76]. A recent report shows that a RORγt antagonist leads to the loss of Th17 cells but not of ILC3s[77].

Thymocytes and Th17 cells

RORγt is required for the survival of immature $CD4^+CD8^+$ thymocytes by regulating the level of the anti-apoptotic factor Bcl-x_L[10]. In the absence of RORγt, immature thymocytes can spend less time at the $CD4^+CD8^+$ stage and therefore, recombination of the genes coding for the TCRα chain is biased towards proximal Vα to Jα re-arrangements[78]. The thymus of a RORγt-deficient mouse contains half the number of cells found in the thymus of a wild-type animal[10]. In the periphery, control of anti-apoptotic genesby RORγt has not been reported.

Expression of RORγt by T cells remained confined to immature thymocytes, until Th17 cells develop that require RORγt[18]. Naive $CD4^+$ T cells are induced into

the Th17 pathway by IL-1β[79], IL-23[17, 80], or the combination of IL-6 and TGFβ[81-83]. IL-23 and IL-6 induce the phosphorylation of STAT-3, which in turn induces the expression of RORγt[84]. The characterization of Th17 cells was initially reported in the context of EAE as the cells that are induced by IL-23 to express IL-17 and provoke autoimmunity[17], as well as in arthritis[85, 86] and IBD[87, 88]. Therefore, RORγtwas firstperceived as a public enemy that must be targeted to block the progression of autoimmune inflammation. Another line of research nevertheless showed the importance of Th17 cells in mucosal immunity to pathogens and their role in the containment of the microbiota through both the production of IL-17 and IL-22[89, 90].

Caught in the middle of these confusing perceptions, IL-17 and IL-22 have been sometimes described as both pro- and anti-inflammatory, which only reflects the homeostatic or pathologic context in which these cytokines were studied. In contrast, ILC3s, also acting both during intestinal homeostasis and pathology, were first characterized in the context of intestinal homeostasis and thus as primarily "beneficial" cells[39, 43]. Nevertheless, the distinctive role of Th17 cells and ILC3s in homeostasis, defense and pathology has been difficult to pull apart, as models to ablate Th17 cells or ILC3s, individually, have to be improved [19, 60]. Based on their innate versus adaptive nature, early responses against pathogens have generally been "assigned"to ILC3s, while chronic responses, such as autoimmune inflammation, have been assignedto Th17 cells.

The mechanisms by which microbes induce Th17 cells, and more generally type 3 immune responses,remain a hard nut to crack. Whereas it is clear that IL-1β and IL-23 play a central role in the cascade of events that lead to the generation of Th17 cells[17, 79, 80, 91], the signals that induce IL-23 are not known. It has been shown that ATP, produced by bacteria, activates a subset of intestinal lamina propria DCs through the P2X and P2Y receptors to produce IL-23[92], but this mechanism remains to be validated in the context ofbacteria that adhere to epithelial cells, such as segmented filamentous bacteria (SFB)[93, 94] and pathogenic strains of *E. coli*, which efficiently induce the generation of Th17 cells[95]. The pathway by which these adherent bacteria induce Th17 cells remains unknown.

Type 3 Tregs

FoxP3 is the signature transcription factor for regulatory T (Treg) cells. Therefore, it came as a surprise that a significant proportion of intestinal FoxP3$^+$ T cells also express RORγt[96]. It was suggested that RORγt$^+$ FoxP3$^+$ cells are not Tregs, but rather precursor T cells that express both transcription factors until expression of one is promoted over the other to generate Tregs or Th17 cells[83]. This hypothesis was derived form the observation that 25% of intestinal Th17 cells had expressed FoxP3 at some stage of their development, as determined by genetic fate mapping of FoxP3$^+$ cells. It was also suggested that Tregs acquire characteristics of effectors cells, induced by the inflammatory state of the tissue[97]. For example, colitis induces the expression of IL-23 by macrophages and DCs, which in turn induces the expression of RORγt in developing effector T cells as well as in Tregs. This leads to the expression of the chemokine receptor CCR6 on both mature Th17 and RORγt$^+$ Tregs[96], and thus co-localization of both the effector and the regulator cells to augment the efficacy of immune regulation[98]. Furthermore, a small proportion of RORγt$^+$ Tregs was found to be "perverted"into genuine effector cells, induced by chronic inflammationinto the expression of IL-17 [99].

