Published in final edited form as:

Semin Immunopathol. 2013 November ; 35(6): 657–670. doi:10.1007/s00281-013-0393-5.

Aryl Hydrocarbon Receptor Promotes ROR γ ⁺ ILCs and Controls Intestinal Immunity and Inflammation

Ju Qiu^{1,2} and Liang Zhou^{1,2}

¹Department of Pathology, Feinberg School of Medicine, Northwestern University, 300 E. Superior Street, Chicago, IL 60611

²Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, 300 E. Superior Street, Chicago, IL 60611

Abstract

Unlike adaptive immune cells that require antigen recognition and functional maturation during infection, innate lymphoid cells (ILCs) usually respond to pathogens promptly and serve as the first line of defense in infectious diseases. RAR-related orphan receptors (ROR γ)⁺ ILCs are one of the innate cell populations that have recently been intensively studied. During the fetal stage of development, ROR γ ⁺ ILCs (e.g., lymphoid tissue inducer-LTi cells) are required for lymphoid organogenesis. In adult mice, ROR γ ⁺ ILCs are abundantly present in the gut to exert immune defensive functions. Under certain circumstances, however, ROR γ ⁺ ILCs can be pathogenic and contribute to intestinal inflammation. Aryl hydrocarbon receptor (Ahr), a ligand-dependent transcriptional factor, is widely expressed by various immune and non-immune cells. In the gut, the ligand for Ahr can be derived/generated from diet, microflora, and/or host cells. Ahr has been shown to regulate different cell populations in the immune system including ROR γ ⁺ ILCs, T helper (Th)17/22 cells, T cells, regulatory T cells (Tregs), Tr1 cells, and antigen presenting cells (APCs). In this review, we will focus on the development and function of ROR γ ⁺ ILCs, and discuss the role of Ahr in intestinal immunity and inflammation in mice and in humans. Better understanding the function of Ahr in the gut is important for developing new therapeutic means to target Ahr in future treatment of infectious and autoimmune diseases.

Keywords

Innate lymphoid cell; Aryl hydrocarbon receptor; Intestinal immunity and inflammation

Introduction

The mammalian intestinal tract harbors trillions of commensal bacteria. The crosstalk between the microflora and the immune cells is important for the development of intestinal immune system and the maintenance of a healthy environment in the gut. The innate and adaptive immune systems exhibit surveillance functions to fight against invasive pathogens that can cause infectious diseases. However, excessive inflammatory responses can also result in damage in the intestinal tract and cause colitis. In the gut, Th17 cells, Th22 cells, T cells, and ROR γ ⁺ ILCs are main producers of effector cytokines interleukin (IL)-22 and/or IL-17A (hereafter referred to as IL-17). Th17 cells expressing the nuclear receptor

Correspondence: Liang Zhou, M.D., Ph.D. Assistant Professor, Department of Pathology, Department of Microbiology and Immunology, Northwestern University, Feinberg School of Medicine, 300 E Superior Street, Tarry 3-703/3-710, Chicago, Illinois 60611, l-zhou@northwestern.edu, Office Phone: (312) 503-3182, Lab Phone: (312) 503-3186, Fax: (312) 503-8240.

This article is published as part of the Special Issue on “Roles of Aryl Hydrocarbon Receptor in Controlling Immunity”

ROR γ t (encoded by the *Rorc* gene) are one of the T helper cell subsets that mediates extracellular pathogen clearance but also causes autoimmunity when dysregulated (1–5). Th17 cells with both anti-microbial and pro-inflammatory properties are enriched in the intestinal lamina propria layer and produce signature cytokines IL-17 and IL-22. Th22 cells were originally identified in humans (6, 7). It has recently been shown in mice that Th22 cells can be skewed by IL-6 *in vitro* and produce mainly IL-22 but little IL-17 (8). Although it remains to be determined whether Th22 and Th17 cells belong to the same subset of T helper cells with different effector cytokine properties, Th22 cells appear to be more effective than Th17 cells during the clearance of *Citrobacter rodentium*, a murine pathogen that models human enterohemorrhagic *E. coli* and enteropathogenic *E. coli* infections responsible for the deaths of several hundred thousand children in developing countries each year. Consistent with the protective role of Th22/Th17 cells, IL-22 has been shown to be an effector cytokine essential for *Citrobacter rodentium* clearance (3, 9). T cells are relatively rare in the lamina propria but are more enriched in the small and large intestinal intraepithelial lymphocytes, which mainly consist of TCR $\alpha\beta$ and TCR $\gamma\delta$ CD8 $^{+}$ cells (10, 11).

T cells use different V γ gene segments to encode TCRs at different peripheral sites (12). The intestinal T cells that participate in early host defense against pathogens predominantly express TCRV γ 5 and can pair with multiple TCRV α chains (13). A subset of innate lymphoid cells (ILCs) expressing ROR γ t is essential for gut immunity. ROR γ t $^{+}$ ILCs and Th17 cells share a number of common features (e.g., transcription factor requirement, cytokine profile, and anatomic location). Given their production of IL-17 and/or IL-22, ROR γ t $^{+}$ ILCs are also known as ILC17 or ILC22. In this review, we use the term ROR γ t $^{+}$ ILCs to denote this population of cells. Aryl hydrocarbon receptor (Ahr) is a ligand-dependent transcriptional factor, which functions as an environmental sensor to recognize xenobiotic and/or endogenous compounds. Ahr has been implicated in the development and/or function of all the aforementioned cell populations. In this review, we discuss the development and function of ROR γ t $^{+}$ ILCs as well as the crosstalk between ROR γ t $^{+}$ ILCs and other cell populations in the gut. We focus on how Ahr regulates intestinal ROR γ t $^{+}$ ILC development/maintenance and function, and discuss the potential role of Ahr in human intestinal diseases.

Various cell populations that express ROR γ t in the gut

Three major cell populations in the gut that express the transcription factor ROR γ t are Th17/Th22 cells, T cells and ROR γ t $^{+}$ ILCs. These populations of cells share similar cytokine profiles characterized by the production of IL-17 and IL-22. Th17/Th22 cells are abundantly present in the gut under the steady state especially in the small intestinal lamina propria (1, 8, 14). Segmented filamentous bacteria (SFB), a type of commensal bacteria, have been reported to be a potent inducer for Th17 cell differentiation in the small intestines (1). Th17 cells are critical for controlling bacterial intrusion and fungi infection. However, Th17 cells have also been considered to be pathogenic in autoimmune diseases, such as human inflammatory bowel disease (IBD), by secreting pro-inflammatory cytokines such as IL-17, IL-17F, and IL-22 that contribute to tissue inflammation and damage (15–17). Interestingly, IL-17 has also been reported to be protective in CD45RB hi T cell transfer colitis by inhibiting Th1 cytokines (e.g., IFN- γ), suggesting the intricate regulatory network among the cytokine-induced signaling pathways in the gut (18). IL-22 has dual functions in regard to either protective immunity or pathogenic inflammation in different disease settings. For example, IL-22 acts on gut epithelial cells to promote anti-microbial peptide secretion to clear certain bacterial infections. However, IL-22 can also promote severe inflammation in CD45RB low T cell-induced colitis by causing mucosal thickening and epithelial hyperplasia (19). IL-17 has been reported to promote the intestinal tumorigenesis in mice bearing a

heterozygous mutation in the adenomatous polyposis coli (Apc) gene (Apc(Min/+) mice) (20). Further study showed that IL-23 produced by antigen presenting cells upon microbial product stimulation induces IL-17 production from Th17 cells, which causes barrier deterioration and triggers inflammation and tumor growth (20, 21). T cells have been shown to play a dual role in intestinal inflammation (22). It has been reported that mice deficient in TCR have worsened DSS-induced colitis and TNBS-induced colitis, suggesting a protective role of T cells in gut inflammation (23, 24). However, it has also been shown that T cells can produce large amounts of IFN- in colitic *TCR*^{-/-} mice (25). Although T cells are dispensable for initiating colitis in *TCR*^{-/-} mice, T cells in the *TCR*^{-/-} mice are essential for the colitis progression (26). Various immune cells in the gut form an intricate network to combat pathogen infections, while keeping autoimmunity at bay (Figure 1). Here, we will review the recent progress in understanding the development and function of ROR^γ⁺ ILCs in the context of intestinal immunity and inflammation.