Nevertheless, the majority of RORγt$^+$ Tregs produces IL-10 at levels that exceed the levels produced by other subsets of Tregs, and express high levels of other attributes of Tregs, such as ICOS, CTLA-4, CD39 and CD73[100], and exert regulatory functions *in vitro* and *in vivo*[96, 98]. Moreover, IL-10-producing RORγt$^+$ Tregs dramatically expand during intestinal and lung inflammation, presumably to avoid exponential growth of the inflammation[96]. Nevertheless, it is possible that the phenomenon of Treg "perversion" expandsduring chronic long-term inflammation, conditions found in the intestine of IBD and colon cancer patients[99]- but rarely obtained in mouse models.

Recently, it was demonstrated that microbiota- and antigen-induced Tregs express RORγt, whereas microbiota-independent Tregs include asubset expressing Gata3[100-103]. The generation of RORγt$^+$ Tregs is dependent on antigen presentation by DCs and macrophages, as well as on the activation of STAT3[100]. Surprisingly, the "pro-Th17" cytokines IL-6 and IL-23, rather than IL-10, activate STAT3 for the generation of RORγt$^+$ Tregs. The eventual lineage choice to generate RORγt$^+$ Tregs instead of Th17 cells is dependent on the metabolism of vitamin A into retinoic acid

(RA), as an absence of vitamin A, or the inhibition of the RA receptor, tips the balance in favor of Th17 cells[100]. This suggests that the normal metabolism of vitamin A by DCs, stromal cells and neurons, rather than microbes, determines the balance between RORγt$^+$ Tregs and Th17, or in other words, between anti- and pro-inflammatory (type 3) responses. I therefore propose that RA is a "normo-signal", required in many physiological processes, which is used by the immune system to monitor the health state of a tissue. In the context of tissue damage,upon infection or injury, the production of RA is decreased and the balance between RORγt$^+$ Tregs and Th17 cells is shifted in favor of Th17 cells. During homeostasis in the intestine, this balance is regulated to the level of type 3 immunity required to contain the symbiotic microbiota.

In one study, the absence of RORγt$^+$ Tregs lead to the increase in Th1 and Th17 cells, and the exacerbation of colitis[101]. However, in another study, RORγt$^+$ Tregs were not found to control Th1 and Th17 cells, but rather Th2 cells[100]. It was proposed that bacteria induce type 3 responses, mediated by Th17 cells and RORγt$^+$ Tregs, which collectively repress competing type 1 or type 2 responses, a phenomenon described by the equilibrium model of immunity[104]. As a consequence, in mice that lack RORγt$^+$ Tregs or Th17 cells, anti-helminth responses are increasedand allergic inflammation is exacerbated.Thus microbiota regulates allergy through the induction of type 3 responses[100]. The exacerbated Th17 responses observed in the absence of RORγt$^+$ Tregs, reported in the first study[101], may be the consequence of an intestinal microbiota that is more potently inducing type 3 responses than in the second study [100].

The developmental origin of RORγt$^+$ Tregs remains debated and is difficult to address. Do they derive from Th17 cells, RORγt$^-$ FoxP3$^+$ Tregs, or naïve T cells? Genetic fate mapping, using *Foxp3* or *Rorc(γt),* cannot be conclusive, as FoxP3 is continuously expressed in the Treg lineage, whereas RORγt is already expressed by immature CD4$^+$CD8$^+$ T cells in the thymus[39]. Resolution of the differentiation pathway of RORγt$^+$ Tregs requires the transfer of single T cells, or individual T cell barcoding.

Other cells expressing RORγt

RORγt is expressed exclusively by lymphoid cells. Or so we thought. Populations of bone marrow and human blood neutrophils express RORγt, as well as IL-17 upon stimulation with IL-6 and IL-23[105]. A similar subset of RORγt$^+$ neutrophils was identified in mice that expresses IL-17 upon *Aspergillus fumigatus* infection of the lungs[106]. These observations are reminiscent of older data showing that LPS induces lung neutrophilia, the recruitment of which is induced by CXC chemokines produced by stromal and epithelial cells. These chemokines are in turn induced by IL-17 produced by neutrophils and T cells[107].

RORγt as a molecule

The natural ligand for RORγt has remained elusive for long (**Figure 2**). The related RORα was co-crystallized with cholesterol and cholesterol sulfate[108]. Recently, it was found that cholesterol biosynthetic intermediates, such as oxysterols, are involved in the function of RORγt during the development of lymph nodes and the differentiation of Th17 cells and IL-17$^+$ Tγδcells [29, 109]. These sterols are likely to be endogenous metabolites, rather than compounds derived from the microbiota, as lymph nodes and ILC3s develop in germ free animals. Interestingly, 7α,27-hydroxycholesterol, is both a ligand for RORγt and EBI2, a G protein-coupled receptor involved in the positioning of activated B cells in lymph nodes. The functional link between RORγt and EBI2 remains unexplored[30].

Synthetic inverse agonists of RORγt have been identified that derive from LXR agonists [75]or sterols such as digoxin [76] and ursolic acid [110, 111]. Such compounds trigger intense interests from the pharmaceutic industry as drugs to block type 3 responses during autoimmunity and IBD. Evidently, given the multiple cells and pathways that depend on RORγt, including the containment of the intestinal microbiota and the regulation of competing immune responses such as allergic responses, such a strategy may cause important collateral damage, or be inefficient in the intestine[46, 100]. It is possible that for similar reasons, anti-IL-23 treatment is inefficient or even deleterious in the context of IBD, even though it is very efficient in the context of skin disease[112]. Interestingly, it appears that Th17 cells are more sensitive to transient RORγt inhibition than ILC3s[77], indicating that partial blockage of type 3 responses can be achieved with RORγt inhibitors that may preserve the activity of ILC3s and some level of microbial containment.

Regulation of RORγt also occurs at the protein-protein level. Itch is an E3 ubiquitin ligase that binds RORγt and targets it for ubiquitination and degradation[113]. In the absence of Itch, mice develop IL-17-dependent intestinal inflammation and tumorigenesis. Nitric oxide (NO) also regulates the activity of RORγt through nitration of tyrosine residues[114]. As a consequence, iNOS-deficient mice develop enhanced Th17 differentiation. At the transcription factor level, FoxP3 binds directly to RORγtand appears to impose its anti-inflammatory programin RORγt^{+} Tregs over the pro-inflammatory program induced by RORγt[83, 96]. Gata3, the signature transcription factor of type 2 responses, directly binds to and inhibits the *Rorc* promoter[103]. Finally, T-bet, the signature transcription factor of type 1 responses, prevents Runx1 to mediate transactivation of *Rorc*[115].

An interesting twist in the regulation of RORγt expression involves Nfil3. This basic leucine zipper transcription factor is required for the development of NK cells[116], as well as more generally for the development of ILCs[117-119]. Nfil3 is negatively regulated by the clock protein Rev-Erbα, and in turn, negatively regulates expression of RORγt by directly binding to the *Rorc* promoter[28]. As a consequence, the expression of RORγt, and the amplitude of type 3 responses, is regulated by the circadian clock, and disruption of the circadian rhythm leads to increased susceptibility to type 3 inflammatory pathology.

RORγt and RORγ

RORγ directly regulates the expression of clock genes through binding to the promoter of Cry1, Bmal1, Rev-Erbα and also Nfil3[120]. Thus, RORγ regulates clock genes, and one of those clock genes, Nfil3, regulates RORγt[28]. However, RORγ and RORγt are not usually co-expressed in the same type of cells. Whereas RORγt is expressed in lymphoid cells (and neutrophils) exclusively, RORγ is not expressed in hematopoietic cells, but in many other types of parenchymal cells, such as hepatocytes and muscle cells[9]. Therefore, it is possible that RORγ and RORγt have similarbiology in different cells, both connected to circadian clocks and oxysterol ligands[29, 109], but have different functions through the regulation of distinct set of tissue-specific genes.