ROR^γ⁺ ILCs: a subset of innate lymphoid cells

The innate lymphoid cells (ILCs) are referred to as cells that lack rearranged antigen receptors, and respond to pathogens in the first place without cell contact-based signals from antigen presenting cells. There are at least three types of ILCs: ILC1, ILC2, and ROR^γ⁺ ILCs (27–29). ILC1 cells contain NK cells, which secrete IFN- and function similarly to Th1 cells. NK cells are crucial for the clearance of intracellular pathogens (e.g., virus), requiring IL-15 signal for their development. T-bet is the transcription factor that controls the function of ILC1. ILC2 cells include natural helper (NH) cells, nuocytes, and innate helper type 2 (Ih2) cells. ILC2s are lineage marker negative but express CD117, Sca-1 and CD127. Human ILC2 cells express CRTH2, while mouse mature ILC2s express KLRG1 (30, 31). ILC2s are IL-17RB⁺IL-33R⁺, thus highly responsive to IL-25 and IL-33 stimulation, respectively. GATA3 is required for the differentiation and maintenance of ILC2 cells that secrete high levels of Th2-associated cytokines IL-5 and IL-13, suggesting a striking similarity between ILC2 and Th2 cells (32). ILC2 cells are enriched in mucosal tissues (e.g., the intestine, mesenteric lymph nodes, and the lung), playing a protective role in small intestinal helminth infection and contributing to allergic airway inflammation such as asthma (27).

ROR^γ⁺ ILCs were discovered about two decades ago with the characterization of prototypical lymphoid tissue-inducer (LTi) cells that are involved in secondary lymphoid organogenesis during the fetal stage of development (33, 34). Fetal LTi cells are developed from the common lymphoid progenitors (CLPs) residing in the fetal liver and are characterized as CD4⁺CD3⁺Sca-1⁺c-kit^{int}IL-7R⁺ cells (35). Fetal LTi cells localize in the spleen, blood and lymph node anlagen as early as E12.5, essential for the development of lymph nodes, Peyer's patches, and nasal-associated lymphoid tissues (36). Bone marrow contains the progenitors for postnatal ROR^γ⁺ ILCs (37). Although they can be found in the mouse thymus and spleen (38, 39), the adult ROR^γ⁺ ILCs are mainly present in the intestinal lamina propria, where they are important for the development of cryptopatches and isolated lymphoid follicles (ILFs). Cryptopatches are clusters of cells that are mostly composed of ROR^γ⁺ ILCs. Microbiota-derived signals can convert cryptopatches to ILFs by accumulating T cells, B cells, and dendritic cells in the cluster. The formation of ILFs helps the generation of IgA-producing plasma cells for the clearance of microflora. Fetal ROR^γ⁺ ILCs can differentiate *in situ* in the fetal liver while adult CLPs can only differentiate into mature ROR^γ⁺ ILCs in the lamina propria environment (37).

There are at least two types of ROR^γ⁺ ILCs: LTi cells and NKR-LTi cells. Similar to fetal LTi, adult LTi cells are lineage marker negative with (LTi4) or without the expression of CD4 (LTi0) (40). One sub-population of ROR^γ⁺ ILCs described recently expresses NK cell

receptors such as NKp46 in mice and NKp44 in humans (41–44). This population of cells has been given different names such as NKR-LTi, NKp46⁺ ILC, NCR-22, and NK-22 (28, 40). The precursors of ROR γ ⁺ ILCs (i.e., LTi cells and NKR-LTi cells) seem to have differential expression of a gut-homing integrin called $\alpha 4 \beta 7$ (40). ROR γ ⁺ fetal liver cells tend to develop into LTi cells, whereas ROR γ ⁺ progenitors mainly generate NKR-LTi cells (40). LTi cells and NKR-LTi cells are also distributed differently in the gut-associated tissues. Unlike LTi cells that reside in the cryptopatches and ILFs of the gut, NKR-LTi cells are found in the intestinal lamina propria, gut intraepithelial compartment, mesenteric lymph nodes, and Peyer's patches (41, 45). Although both LTi cells and NKR-LTi cells can produce similar effector cytokine (e.g., IL-22), it remains to be determined whether these two types of ROR γ ⁺ ILCs exert differential functions in gut immunity and inflammation.

Despite a limited number of studies defining the lineage relationship between NKR-LTi cells and LTi cells, increasing evidence supports that NKR-LTi cells and NK cells represent distinct developmental lineages. Two recent studies using fate-mapping approach showed that NK cells never express ROR γ during their development (46, 47). Furthermore, NKR-LTi cells can be developed from NKR⁺ROR γ ⁺ innate lymphocytes (46). NKR-LTi cells can lose ROR γ expression during development, in which IL-7 or IL-12/IL-15 positively or negatively regulate the maintenance of ROR γ expression, respectively (46). Genome-wide expression profiling analysis further indicates that NKp46⁺ROR γ ⁺ cells are more similar to NKp46⁺ROR γ ⁺ cells (i.e., LTi cells) rather than NKp46⁺ROR γ ⁺ cells (i.e., classical NK cells) (45).

ROR γ ⁺ ILCs secrete effector cytokines IL-22 and/or IL-17, and are responsive to IL-1 and IL-23 stimulations. LTi cells produce both IL-22 and IL-17, but NKR-LTi cells only express IL-22 (27). Despite being a population that constitutes only less than 5% of lamina propria lymphocytes in the gut, ROR γ ⁺ ILCs have been considered to be one of the major sources of IL-22, which plays a crucial role in intestinal immune defense against pathogens.

Cross-regulation between ROR γ ⁺ ILCs and T cells

ROR γ ⁺ ILCs strikingly resemble Th17 cells in their cytokine profile (e.g., production of IL-22 and IL-17). Co-evolution of two systems may be a fail-safe mechanism to implement redundancy in host immunity to certain infections especially at the mucosal surfaces. It has been shown that intestinal Th17 responses are enhanced by *Citrobacter rodentium*. Most recently, our group and others have shown that ILC-produced IL-22 is essential for the clearance of *Citrobacter rodentium* in the intestines (48–50). Interestingly, even in the lymphocyte-replete hosts, mice lacking ROR γ ⁺ ILCs died from the infection, highlighting an essential role for ILCs in gut immunity (9).

The crosstalk between ILCs and adaptive immune system (e.g., T cells) has recently been investigated. Despite rapid early ILC2 expansion after infection, analysis of ILC2 numbers in *N. brasiliensis*-infected *Rag2*^{−/−} mice showed that ILC2 numbers were not maintained in the absence of T cells, suggesting that T cells mediate prolonged ILC2 expansion, migration or survival through an as yet unknown mechanism (51). Compared to those in the immune competent mice, ROR γ ⁺ ILCs in *Rag*-deficient mice that lack both T and B cells produce higher level of IL-22, suggesting that the adaptive immunity may suppress the function of ROR γ ⁺ ILCs (52). It will be interesting to study the cross-regulation between ROR γ ⁺ ILCs and Th17/Th22 cells under the steady-state conditions and/or during infection/inflammation. Better characterization of different populations of ROR γ ⁺ ILCs will help elucidation of the interactions between ROR γ ⁺ ILCs and Th17/Th22 cells.

ROR γ ⁺ ILCs, antigen presenting cells, and gut microflora

In sharp contrast to an absolute requirement of gut flora in the small intestinal Th17 cell differentiation, the development of ROR γ ⁺ ILCs seems to be independent of gut microflora. Early reports suggest that microflora are required for the maintenance of NKR-LTi cells by stabilizing ROR γ expression in NKR-LTi cells (41, 46). In addition, microflora can induce IL-7 expression, which seems to be specifically required for the development of NKR-LTi cells (46). Consistent with these data, NKR-LTi cells are absent in the fetal or newborn intestines but only appear after weaning when mice start to be populated by gut flora (40). However, recent data indicate that ROR γ ⁺ ILCs exist in mice treated with broad-spectrum antibiotics as well as in germ-free mice, suggesting that commensal bacteria are at least not necessary for the development of ROR γ ⁺ ILCs (40, 45). Indeed, IL-22-producing LTi cells are abundantly present in the fetal intestines that are sterile and free of indigenous bacteria. Unexpectedly, by inducing epithelial expression of IL-25, microflora have been shown to inhibit the function of ROR γ ⁺ ILCs (i.e., the production of IL-22) (52). This effect of inhibition is indirect because ROR γ ⁺ ILCs do not express IL-25R (i.e., IL-17BR) and requires the presence of CD11C⁺ dendritic cells (DCs) in a cell contact-dependent manner (52).