Interestingly, the other members of the ROR family, RORα and RORβ, also play important roles in the circadian rhythms, as both RORα-deficient mice and RORβ-deficient mice show alterations in circadian oscillations[121].

Concluding remarks

RORγt has turned out to be an extraordinary nuclear receptor and transcription factor, which controls type 3 immunity, a critical branch of immune responses that contains the symbiotic microbiota at mucosal surfaces and fights bacterial and fungal pathogens, but also leads to autoimmunity and cancer. The links between RORγt, oxysterols and clock genes makes RORγt a potential node connecting immunity with metabolism and circadian rhythms. The biology of RORγt blossoms at an exciting time as immunology expands into a more transversal field of research connecting different physiological systems.

Acknowledgements

This work has been supported by the Institut Pasteur.

References

1. Becker-Andre, M., Andre, E. & DeLamarter, J.F. Identification of nuclear receptor mRNAs by RT-PCR amplification of conserved zinc-finger motif sequences. *Biochem Biophys Res Commun***194**, 1371-9 (1993).
2. Carlberg, C., Hooft van Huijsduijnen, R., Staple, J.K., DeLamarter, J.F. & Becker-Andre, M. RZRs, a new family of retinoid-related orphan receptors that function as both monomers and homodimers. *Mol Endocrinol***8**, 757-770. (1994).
3. Giguere, V. et al. Isoform-specific amino-terminal domains dictate DNA-binding properties of ROR alpha, a novel family of orphan hormone nuclear receptors. *Genes Dev***8**, 538-53 (1994).
4. Hirose, T., Smith, R.J. & Jetten, A.M. RORγ: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. *Biochem Biophys Res Commun***205**, 1976-1983. (1994).
5. Medvedev, A., Yan, Z.H., Hirose, T., Giguere, V. & Jetten, A.M. Cloning of a cDNA encoding the murine orphan receptor RZR/RORγ and characterization of its response element. *Gene***181**, 199-206 (1996).
6. Becker-Andre, M. et al. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. *J Biol Chem***269**, 28531-28534 (1994).
7. Wiesenberg, I., Missbach, M., Kahlen, J.P., Schrader, M. & Carlberg, C. Transcriptional activation of the nuclear receptor RZR α by the pineal gland hormone melatonin and identification of CGP 52608 as a synthetic ligand. *Nucleic Acids Res***23**, 327-333. (1995).
8. Carlberg, C. & Wiesenberg, I. The orphan receptor family RZR/ROR, melatonin and 5-lipoxygenase: an unexpected relationship. *J Pineal Res***18**, 171-178 (1995).
9. He, Y.W., Deftos, M.L., Ojala, E.W. & Bevan, M.J. RORγt, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. *Immunity***9**, 797-806 (1998).
10. Sun, Z. et al. Requirement for RORγ in thymocyte survival and lymphoid organ development. *Science***288**, 2369-2373 (2000).
11. Kurebayashi, S. et al. Retinoid-related orphan receptor γ (RORγ) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. *Proc Natl Acad Sci U S A***97**, 10132-10137 (2000).
12. Eberl, G. et al. An essential function for the nuclear receptor RORγt in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol***5**, 64-73 (2004).
13. Mebius, R.E., Streeter, P.R., Michie, S., Butcher, E.C. & Weissman, I.L. A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits $CD4^+CD3^-$ cells to colonize lymph nodes. *Proc Natl Acad Sci U S A***93**, 11019-11024 (1996).
14. Mebius, R.E., Rennert, P. & Weissman, I.L. Developing lymph nodes collect $CD4^+CD3^-LT\beta^+$ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity***7**, 493-504 (1997).
15. Adachi, S., Yoshida, H., Kataoka, H. & Nishikawa, S. Three distinctive steps in Peyer's patch formation of murine embryo. *Int Immunol***9**, 507-514 (1997).
16. Cua, D.J. et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature***421**, 744-8 (2003).
17. Langrish, C.L. et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med***201**, 233-40 (2005).

18. Ivanov, II et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17$^+$ T helper cells. *Cell***126**, 1121-1133 (2006).
19. Satoh-Takayama, N. et al. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity***29**, 958-70 (2008).
20. Luci, C. et al. Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. *Nat Immunol***10**, 75-82 (2009).
21. Sanos, S.L. et al. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat Immunol***10**, 83-91 (2009).
22. Cella, M. et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature***457**, 722-5 (2009).
23. Spits, H. et al. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol***13**, 145-9 (2013).
24. Spits, H. & Di Santo, J.P. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol***12**, 21-7 (2011).
25. Eberl, G., Colonna, M., Di Santo, J.P. & McKenzie, A.N. Innate lymphoid cells: a new paradigm in immunology. *Science***348**, aaa6566 (2015).
26. Preitner, N. et al. The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell***110**, 251-260. (2002).
27. Ueda, H.R. et al. A transcription factor response element for gene expression during circadian night. *Nature***418**, 534-539. (2002).
28. Yu, X. et al. TH17 cell differentiation is regulated by the circadian clock. *Science***342**, 727-30 (2013).
29. Santori, F.R. et al. Identification of natural RORgamma ligands that regulate the development of lymphoid cells. *Cell Metab***21**, 286-97 (2015).
30. Cyster, J.G., Dang, E.V., Reboldi, A. & Yi, T. 25-Hydroxycholesterols in innate and adaptive immunity. *Nat Rev Immunol***14**, 731-43 (2014).
31. Yoshida, H. et al. IL-7 receptor α^+ CD3$^-$ cells in the embryonic intestine induces the organizing center of Peyer's patches. *Int Immunol***11**, 643-655 (1999).
32. Honda, K. et al. Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. *J Exp Med***193**, 621-630 (2001).
33. Kim, D. et al. Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. *J Exp Med***192**, 1467-1478 (2000).
34. De Togni, P. et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science***264**, 703-707 (1994).
35. Rennert, P.D., Browning, J.L., Mebius, R., Mackay, F. & Hochman, P.S. Surface lymphotoxin α/β complex is required for the development of peripheral lymphoid organs. *J Exp Med***184**, 1999-2006 (1996).
36. Alimzhanov, M.B. et al. Abnormal development of secondary lymphoid tissues in lymphotoxin β-deficient mice. *Proc Natl Acad Sci U S A***94**, 9302-9307. (1997).
37. Dejardin, E. et al. The lymphotoxin-β receptor induces different patterns of gene expression via two NF-kappaB pathways. *Immunity***17**, 525-535 (2002).
38. Kanamori, Y. et al. Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit$^+$ IL-7R$^+$ Thy1$^+$ lympho-hemopoietic progenitors develop. *J Exp Med***184**, 1449-1459 (1996).