Microflora can also positively affect the function of ROR γ ⁺ ILCs through regulation of antigen presenting cells (APCs), especially DCs. Of note, neither the development of ROR γ ⁺ ILCs nor the production of IL-22 by ILCs under the steady state is dependent on IL-23 ((52); Qiu, Hao, and Zhou, unpublished). However, during infection (e.g., *Citrobacter rodentium*), enhanced production of IL-22 by ROR γ ⁺ ILCs requires IL-23 (9). Consistently, highly elevated expression of IL-22 by intestinal ROR γ ⁺ ILCs was observed upon *in vitro* IL-23 stimulation (41, 48, 53, 54). Furthermore, bacterial component flagellin has been found to induce IL-22 expression in ROR γ ⁺ ILCs through action of IL-23 produced by intestinal CD103⁺CD11b⁺ DCs, a population of DCs previously known to induce intestinal tolerance (55, 56). Under the steady state, commensal bacteria can induce the production of IL-1 β by macrophages, which enhances IL-22 expression by ROR γ ⁺ ILCs (57, 58). IL-1 β has also been shown to be important for the accumulation of pathogenic ILCs during innate colitis (59). IL-1 β and IL-23 can synergize with each other to induce IL-17 production by ROR γ ⁺ ILCs in *Helicobacter hepaticus*-triggered intestinal inflammation (59). TNF α produced by CD103⁺CD11b⁺ DCs can potentiate IL-23-induced innate IL-17 production by ILCs (60). Most recently, CX₃CR₁⁺ phagocytes (DCs and macrophages) have been shown to promote IL-22 production by ROR γ ⁺ ILCs and *Cx₃cr1*^{-/-} mice died of *Citrobacter rodentium* infection due to reduced number of ROR γ ⁺ ILCs and decreased expression of IL-22 (61). Together, these data highlight the importance of cellular crosstalk between APCs and ILCs in gut immunity and inflammation.

Toll-like receptors (TLRs) are expressed by many innate cell populations (e.g., APCs) and can directly recognize structures of microbes. It has been reported that TLR2 protein is expressed by human CD127⁺ROR γ ⁺ and CD56⁺CD127⁺ LTi-like ILCs (62). Human LTi-like cells proliferated and enhanced the production of IL-22 in response to TLR2 ligand Pam3Cys stimulation (62). Besides TLR2, TLR1, 5, 6, 7, and 9 mRNA transcripts have also been detected in human LTi-like cells (62). These data raise an intriguing possibility that ROR γ ⁺ ILCs may also respond to pathogens directly, consistent with the prompt acting properties of ILCs during infection.

The transcriptional control of ROR γ ⁺ ILC development

The ROR family members include ROR α , ROR β , and ROR γ . While ROR α is not expressed in immune cells, ROR β is highly expressed by Th17 cells and contributes to Th17 cell

differentiation and function (63). Recent findings also showed that ROR γ is important for the ILC2 development (64, 65). The *Rorc* gene encodes both ROR γ and ROR γ t with distinct promoters (66, 67). While ROR γ is widely expressed by brain, liver, muscle, and adipose tissues, ROR γ t expression is restricted to lymphoid lineages of the immune system (68). Th17 cells and ROR γ t⁺ ILCs have many similarities in their cytokine profile (e.g., production of IL-22 and/or IL-17) and signaling pathways (e.g., functional enhancement by IL-23 stimulation), and share transcription factor ROR γ t as the “master-regulator”. ROR γ t is exclusively expressed by fetal LTi cells and is essential for their development. Accordingly, ROR γ t-deficient mice lack lymph nodes and Peyer’s patches. In adult mice, IL-22-producing intestinal ILCs are absent without ROR γ t. Recent studies demonstrate that IL-22 is mainly produced by ROR γ t⁺ cells but not ROR γ t⁻ cells in the innate cell populations in the gut (48). Thus, ROR γ t may represent a unique marker for the IL-22/IL-17-producing ILCs.

The inhibitor of DNA binding 2 (Id2) has been shown to be important for the development of fetal LTi cells (36, 69, 70). E proteins encoded by E2A gene, E12 and E47, are required for the development of committed B lymphocyte progenitors (71). Id2, which contains the helix-loop-helix (bHLH) domain but lacks basic DNA binding domain can form dimers with E proteins and inhibit the function of E protein, allowing the cells to adopt NK and LTi progenitor fates (72). *Id2*-deficient mice have no lymph nodes and Peyer’s patches (69). *Id2*-deficient mice lack ROR γ t⁺ LTi cells at E16.5, indicating a pivotal role for Id2 in controlling early lineage commitment of LTi cells. Although there might be other targets of Id2 (73, 74), lowering E protein activity in *Id2*^{-/-} mice by deletion of E2A restores LTi cells in the embryos (72), suggesting that E protein is a major target for Id2 to promote the development of LTi cells.

Runx-related transcription factor 1/Core binding factor 2 (Runx1/Cbf 2) has also been reported to be critical for the development of fetal LTi (75). Runx complexes are composed of a DNA-binding Runx protein and its binding partner Cbf 2. Runx complexes have been shown to play a role in the development of many hematopoietic lineages including B cells, NKT cells, and T cells (76–79). In the fetal liver, the $\alpha\beta$ ⁺ LTi progenitors can be separated into two populations based on the level of IL-7R expression (75). The $\alpha\beta$ ⁺IL-7R^{high} cells are the population that expresses ROR γ t, and their development is regulated by ROR γ t and Id2 (75). The $\alpha\beta$ ⁺IL-7R^{high} fetal LTi progenitors can be converted from $\alpha\beta$ ⁺IL-7R^{mid} cells, a process requiring Runx1/Cbf 2. Notably, *Cbf 2*-deficient mice lack LTi cells but have NK cells, in agreement with the data showing that Cbf 2 itself regulates the expression of ROR γ t in fetal LTi cells. As a result, both the *Runx1-mutant* mice and *Cbf 2-mutant* mice lack lymph nodes and Peyer’s patches (75).

Thymocyte selection-associated high-mobility group box protein (Tox), has recently been shown to control the development of the NK and LTi lineages (80, 81). Tox is expressed by NK cells at specific stages and LTi cells (81). *Tox*^{-/-} mice have fewer small intestinal LTi cells at E18, resulting in defective lymphoid organogenesis at later stage of the development manifested by the absence of lymph nodes and decreased/smaller Peyer’s patches (81). Tox does not seem to function by regulating ROR γ t expression directly since the expression of ROR γ t mRNA is normal in the thymus of *Tox*^{-/-} mice. Notably, Id2 was decreased in mature NK cells in the bone marrow of *Tox*^{-/-} mice, suggesting that Id2 may be one of the targets of Tox in regulating LTi cell development (81). However, overexpression of Id2 in *Tox*^{-/-} bone marrow cells was not sufficient to restore the LTi population (81), arguing that Tox may also act downstream of Id2 for the development of NK and LTi cells, and/or other targets of Tox besides Id2 can regulate the development of NK and LTi cells.

The Notch receptor regulates different stages of T cell development in the thymus (82). Recently, a differential requirement of Notch in the development of bone marrow or fetal liver ILC progenitors has been reported (37, 70). Notch-2 but not Notch-1 is required for the adult but not fetal ROR γ ⁺ ILC development (37). Inhibition of Notch signaling by a γ -secretase inhibitor DAPT ablates the differentiation of bone marrow CLPs to mature ROR γ ⁺ ILCs *in vitro*, while fetal CLPs can be differentiated into ROR γ ⁺ ILCs normally in the presence of DAPT (37). RBP-J is a molecule that associates with the soluble intracellular domain of Notch (NICD) and mediates the transcriptional output of Notch signaling (83). Selectively decreased NKR-LTi cells but less affected NKp46⁺-ROR γ ⁺ ILCs was reported in *RBP-J*-deficient mice (49), suggesting a differential requirement of Notch in programming certain ROR γ ⁺ ILC populations.

Ikaros is a member of the Kruppel family of zinc finger DNA-binding proteins, which regulates the development of various immune cell populations. Mice that express a dominant negative form of Ikaros lack mature T, B, and NK cells (84). Ikaros-null mutant mice have selective defects in the development of fetal and adult lymphoid systems. T and B lymphocytes are absent at the fetal stage, while postnatal thymocytes differentiate into aberrant CD4⁺ T cell with clonal expansion in the absence of functional Ikaros (85). *Ikaros*-null mice also lack lymph nodes, Peyer's patches and lymphoid follicles in the gastrointestinal tract, suggesting a critical role of Ikaros in regulating the development of ROR γ ⁺ ILCs (e.g., fetal LTi cells). Considering the role of Ikaros in Notch signaling (86), the mechanism of the action of Ikaros in regulation of ILC development needs to be further investigated.

Other signaling pathways that regulate the development of ROR γ ⁺ ILCs

IL-7R is expressed by ROR γ ⁺ ILCs and IL-7/IL-7R signaling pathway is required for the formation of secondary lymphoid structures (87, 88). Enhanced IL-7 expression in the IL-7 transgenic mice results in accumulation of LTi cells and a five-fold increase of Peyer's patch numbers (89). IL-7 also promotes *de novo* generation of adult ROR γ ⁺ ILCs from bone marrow cells and provides survival and proliferation signals to ROR γ ⁺ ILCs (46, 89). It has recently been shown that microflora are important for IL-7 production in the gut, which can stabilize the expression of ROR γ in NKR-LTi cells (46). These findings suggest IL-7/IL-7R signaling is crucial for the development/maintenance of ROR γ ⁺ ILCs.

At the fetal stage, ROR γ ⁺ ILCs are important for the formation of secondary lymphoid organs including lymph nodes and Peyer's patches. The TNF superfamily members lymphotoxin- α (LT α) and lymphotoxin- β (LT β) expressed by ROR γ ⁺ ILCs are involved in this process (90). The heterotrimeric complex LT α 2LT β 1 is the main ligand for LT R, which is expressed on stromal organizer cells. The interaction between LT α 2LT β 1 and LT R can cause the increase of cell adhesion and chemokine molecules VCAM1, ICAM1, MADCAM1, CCL19, CCL21, and CXCL13, required for the recruitment of hematopoietic cells to the developing lymph nodes. Recently, it has been shown that LT R signaling is required for the production of IL-22 by ROR γ ⁺ ILCs during *Citrobacter Rodentium* infection and mice genetically deficient in molecules of the lymphotoxin pathway died at the early stage of infection (91–93). The mechanism underlying the protective effect of lymphotoxin signaling involves an LT-driven positive feedback loop controlling IL-22 production by ROR γ ⁺ ILCs via LT R signaling in DCs. Specifically, the expression of LT R in dendritic cells is essential for the production of IL-23, a key cytokine to facilitate ILC function to enhance IL-22 production (93).

CXC chemokine receptor-5 (CXCR5), is needed for B cell homing to follicles in lymph nodes as well as in spleen. *Cxcr5*^{-/-} mice lack almost all Peyer's patches and isolated

lymphoid follicles (94). Adoptive transfer of fetal LTi cells restored the secondary lymphoid structures in *Cxcr5*^{-/-} mice (95). It has been shown that adoptive transfer of CD4⁺CD3⁻ cells purified from wildtype splenocytes that contain adult LTi cells has similar lymphoid structure-rescuing effect in *Cxcr5*^{-/-} mice, suggesting that compared to the fetal LTi cells, adult ROR⁺ ILCs can exert similar functions to regulate lymphoid organogenesis (88). Mice infected with lymphocytic choriomeningitis virus (LCMV) have disrupted lymphoid organ structures due to damages caused by cytotoxic T cell responses (96). Restoration of the lymphoid structures requires the proliferation and accumulation of adult LTi cells to the damage sites during LCMV infection (96). This process is also dependent on LT R signaling pathway, which mediates the interaction between LTi cells and stroma cells.

CCR6, a chemokine receptor, is expressed by LTi cells clustered in cryptopatches (97, 98) but not by NKR-LTi cells that are present outside cryptopatches (54, 98). Accordingly, *Ccr6*-deficient mice develop abnormal cryptopatches and ILFs (97). Although the number/development of ROR⁺ ILCs is unaltered in the absence of CCR6, IL-22 expression is significantly increased in *Ccr6*-deficient ROR⁺ ILCs, indicating that CCL20-CCR6 signaling axis may also regulate ILC function (see below).

Effector functions of ROR⁺ ILCs in the gut

One of the most important effector molecules produced by ROR⁺ ILCs is IL-22. IL-22 receptor (IL-22R) is expressed exclusively by non-hematopoietic cells such as intestinal epithelial cells. Engagement of IL-22R signaling leads to secretion of anti-microbial peptides such as α -defensin, RegIII γ , RegIII β , S100A8, and S100A9 that can control pathogenic bacterial infections (2, 3, 99). *Il22*-deficient mice succumbed to *Citrobacter rodentium* infection due to impaired production of RegIII γ and RegIII β by gut epithelial cells. Accordingly, *RegIII γ* -deficient mice have increased bacterial colonization at the intestinal epithelial surface, resulting in enhanced production of IFN- γ by CD4⁺ T cells and increased IgA level (100). IL-22 has recently been shown to have anti-parasite effect in the gut as well (101). IFN- γ is required for the control of intracellular apicomplexan parasite *Eimeria falciformis* (102). In *Ifng*-deficient mice, neutralization of IL-22 alone can increase the parasite burden, suggesting an important anti-parasitic role of IL-22. Control of *Candida albicans* infection in the gastrointestinal tract has also been shown to require early activation of the IL-23-IL-22 axis in ILCs (103). Thus, IL-22 is an essential effector cytokine that promotes gut immunity.

In addition to its anti-microbial function, IL-22 is also critical for intestinal epithelial cell integrity mediated by mucin (e.g., Muc2). *Muc2*-deficient mice developed enhanced inflammation and cancer due to bacterial accumulation in epithelial cell layer and far down in the crypts, consistent with a notion that mucin can build a mucus barrier to prevent bacterial intrusion to the gut epithelia (104). The intestinal stem cells have been found to be a target of IL-22 (105). In a graft versus host disease (GVHD) model, recipient-derived IL-22 secreted by intestinal ROR⁺ ILCs is crucial to protect the recipients from death, however no difference in inflammatory responses was observed between wildtype and *Il22*^{-/-} recipients (105). Immunohistochemistry staining suggested that IL-22R are expressed by intestinal stem cells at the crypt base between Paneth cells, suggesting a direct effect of IL-22 on the regeneration of epithelial cells (105). IL-22 can protect mice from DSS-induced colitis (106), in which IL-22 is upregulated. Consistently, IL-22 but not IL-6 acts through STAT3 to regulate the apoptosis and wound healing pathways in intestinal epithelial cells (107). *Rorc*-deficient mice, which lack ROR⁺ ILCs, are susceptible to chronic DSS-induced colitis (108). This susceptibility is mainly due to increased number of tertiary lymphoid tissues (tLTs) that leads to inflammatory pathology upon epithelial cell damage. It is thought that in the absence of ROR⁺ ILCs, compensatory elevation of tLTs is

needed in *Rorc*-deficient mice to control the bacterial expansion after epithelial insult, while causing deregulated B cell responses and aggravated inflammation (108).

Besides their protective role in gut immunity against pathogen infections, certain ROR t^+ ILCs can also exert pathogenic functions and are involved in inflammatory responses. Among them, Thy1^{high}Sca1⁺ROR t^+ IL-23R⁺ innate lymphoid cells are enriched in the inflamed gut (109). *Rag2*^{-/-} mice infected with *Helicobacter hepaticus* or administered with anti-CD40 mAb develop colitis mediated by innate cells (109). Thy1^{high}Sca1⁺ROR t^+ IL-23R⁺ cells secrete large amounts of IL-17 and IFN- γ in response to IL-23, which is a key cytokine produced by dendritic cells to drive the intestinal inflammation in this model (109). Depletion of Thy1⁺ cells in *Rag2*^{-/-} mice completely abolished the innate colitis, indicating the pivotal role of ILCs in disease pathogenesis. Such IL-23-responsive ILCs are also present in the human intestines and play a pathogenic role in human Crohn's disease (CD) (110). The *Tbx21*^{-/-}*Rag2*^{-/-} (TRUC) mice develop spontaneous colitis that is dependent on gut flora (111). The IL-23 responsive ROR t^+ ILCs have recently been shown to be responsible for the disease pathogenesis in TRUC mice (60). T-bet is required for the colitogenic IFN- γ production by ILCs, however, without the expression of T-bet in TRUC mice, ROR t^+ ILCs preferentially express pathogenic IL-17, thus leading to gut inflammation (60). IL-7R signaling is also crucial for the colitis pathogenesis in TRUC mice. T-bet suppresses the expression of IL-7R that is required for gut ILC homeostasis probably through directly binding to *Il7ra* gene locus. Consistent with the notion that IL-7R provides survival signal to ROR t^+ ILCs (46, 89), IL-7R blockade dramatically reduced the severity of colitis in TRUC mice (60). Together, these data suggest that T-bet can play a protective role in controlling the pathogenicity of ROR t^+ ILCs during certain innate immune cell-mediated colitis.

Ahr, an environmental sensor, functions in the immune system

Ahr is a ligand-dependent transcription factor that belongs to the basic helix-loop-helix transcription factor family members. The ligand for Ahr can come from environmental contaminants, therapeutics, naturally occurring chemicals, or small molecules from mammalian cells/tissues (112). Ahr ligands can be categorized into two major groups: xenobiotic and endogenous ligands. The most potent xenobiotic ligand for Ahr is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has been used in various *in vitro* and *in vivo* studies (113, 114). Other xenobiotics known to be Ahr ligands include polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAH). Besides xenobiotics, endogenous Ahr ligands such as tryptophan derivative 6-formylindolo[3,2-b]carbazole (FICZ) and 2-(1 H-Indole-3 -carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) are also present (115, 116). Ahr protein is composed of a bHLH domain for DNA binding, the Per-Arnt-Sim (PAS) domain for ligand binding, and a Q-rich domain for co-activator recruitment and transactivation (117, 118). Without ligands, Ahr is retained in the cytoplasm and binds to the chaperone Hsp90, as well as co-chaperones ARA9 and p23. Binding to ligands leads to the conformational change of Ahr and its dissociation from chaperones. This event exposes the nuclear localization signal of Ahr. As a result, Ahr translocates to the nucleus and heterodimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT). The heterodimerization enhances the binding capacity of Ahr to the DNA elements called "dioxin responsive elements" (DRE) at the promoter and other regulatory loci of target genes. The most well-characterized target genes of Ahr include the cytochrome P450 family members *Cyp1a1* and *Cyp1a2* (119).