39. Eberl, G. & Littman, D.R. Thymic origin of intestinal αβ T cells revealed by fate mapping of RORγt^{+} cells. *Science***305**, 248-251 (2004).
40. Hamada, H. et al. Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J Immunol***168**, 57-64 (2002).
41. Lorenz, R.G., Chaplin, D.D., McDonald, K.G., McDonough, J.S. & Newberry, R.D. Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin β receptor, and TNF receptor I function. *J Immunol***170**, 5475-5482 (2003).
42. Tsuji, M. et al. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin a generation in the gut. *Immunity***29**, 261-271 (2008).
43. Bouskra, D. et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature***456**, 507-10 (2008).
44. Girardin, S.E. et al. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science***300**, 1584-1587 (2003).
45. McDonald, K.G. et al. CC chemokine receptor 6 expression by B lymphocytes is essential for the development of isolated lymphoid follicles. *Am J Pathol***170**, 1229-1240 (2007).
46. Lochner, M. et al. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORγt and LTi cells. *J Exp Med***208**, 125-134. (2011).
47. Aloisi, F. & Pujol-Borrell, R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol***6**, 205-17 (2006).
48. Mackley, E.C. et al. CCR7-dependent trafficking of RORgamma(+) ILCs creates a unique microenvironment within mucosal draining lymph nodes. *Nat Commun***6**, 5862 (2015).
49. Sawa, S. et al. Lineage relationship analysis of RORγt+ innate lymphoid cells. *Science***330**, 665-9 (2010).
50. Reynders, A. et al. Identity, regulation and in vivo function of gut NKp46+RORgammat+ and NKp46+RORgammat- lymphoid cells. *EMBO J***30**, 2934-47 (2011).
51. Satoh-Takayama, N. et al. The Chemokine Receptor CXCR6 Controls the Functional Topography of Interleukin-22 Producing Intestinal Innate Lymphoid Cells. *Immunity***41**, 776-788 (2014).
52. Klose, C.S. et al. A T-bet gradient controls the fate and function of CCR6-RORgammat+ innate lymphoid cells. *Nature***494**, 261-5 (2013).
53. Crellin, N.K. et al. Regulation of cytokine secretion in human CD127(+) LTi-like innate lymphoid cells by Toll-like receptor 2. *Immunity***33**, 752-64 (2010).
54. Wang, Y. et al. Lymphotoxin beta receptor signaling in intestinal epithelial cells orchestrates innate immune responses against mucosal bacterial infection. *Immunity***32**, 403-13 (2010).
55. Kruglov, A.A. et al. Nonredundant function of soluble LTalpha3 produced by innate lymphoid cells in intestinal homeostasis. *Science***342**, 1243-6 (2013).
56. Magri, G. et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. *Nat Immunol***15**, 354-64 (2014).
57. Fan, X. & Rudensky, A.Y. Hallmarks of Tissue-Resident Lymphocytes. *Cell***164**, 1198-211 (2016).
58. Sun, J.C., Beilke, J.N. & Lanier, L.L. Adaptive immune features of natural killer cells. *Nature***457**, 557-61 (2009).

59. Hernandez, P.P. et al. Interferon-lambda and interleukin 22 act synergistically for the induction of interferon-stimulated genes and control of rotavirus infection. *Nat Immunol***16**, 698-707 (2015).
60. Sonnenberg, G.F. et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science***336**, 1321-5 (2012).
61. Sawa, S. et al. RORgammat(+) innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol***12**, 320-326 (2011).
62. Aparicio-Domingo, P. et al. Type 3 innate lymphoid cells maintain intestinal epithelial stem cells after tissue damage. *J Exp Med***212**, 1783-91 (2015).
63. Lindemans, C.A. et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature***528**, 560-4 (2015).
64. Dudakov, J.A. et al. Interleukin-22 drives endogenous thymic regeneration in mice. *Science***336**, 91-5 (2012).
65. Zenewicz, L.A. et al. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity***27**, 647-59 (2007).
66. Gasteiger, G., Fan, X., Dikiy, S., Lee, S.Y. & Rudensky, A.Y. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science***350**, 981-5 (2015).
67. Kirchberger, S. et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J Exp Med***210**, 917-31 (2013).
68. Buonocore, S. et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature***464**, 1371-5 (2010).
69. Geremia, A. et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med***208**, 1127-33 (2011).
70. Kim, H.Y. et al. Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med***20**, 54-61 (2014).
71. Burcelin, R., Garidou, L. & Pomie, C. Immuno-microbiota cross and talk: the new paradigm of metabolic diseases. *Semin Immunol***24**, 67-74 (2012).
72. Hepworth, M.R. et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature***498**, 113-7 (2013).
73. von Burg, N. et al. Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. *Proc Natl Acad Sci U S A***111**, 12835-40 (2014).
74. Mortha, A. et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science***343**, 1249288 (2014).
75. Solt, L.A. et al. Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature***472**, 491-4 (2011).
76. Huh, J.R. et al. Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgammat activity. *Nature***472**, 486-90 (2011).
77. Withers, D.R. et al. Transient inhibition of ROR-gammat therapeutically limits intestinal inflammation by reducing TH17 cells and preserving group 3 innate lymphoid cells. *Nat Med***22**, 319-23 (2016).
78. Guo, J. et al. Regulation of the TCRα repertoire by the survival window of $CD4^+CD8^+$ thymocytes. *Nat Immunol***3**, 469-476 (2002).
79. Sutton, C., Brereton, C., Keogh, B., Mills, K.H. & Lavelle, E.C. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med***203**, 1685-91 (2006).