Although initial assessment of *Ahr*-deficient mice revealed few defects in the immune system (120), recent data suggest that Ahr plays an important role in both innate and adaptive immune responses. Ahr has been shown to regulate Th1/Th2 differentiation both *in*

vitro and *in vivo* (121). Ahr has also been reported to function in Th17 cells, T cells, Tregs, Tr1 cells, and antigen presenting cells (e.g., dendritic cells and macrophages) (13, 122–127) (See accompanying reviews in the same issue). In the following sections, we will discuss the specific role of Ahr in controlling intestinal immune responses, focusing on ROR γ ⁺ ILCs.

Modulation of gut immune responses by Ahr ligands

Ahr ligands (both agonist and antagonist) have been used to examine the *in vivo* role of Ahr in the gut. Oral administration of Ahr agonist 6-naphthoflavone (6-NF) ameliorates DSS-induced colitis, the mechanism of which may be through inhibition of pro-inflammatory cytokine production such as TNF- α , IL-6, and IL-1 (128). It has been shown that FICZ, a UV photo-product of tryptophan, has a protective effect in various animal models of colitis (e.g., DSS-induced colitis, TNBS-induced colitis, and T cell transfer colitis) (115). The therapeutic effect of FICZ in TNBS-induced colitis is thought to be at least partially dependent on IL-22. Colonic cells produce relatively fewer amounts of IFN- γ , IL-17, and TNF- α in FICZ-treated mice, while blockade of IL-22 can ablate the downregulation of pro-inflammatory cytokines by FICZ (115). Amelioration of DSS-induced colitis may be mediated through epigenetic mechanisms (129). Demethylation of Foxp3 promoter and methylation of IL-17 promoter were observed upon Ahr ligand treatment (129). Of note, although ligand treatment offers exciting promise for future therapies, administration of ligands will inevitably activate Ahr in many tissues/cell types due to the broad expression pattern of Ahr. Furthermore, certain ligands may also exert indirect effects on the immune system through their metabolites (112). Thus, mechanistic insights await selective activation/deletion of Ahr *in vivo* in a cell/tissue-specific manner.

Ahr is well known to mediate the carcinogenicity of a family of environmental contaminants (i.e., xenobiotic ligands) (130). From an evolutionary perspective, Ahr exists as a sensor for endogenous ligands, rather than evolving directly to detect pollutants (e.g., dioxin). Consistent with this notion, we have recently shown *in vivo* efficacy of Ahr ligand (e.g., FICZ) administration to enhance the accumulation of intestinal ROR γ ⁺ ILCs (48). Recent data suggest that mice fed a synthetic diet that is free of phytochemicals (e.g., indole-3-carbinol (I3C) found in cruciferous vegetables) have a low level of *in vivo* Ahr activity as revealed by the decreased number of ROR γ ⁺ ILCs and T cells in the gut (13, 50), consistent with the data showing that tryptophan-derived phytochemicals under the influence of stomach acids can be converted to endogenous Ahr ligands (e.g., indolo[3,2-b]carbazole (ICZ) and 3,3-diindolylmethane (DIM)) (131). Intriguingly, another report suggests that ligands derived from the host cells may be important for the development of ROR γ ⁺ ILCs since deprivation of ligands from mouse diet had a minimal impact on the accumulation of ROR γ ⁺ ILCs in the gut (49). It remains to be determined whether these apparent discrepancies were due to experimental variations (e.g., mouse chow preparation and animal facility difference). Nevertheless, these data indicate the complexity of Ahr endogenous ligands in regulating ROR γ ⁺ ILCs and suggest that the precautions are needed in interpreting data involving Ahr ligand treatment.

Ahr controls the development and maintenance of ROR γ ⁺ ILC

We and others have recently reported that Ahr controls the development and function of ROR γ ⁺ ILCs (48–50). Unlike other transcription factors (e.g., Id2), Ahr seems to only affect the development of adult ROR γ ⁺ ILCs but not fetal ROR γ ⁺ ILCs (48–50). Consistently, *Ahr*-deficient mice show no apparent defects in secondary lymphoid organogenesis, such as the formation of lymph nodes and Peyer's patches. In newborn mice, no difference in the accumulation of intestinal ROR γ ⁺ ILCs was found between *Ahr*^{−/−}

mice and their littermate wildtype controls. The deficiency in ROR⁺ ILCs only appears at the weaning age of *Ahr*^{-/-} mice, revealed by decreased proportion as well as total number of ROR⁺ ILCs in the gut (48, 50). The defect in ROR⁺ ILCs leads to marked reduction of cryptopatches and ILFs in both the small and large intestines of *Ahr*^{-/-} mice (50). Furthermore, mice with *Ahr* deletion specifically in ROR⁺ ILCs showed similar phenotypes to *Ahr*-null mice, suggesting a cell-intrinsic role of *Ahr* in regulating ROR⁺ ILCs ((50), and Qiu and Zhou, unpublished).

Although the precise mechanisms by which *Ahr* programs ROR⁺ ILCs remain elusive, it has been shown that *Ahr* can modulate the development/maintenance of ROR⁺ ILCs in multiple regulatory modes. We have shown that *Ahr* is important for the survival of ROR⁺ ILCs (48). In agreement with the role of IL-7/IL-7R signaling pathway in the survival of ROR⁺ ILCs (46, 89), IL-7R expression in ROR⁺ ILCs is decreased, and IL-7 expression in small intestinal epithelial cells is also reduced in *Ahr*-deficient mice. Consequently, decreased expression of anti-apoptotic genes and enhanced apoptosis were observed in *Ahr*-deficient ROR⁺ ILCs. Interestingly, Kiss et al showed reduced percentage of Ki67⁺ cells in ROR⁺ ILCs from two-week old mice, suggesting that decreased ability of proliferation may also contribute to the defective expansion of NKR-LTi cells in the absence of *Ahr* at least at the early stage of mouse development. *Ahr* also regulates the expression of *Kit*, which can control ROR⁺ ILC development (50). Consistent with this notion, *Kit*^{WvWv} mice, in which *Kit* is functionally impaired, have reduced number of ROR⁺ ILCs. *Kit* expression in mice decreases after birth but is further upregulated after weaning (50). Consistent with a requirement of *Ahr* for the development/maintenance of ROR⁺ ILCs after the weaning age, *Ahr* is essential for the upregulation of *Kit* (50). ChIP experiment further showed that *Ahr* binds to the DREs located at the promoter of *Kit*, suggesting direct transcriptional regulation of *Kit* expression by *Ahr*. *Ahr* may also control the development of ROR⁺ ILCs by regulating Notch expression (49). However, this effect seems to be restricted to NKR-LTi cells rather than NKp46⁺-ROR⁺ ILCs through an as yet unidentified mechanism.

Cooperative action of *Ahr* and ROR γ t in regulation of ROR γ t⁺ ILC function

Besides regulating the development/maintenance of ROR⁺ ILCs, *Ahr* also controls the function of ROR⁺ ILCs (i.e., production of IL-22). *Ahr*-deficient ROR⁺ ILCs express less IL-22 on a per cell basis. Using ChIP assay, we recently demonstrated that binding of *Ahr* to the *Cyp11a1* (a known *Ahr* direct target gene) locus is independent of ROR⁺. In contrast, without ROR⁺, *Ahr* is unable to bind to the *Il22* locus, consistent with multiple DREs that are clustered with ROR responsive elements (ROREs) at the *Il22* locus. With the co-expression of ROR⁺, *Ahr* binds to the *Il22* locus efficiently. Using co-immunoprecipitation (co-IP) assay, we further detected the interaction between *Ahr* and ROR⁺ (48), suggesting that *Ahr* may cooperate with ROR⁺ to promote the *Il22* transcription. Indeed, co-expression of *Ahr* and ROR⁺ synergistically upregulates *Il22*. It remains to be determined whether *Ahr* indirectly binds to ROREs through interaction with ROR⁺ to enhance the *Il22* transcription. Alternatively, facilitated by ROR⁺, *Ahr* may acquire enhanced DNA binding activity, whereby directly binding to the DREs at the *Il22* locus to induce transcription. It is worthy to note that although binding of *Ahr* at the *Il22* locus is facilitated by ROR⁺, we have consistently observed reduction of *Ahr* binding at the *Cyp11a1* locus when ROR⁺ is co-expressed ((48) and Qiu and Zhou, unpublished). It is tempting to speculate that at least two types of *Ahr* target genes are present in ILCs, such as ROR⁺-dependent genes (e.g., IL-22) and ROR⁺-independent genes (e.g., *Cyp11a1*). ROR⁺ may function as a pioneering factor to set a permissive chromatin structure at certain genes (e.g., *Il22*), and subsequent recruitment of *Ahr* by ROR⁺ via protein-protein interaction induces gene transcription.

In addition to the direct mechanism of the action of Ahr in regulating *Il22* gene transcription, the decreased IL-22 production by ROR γ^+ ILCs in *Ahr*-deficient mice could also be attributed to reduced responsiveness to IL-23 (48). Lower *Il23r* expression was consistently detected in *Ahr*-deficient ROR γ^+ ILCs (48). Since *Il23r* is a known direct target gene of ROR γ (132), whether *Il23r* expression in ROR γ^+ ILCs is also regulated by cooperative action of Ahr and ROR γ remains to be further determined.

Ahr and ROR γ^+ ILCs in human colitis

Crohn's disease (CD) and ulcerative colitis (UC) are the two major types of human inflammatory bowel disease (IBD). These two types of IBD have distinct disease pathogenesis in that CD is thought to be mediated by Th1 cells, while UC is Th2 cell-dominant (133). Importantly, Th17 cells are considered to be involved in the pathogenesis of both diseases. Expression of Ahr has been examined in colitis patients (115). Compared to healthy controls, *Ahr* mRNA is decreased in Crohn's disease patients but not in patients with UC (115). Ahr expression was found to be especially downregulated in the inflamed mucosa. Flow cytometry analysis demonstrated at the protein level that Ahr expression was decreased in CD3 $^+$, CD4 $^+$ CD56 $^+$, and CD25 $^+$ lamina propria mononuclear cells (115). These findings indicate that Ahr is likely to play an important immune regulatory role in IBD. In contrast, IL-22 has been shown to be upregulated in both CD and UC patients (134, 135). Moreover, the level of IL-22 correlates with the IBD severity (136). Specifically, increased IL-22, but not IL-17 and IFN- γ was observed in CD patients (137). Despite the protective function of IL-22 in mouse models of colitis, IL-22 can stimulate the secretion of pro-inflammatory cytokines by human colonic sub-epithelial myofibroblasts in human IBD patients (135). Thus, the role of IL-22 in human IBD remains to be further explored.

ROR γ^+ ILCs are generally considered to be protective in gut immunity, presumably through production of IL-22 to promote tissue repair and/or to stimulate anti-microbial function of epithelial cells (2, 3, 99). Recent data have suggested that depletion of IL-22-producing ROR γ^+ ILCs results in the selective dissemination and survival of bacteria *Alcaligenes spp.* in peripheral tissues of mice (138). Considering *Alcaligenes*-specific systemic immune responses associated with Crohn's disease and progressive hepatitis C virus infection in patients, identification of abundant presence of IL-22-producing ROR γ^+ ILCs in the intestine and gut-associated lymphoid tissues (GALTs) of healthy humans suggests an intriguing link between ROR γ^+ ILCs and pathogen control in humans (138). In humans, it has been shown that CD56 $^-$ ILCs express IL-17 and IFN- γ , whereas IL-22 is mainly produced by the CD56 $^+$ ILC compartment. Interestingly, selective increase in CD127 $^+$ CD56 $^-$ ILCs in the inflamed intestine was observed in human colitis patients (110). Given the strong clinical relevance of ROR γ^+ ILCs, it is important to identify and characterize factor(s) that differentially regulate tissue-protective IL-22-producing ILC populations and pathogenic IFN- γ /IL-17-producing ILC populations in intestinal inflammation.

Future directions

The innate and adaptive immune systems in the gut play a fundamental role in eliminating pathogens while maintaining an immune-balanced environment during infections and autoimmune diseases. ILCs, T cells and antigen presenting cells may maintain an active crosstalk with each other and also interact with the gut flora to become educated and functional. Ahr is widely expressed by different types of immune cells in the gut and the intestinal environment is naturally enriched for Ahr ligands originated from food or generated by indigenous microflora, highlighting the gut as an important anatomical site for the physiological action of Ahr.

How Ahr expression and/or function are regulated in ROR γ ⁺ ILCs remains unclear. It is worth mentioning that although *Ahr*-deficient mice have marked reduction of ROR γ ⁺ ILCs, a significant number of ROR γ ⁺ ILCs is still present in the absence of Ahr (48). In the future, it is important to identify and characterize factors/signaling pathways that may bypass the requirement of Ahr in ROR γ ⁺ ILC development and/or maintenance. The cell-intrinsic action of Ahr requires further investigation in a tissue/cell type-specific manner. A dialogue among ILCs, T cells, and APCs needs to be further decoded. The signaling pathways that are regulated by Ahr in ROR γ ⁺ ILCs remain to be determined in the future.

Although microflora are apparently not required for the development of ROR γ ⁺ ILCs, it is unknown whether specific microflora population(s) can contribute to ROR γ ⁺ ILC programming in the gut. The impact of ROR γ ⁺ ILCs on shaping commensal gut flora is also an important research area for future study. The potential endogenous ligands for Ahr remain to be investigated. It is of special interest to understand the role of microflora in generating/modifying Ahr ligands. The intricate effect of various Ahr ligands either from exogenous origin or from diet may affect the strength of Ahr activation, causing differential effects in future clinical applications.

Acknowledgments

We thank all members of the Zhou Laboratory for their helpful discussion. This work was supported by the National Institutes of Health (AI089954 and AI091962 to LZ) and by a Cancer Research Institute Investigator Award (LZ). Liang Zhou is a Pew Scholar in Biomedical Sciences, supported by the Pew Charitable Trusts.

References

1. Ivanov, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell*. 2006; 126:1121–33. [PubMed: 16990136]
2. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med*. 2006; 203:2271–9. [PubMed: 16982811]
3. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, Ouyang W. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med*. 2008; 14:282–9. [PubMed: 18264109]
4. Komiyama Y, Nakae S, Matsuki T, Nambu A, Ishigame H, Kakuta S, Sudo K, Iwakura Y. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol*. 2006; 177:566–73. [PubMed: 16785554]
5. Wu HJ, Ivanov, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, Mathis D. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*. 2010; 32:815–27. [PubMed: 20620945]
6. Duhon T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol*. 2009; 10:857–63. [PubMed: 19578369]
7. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol*. 2009; 10:864–71. [PubMed: 19578368]
8. Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, Hatton RD, Weaver CT. Th22 Cells Are an Important Source of IL-22 for Host Protection against Enteropathogenic Bacteria. *Immunity*. 2012; 37:1061–75. [PubMed: 23200827]
9. Sonnenberg GF, Monticelli LA, Elloso MM, Fouser LA, Artis D. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. *Immunity*. 2011; 34:122–34. [PubMed: 21194981]
10. Goodman T, Lefrancois L. Expression of the gamma-delta T-cell receptor on intestinal CD8⁺ intraepithelial lymphocytes. *Nature*. 1988; 333:855–8. [PubMed: 2968521]