80. Harrington, L.E. et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol***6**, 1123-32 (2005).
81. Mangan, P.R. et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature***441**, 231-4 (2006).
82. Bettelli, E. et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature***441**, 235-8 (2006).
83. Zhou, L. et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature***453**, 236-40 (2008).
84. Harris, T.J. et al. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J Immunol***179**, 4313-7 (2007).
85. Sato, K. et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J Exp Med***203**, 2673-82 (2006).
86. Hirota, K. et al. T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. *J Exp Med***204**, 41-7 (2007).
87. Elson, C.O. et al. Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. *Gastroenterology***132**, 2359-70 (2007).
88. Kastelein, R.A., Hunter, C.A. & Cua, D.J. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol***25**, 221-42 (2007).
89. Aujla, S.J. et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med***14**, 275-81 (2008).
90. Khader, S.A., Gaffen, S.L. & Kolls, J.K. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol***2**, 403-11 (2009).
91. Sano, T. et al. An IL-23R/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses. *Cell***163**, 381-93 (2015).
92. Atarashi, K. et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature***455**, 808-12 (2008).
93. Ivanov, I.I. et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell***139**, 485-498 (2009).
94. Gaboriau-Routhiau, V. et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity***31**, 677-89 (2009).
95. Atarashi, K. et al. Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell***163**, 367-80 (2015).
96. Lochner, M. et al. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORγt+ T cells. *J Exp Med***205**, 1381-93 (2008).
97. Wohlfert, E. & Belkaid, Y. Plasticity of T reg at infected sites. *Mucosal Immunol***3**, 213-5 (2010).
98. Yang, B.H. et al. Foxp3(+) T cells expressing RORgammat represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol***9**, 444-57 (2016).
99. Blatner, N.R. et al. Expression of RORgammat Marks a Pathogenic Regulatory T Cell Subset in Human Colon Cancer. *Sci Transl Med***4**, 164ra159 (2012).
100. Ohnmacht, C. et al. The microbiota regulates type 2 immunity through RORgt+ T cells. *Science***349**, 989-93 (2015).