11. Konkel JE, Maruyama T, Carpenter AC, Xiong Y, Zamarron BF, Hall BE, Kulkarni AB, Zhang P, Bosselut R, Chen W. Control of the development of CD8 α CD4 α ⁺ intestinal intraepithelial lymphocytes by TGF- β . *Nat Immunol.* 2011; 12:312–9. [PubMed: 21297643]
12. Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat Rev Immunol.* 2002; 2:336–45. [PubMed: 12033739]
13. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, Veldhoen M. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell.* 2011; 147:629–40. [PubMed: 21999944]
14. Ivanov, de Frutos RL, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe.* 2008; 4:337–49. [PubMed: 18854238]
15. Kobayashi T, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R, Kitazume MT, Nakazawa A, Sugita A, Koganei K, Isobe K, Hibi T. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. *Gut.* 2008; 57:1682–9. [PubMed: 18653729]
16. Rovedatti L, Kudo T, Biancheri P, Sarra M, Knowles CH, Rampton DS, Corazza GR, Monteleone G, Di Sabatino A, Macdonald TT. Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. *Gut.* 2009; 58:1629–36. [PubMed: 19740775]
17. Kamada N, Hisamatsu T, Okamoto S, Chinen H, Kobayashi T, Sato T, Sakuraba A, Kitazume MT, Sugita A, Koganei K, Akagawa KS, Hibi T. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN- γ axis. *J Clin Invest.* 2008; 118:2269–80. [PubMed: 18497880]
18. O'Connor W Jr, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat Immunol.* 2009; 10:603–9. [PubMed: 19448631]
19. Kamanaka M, Huber S, Zenewicz LA, Gagliani N, Rathinam C, O'Connor W Jr, Wan YY, Nakae S, Iwakura Y, Hao L, Flavell RA. Memory/effector (CD45RB^{lo}) CD4 T cells are controlled directly by IL-10 and cause IL-22-dependent intestinal pathology. *J Exp Med.* 2011; 208:1027–40. [PubMed: 21518800]
20. Chae WJ, Gibson TF, Zelterman D, Hao L, Henegariu O, Bothwell AL. Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. *Proc Natl Acad Sci U S A.* 2010; 107:5540–4. [PubMed: 20212110]
21. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature.* 2012; 491:254–8. [PubMed: 23034650]
22. Nanno M, Shiohara T, Yamamoto H, Kawakami K, Ishikawa H. gammadelta T cells: firefighters or fire boosters in the front lines of inflammatory responses. *Immunol Rev.* 2007; 215:103–13. [PubMed: 17291282]
23. Tsuchiya T, Fukuda S, Hamada H, Nakamura A, Kohama Y, Ishikawa H, Tsujikawa K, Yamamoto H. Role of gamma delta T cells in the inflammatory response of experimental colitis mice. *J Immunol.* 2003; 171:5507–13. [PubMed: 14607957]
24. Inagaki-Ohara K, Chinen T, Matsuzaki G, Sasaki A, Sakamoto Y, Hiromatsu K, Nakamura-Uchiyama F, Nawa Y, Yoshimura A. Mucosal T cells bearing TCR γ delta play a protective role in intestinal inflammation. *J Immunol.* 2004; 173:1390–8. [PubMed: 15240735]
25. Kohyama M, Nanno M, Kawaguchi-Miyashita M, Shimada S, Watanabe M, Hibi T, Kaminogawa S, Ishikawa H. Cytolytic and IFN- γ -producing activities of gamma delta T cells in the mouse intestinal epithelium are T cell receptor-beta-chain dependent. *Proc Natl Acad Sci U S A.* 1999; 96:7451–5. [PubMed: 10377435]
26. Kawaguchi-Miyashita M, Shimada S, Kurosu H, Kato-Nagaoka N, Matsuoka Y, Ohwaki M, Ishikawa H, Nanno M. An accessory role of TCR γ delta(+) cells in the exacerbation of inflammatory bowel disease in TCR α mutant mice. *Eur J Immunol.* 2001; 31:980–8. [PubMed: 11298322]

27. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol.* 2012; 30:647–75. [PubMed: 22224763]
28. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol.* 2011; 12:21–7. [PubMed: 21113163]
29. Tait Wojno ED, Artis D. Innate lymphoid cells: balancing immunity, inflammation, and tissue repair in the intestine. *Cell Host Microbe.* 2012; 12:445–57. [PubMed: 23084914]
30. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, Voehringer D, Busslinger M, Diefenbach A. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity.* 2012; 37:634–48. [PubMed: 23063333]
31. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, Fokkens WJ, Cupedo T, Spits H. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR4 and CD161. *Nat Immunol.* 2011; 12:1055–62. [PubMed: 21909091]
32. Zhou L. Striking similarity: GATA-3 regulates ILC2 and Th2 cells. *Immunity.* 2012; 37:589–91. [PubMed: 23084352]
33. Kelly KA, Scollay R. Seeding of neonatal lymph nodes by T cells and identification of a novel population of CD3–CD4+ cells. *Eur J Immunol.* 1992; 22:329–34. [PubMed: 1347010]
34. Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4+CD3– LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity.* 1997; 7:493–504. [PubMed: 9354470]
35. Mebius RE, Miyamoto T, Christensen J, Domen J, Cupedo T, Weissman IL, Akashi K. The fetal liver counterpart of adult common lymphoid progenitors gives rise to all lymphoid lineages, CD45+CD4+CD3– cells, as well as macrophages. *J Immunol.* 2001; 166:6593–601. [PubMed: 11359812]
36. Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol.* 2004; 5:64–73. [PubMed: 14691482]
37. Possot C, Schmutz S, Chea S, Boucontet L, Louise A, Cumano A, Golub R. Notch signaling is necessary for adult, but not fetal, development of RORgamma(t) innate lymphoid cells. *Nat Immunol.* 2011; 12:949–58. [PubMed: 21909092]
38. Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, McConnell FM, Scott HS, Penninger JM, Jenkinson EJ, Lane PJ, Anderson G. RANK signals from CD4(+)3(–) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J Exp Med.* 2007; 204:1267–72. [PubMed: 17502664]
39. Takatori H, Kanno Y, Watford WT, Tato CM, Weiss G, Ivanov, Littman DR, O’Shea JJ. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J Exp Med.* 2009; 206:35–41. [PubMed: 19114665]
40. Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F, Di Santo JP, Eberl G. Lineage relationship analysis of RORgamma(t) innate lymphoid cells. *Science.* 2010; 330:665–9. [PubMed: 20929731]
41. Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johnen C, Diefenbach A. RORgamma(t) and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat Immunol.* 2009; 10:83–91. [PubMed: 19029903]
42. Cupedo T, Crellin NK, Papazian N, Rombouts EJ, Weijer K, Grogan JL, Fibbe WE, Cornelissen JJ, Spits H. Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. *Nat Immunol.* 2009; 10:66–74. [PubMed: 19029905]
43. Luci C, Reynders A, Ivanov, Cognet C, Chiche L, Chasson L, Hardwigen J, Anguiano E, Banchereau J, Chaussabel D, Dalod M, Littman DR, Vivier E, Tomasello E. Influence of the transcription factor RORgamma(t) on the development of NKp46+ cell populations in gut and skin. *Nat Immunol.* 2009; 10:75–82. [PubMed: 19029904]
44. Takayama T, Kamada N, Chinen H, Okamoto S, Kitazume MT, Chang J, Matuzaki Y, Suzuki S, Sugita A, Koganei K, Hisamatsu T, Kanai T, Hibi T. Imbalance of NKp44(+)NKp46(–) and NKp44(–)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn’s disease. *Gastroenterology.* 2010; 139:882–92. 92e1–3. [PubMed: 20638936]

45. Reynders A, Yessaad N, Vu Manh TP, Dalod M, Fenis A, Aubry C, Nikitas G, Escaliere B, Renaud JC, Dussurget O, Cossart P, Lecuit M, Vivier E, Tomasello E. Identity, regulation and in vivo function of gut NKp46+RORgammat+ and NKp46+RORgammat- lymphoid cells. *EMBO J*. 2011; 30:2934–47. [PubMed: 21685873]
46. Vonarbourg C, Mortha A, Bui VL, Hernandez PP, Kiss EA, Hoyler T, Flach M, Bengsch B, Thimme R, Holscher C, Honig M, Pannicke U, Schwarz K, Ware CF, Finke D, Diefenbach A. Regulated expression of nuclear receptor RORgammat confers distinct functional fates to NK cell receptor-expressing RORgammat(+) innate lymphocytes. *Immunity*. 2010; 33:736–51. [PubMed: 21093318]
47. Satoh-Takayama N, Lesjean-Pottier S, Vieira P, Sawa S, Eberl G, Vosschenrich CA, Di Santo JP. IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors. *J Exp Med*. 2010; 207:273–80. [PubMed: 20142427]
48. Qiu J, Heller JJ, Guo X, Chen ZM, Fish K, Fu YX, Zhou L. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity*. 2012; 36:92–104. [PubMed: 22177117]
49. Lee JS, Cella M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, Mantovani A, Kopan R, Bradfield CA, Newberry RD, Colonna M. AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nat Immunol*. 2012; 13:144–51. [PubMed: 22101730]
50. Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, Diefenbach A. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science*. 2011; 334:1561–5. [PubMed: 22033518]
51. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, Jolin HE, McKenzie AN. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010; 464:1367–70. [PubMed: 20200518]
52. Sawa S, Lochner M, Satoh-Takayama N, Dulauroy S, Berard M, Kleinschek M, Cua D, Di Santo JP, Eberl G. RORgammat(+) innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol*. 2011; 12:320–6. [PubMed: 21336274]
53. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*. 2009; 457:722–5. [PubMed: 18978771]
54. Satoh-Takayama N, Vosschenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O, Eberl G, Di Santo JP. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity*. 2008; 29:958–70. [PubMed: 19084435]
55. Van Maele L, Carnoy C, Cayet D, Songhet P, Dumoutier L, Ferrero I, Janot L, Erard F, Bertout J, Leger H, Sebbane F, Benecke A, Renaud JC, Hardt WD, Ryffel B, Sirard JC. TLR5 signaling stimulates the innate production of IL-17 and IL-22 by CD3(neg)CD127+ immune cells in spleen and mucosa. *J Immunol*. 2010; 185:1177–85. [PubMed: 20566828]
56. Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, Flavell RA, Littman DR, Pamer EG. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity*. 2012; 36:276–87. [PubMed: 22306017]
57. Shaw MH, Kamada N, Kim YG, Nunez G. Microbiota-induced IL-1beta, but not IL-6, is critical for the development of steady-state TH17 cells in the intestine. *J Exp Med*. 2012; 209:251–8. [PubMed: 22291094]
58. Hughes T, Becknell B, Freud AG, McClory S, Briercheck E, Yu J, Mao C, Giovenzana C, Nuovo G, Wei L, Zhang X, Gavrilin MA, Wewers MD, Caligiuri MA. Interleukin-1beta selectively expands and sustains interleukin-22+ immature human natural killer cells in secondary lymphoid tissue. *Immunity*. 2010; 32:803–14. [PubMed: 20620944]
59. Coccia M, Harrison OJ, Schiering C, Asquith MJ, Becher B, Powrie F, Maloy KJ. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. *J Exp Med*. 2012; 209:1595–609. [PubMed: 22891275]