101. Sefik, E. et al. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science***349**, 993-7 (2015).
102. Schiering, C. et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature***513**, 564-8 (2014).
103. Wohlfert, E.A. et al. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. *J Clin Invest***121**, 4503-15 (2011).
104. Eberl, G. Immunity by Equilibrium. *Nat Rev Immunol***doi: 10.1038/nri.2016.75** (2016).
105. Taylor, P.R. et al. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORgammat and dectin-2. *Nat Immunol***15**, 143-51 (2014).
106. Savers, A. et al. Infection-Mediated Priming of Phagocytes Protects against Lethal Secondary Aspergillus fumigatus Challenge. *PLoS One***11**, e0153829 (2016).
107. Ferretti, S., Bonneau, O., Dubois, G.R., Jones, C.E. & Trifilieff, A. IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol***170**, 2106-12 (2003).
108. Kallen, J.A. et al. X-ray structure of the hRORalpha LBD at 1.63 A: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORalpha. *Structure***10**, 1697-707 (2002).
109. Soroosh, P. et al. Oxysterols are agonist ligands of RORgammat and drive Th17 cell differentiation. *Proc Natl Acad Sci U S A***111**, 12163-8 (2014).
110. Xu, T. et al. Ursolic acid suppresses interleukin-17 (IL-17) production by selectively antagonizing the function of RORgamma t protein. *J Biol Chem***286**, 22707-10 (2011).
111. Huh, J.R. & Littman, D.R. Small molecule inhibitors of RORgammat: targeting Th17 cells and other applications. *Eur J Immunol***42**, 2232-7 (2012).
112. Teng, M.W. et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat Med***21**, 719-29 (2015).
113. Kathania, M. et al. Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-gammat ubiquitination. *Nat Immunol***10.1038/ni.3488** (2016).
114. Jianjun, Y. et al. T cell-derived inducible nitric oxide synthase switches off Th17 cell differentiation. *J Exp Med***210**, 1447-62 (2013).
115. Lazarevic, V. et al. T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgammat. *Nat Immunol***12**, 96-104 (2011).
116. Gascoyne, D.M. et al. The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol***10**, 1118-24 (2009).
117. Geiger, T.L. et al. Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. *J Exp Med***211**, 1723-31 (2014).
118. Seillet, C. et al. Nfil3 is required for the development of all innate lymphoid cell subsets. *J Exp Med***211**, 1733-40 (2014).
119. Xu, W. et al. Nfil3 orchestrates the emergence of common helper innate lymphoid cell precursors. *Cell Reports***in press** (2015).
120. Takeda, Y., Jothi, R., Birault, V. & Jetten, A.M. RORgamma directly regulates the circadian expression of clock genes and downstream targets in vivo. *Nucleic Acids Res***40**, 8519-35 (2012).

121. Kojetin, D.J. & Burris, T.P. REV-ERB and ROR nuclear receptors as drug targets. *Nat Rev Drug Discov***13**, 197-216 (2014).

Figure legends

Figure 1. The RORγt$^+$ cells.

RORγt$^+$ cells include type 3 innate lymphoid cells (ILC3s) and several subsets of T cells (Th17 cells, invariant NKT cells, Tγδ cells). Not mentioned is a subset of IL-17-producing neutrophils that has been documented in mouse and man (see text). All RORγt$^+$ cells can express the effector cytokines IL-17 and IL-22 to varying degrees, except most RORγt$^+$ regulatory T cells (microbiota-induced or iTregs) that express IL-10. A subset of ILC3s, named lymphoid tissue inducer (LTi) cells, expresses soluble ($LT\alpha_3$) and membrane-bound ($LT\alpha_1\beta_2$) lymphotoxin, as well as TRANCE and TRANCE ligand, which are involved in the development of lymphoid tissues in the fetus and after birth in the intestinal lamina propria and the activation of B cells. IL-17, as well as $LT\alpha_1\beta_2$, induces the recruitment of neutrophils, IL-22 prevents apoptosis of epithelial stem cells, and both IL-17 and IL-22 induce the production of anti-microbial peptides (AMPs) by epithelial cells. RORγt$^+$ Tregs are induced by the symbiotic microbiota and regulate competing type 1 and type 2 responses.

Figure 2. The RORγt molecule.

The type 3 inducer cytokines IL-23 and IL-6 induce the phosphorylation of the transcription factor STAT3, which then activates *Rorc(γt)*, the gene coding for RORγt that is essential for the generation of ILC3s and RORγt$^+$ T cells. RORγt then induces the expression of the type 3 effector cytokines IL-17 and IL-22. FoxP3, the signature transcription factor of Treg cells, binds RORγt and generally imposes a regulatory phenotype to FoxP3$^+$ RORγt$^+$(Treg) cells. Similarly, Gata3 and T-bet, the signature transcription factor of type 2 and type 1 lymphoid cells, respectively, block the expression of RORγt. The expression of RORγt is also under control of the circadian rhythm, as Nfil3, regulated by clock genes, represses *Rorc(γt)*. RORγ is involved in the regulation of circadian clocks, but is not normally expressed by hematopoietic cells. Natural ligands of RORγt have recently been identified as oxysterols, the biology of which remains to be fully understood.