60. Powell N, Walker AW, Stolarczyk E, Canavan JB, Gokmen MR, Marks E, Jackson I, Hashim A, Curtis MA, Jenner RG, Howard JK, Parkhill J, MacDonald TT, Lord GM. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor⁺ innate lymphoid cells. *Immunity*. 2012; 37:674–84. [PubMed: 23063332]
61. Manta C, Heupel E, Radulovic K, Rossini V, Garbi N, Riedel CU, Niess JH. CX(3)CR1(+) macrophages support IL-22 production by innate lymphoid cells during infection with *Citrobacter rodentium*. *Mucosal Immunol*. 2013; 6:177–88. [PubMed: 22854708]
62. Crellin NK, Trifari S, Kaplan CD, Satoh-Takayama N, Di Santo JP, Spits H. Regulation of cytokine secretion in human CD127(+) LTi-like innate lymphoid cells by Toll-like receptor 2. *Immunity*. 2010; 33:752–64. [PubMed: 21055975]
63. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS, Tian Q, Jetten AM, Dong C. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity*. 2008; 28:29–39. [PubMed: 18164222]
64. Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, Camelo A, Barlow JL, Neill DR, Panova V, Koch U, Radtke F, Hardman CS, Hwang YY, Fallon PG, McKenzie AN. Transcription factor RORalpha is critical for nuocyte development. *Nat Immunol*. 2012; 13:229–36. [PubMed: 22267218]
65. Halim TY, MacLaren A, Romanish MT, Gold MJ, McNagny KM, Takei F. Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity*. 2012; 37:463–74. [PubMed: 22981535]
66. He YW, Deftos ML, Ojala EW, Bevan MJ. RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. *Immunity*. 1998; 9:797–806. [PubMed: 9881970]
67. Ruan Q, Kameswaran V, Zhang Y, Zheng S, Sun J, Wang J, DeVirgiliis J, Liou HC, Beg AA, Chen YH. The Th17 immune response is controlled by the Rel-RORgamma-RORgamma T transcriptional axis. *J Exp Med*. 2011; 208:2321–33. [PubMed: 22006976]
68. 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]
69. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, Gruss P. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature*. 1999; 397:702–6. [PubMed: 10067894]
70. Cherrier M, Sawa S, Eberl G. Notch, Id2, and RORgamma sequentially orchestrate the fetal development of lymphoid tissue inducer cells. *J Exp Med*. 2012; 209:729–40. [PubMed: 22430492]
71. Beck K, Peak MM, Ota T, Nemazee D, Murre C. Distinct roles for E12 and E47 in B cell specification and the sequential rearrangement of immunoglobulin light chain loci. *J Exp Med*. 2009; 206:2271–84. [PubMed: 19752184]
72. Boos MD, Yokota Y, Eberl G, Kee BL. Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity. *J Exp Med*. 2007; 204:1119–30. [PubMed: 17452521]
73. Iavarone A, King ER, Dai XM, Leone G, Stanley ER, Lasorella A. Retinoblastoma promotes definitive erythropoiesis by repressing Id2 in fetal liver macrophages. *Nature*. 2004; 432:1040–5. [PubMed: 15616565]
74. Stinson J, Inoue T, Yates P, Clancy A, Norton JD, Sharrocks AD. Regulation of TCF ETS-domain transcription factors by helix-loop-helix motifs. *Nucleic Acids Res*. 2003; 31:4717–28. [PubMed: 12907712]
75. Tachibana M, Tenno M, Tezuka C, Sugiyama M, Yoshida H, Taniuchi I. Runx1/Cbfbeta2 complexes are required for lymphoid tissue inducer cell differentiation at two developmental stages. *J Immunol*. 2011; 186:1450–7. [PubMed: 21178013]
76. Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Ogawa S, Kurokawa M, Hirai H. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med*. 2004; 10:299–304. [PubMed: 14966519]

77. Setoguchi R, Tachibana M, Naoe Y, Muroi S, Akiyama K, Tezuka C, Okuda T, Taniuchi I. Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development. *Science*. 2008; 319:822–5. [PubMed: 18258917]
78. Egawa T, Eberl G, Taniuchi I, Benlagha K, Geissmann F, Hennighausen L, Bendelac A, Littman DR. Genetic evidence supporting selection of the Valpha14i NKT cell lineage from double-positive thymocyte precursors. *Immunity*. 2005; 22:705–16. [PubMed: 15963785]
79. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 1996; 84:321–30. [PubMed: 8565077]
80. Aliahmad P, Kaye J. Development of all CD4 T lineages requires nuclear factor TOX. *J Exp Med*. 2008; 205:245–56. [PubMed: 18195075]
81. Aliahmad P, de la Torre B, Kaye J. Shared dependence on the DNA-binding factor TOX for the development of lymphoid tissue-inducer cell and NK cell lineages. *Nat Immunol*. 2010; 11:945–52. [PubMed: 20818394]
82. Deftos ML, Bevan MJ. Notch signaling in T cell development. *Curr Opin Immunol*. 2000; 12:166–72. [PubMed: 10712939]
83. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*. 2009; 137:216–33. [PubMed: 19379690]
84. Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S, Sharpe A. The Ikaros gene is required for the development of all lymphoid lineages. *Cell*. 1994; 79:143–56. [PubMed: 7923373]
85. Wang JH, Nichogiannopoulou A, Wu L, Sun L, Sharpe AH, Bigby M, Georgopoulos K. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. *Immunity*. 1996; 5:537–49. [PubMed: 8986714]
86. Chari S, Winandy S. Ikaros regulates Notch target gene expression in developing thymocytes. *J Immunol*. 2008; 181:6265–74. [PubMed: 18941217]
87. Adachi S, Yoshida H, Honda K, Maki K, Saijo K, Ikuta K, Saito T, Nishikawa SI. Essential role of IL-7 receptor alpha in the formation of Peyer's patch anlage. *Int Immunol*. 1998; 10:1–6. [PubMed: 9488150]
88. Schmutz S, Bosco N, Chappaz S, Boyman O, Acha-Orbea H, Ceredig R, Rolink AG, Finke D. Cutting edge: IL-7 regulates the peripheral pool of adult ROR gamma+ lymphoid tissue inducer cells. *J Immunol*. 2009; 183:2217–21. [PubMed: 19635901]
89. Meier D, Bornmann C, Chappaz S, Schmutz S, Otten LA, Ceredig R, Acha-Orbea H, Finke D. Ectopic lymphoid-organ development occurs through interleukin 7-mediated enhanced survival of lymphoid-tissue-inducer cells. *Immunity*. 2007; 26:643–54. [PubMed: 17521585]
90. Fu YX, Chaplin DD. Development and maturation of secondary lymphoid tissues. *Annu Rev Immunol*. 1999; 17:399–433. [PubMed: 10358764]
91. Spahn TW, Maaser C, Eckmann L, Heidemann J, Luger A, Newberry R, Domschke W, Herbst H, Kucharzik T. The lymphotoxin-beta receptor is critical for control of murine *Citrobacter rodentium*-induced colitis. *Gastroenterology*. 2004; 127:1463–73. [PubMed: 15521015]
92. Wang Y, Koroleva EP, Kruglov AA, Kuprash DV, Nedospasov SA, Fu YX, Tumanov AV. Lymphotoxin beta receptor signaling in intestinal epithelial cells orchestrates innate immune responses against mucosal bacterial infection. *Immunity*. 2010; 32:403–13. [PubMed: 20226692]
93. Tumanov AV, Koroleva EP, Guo X, Wang Y, Kruglov A, Nedospasov S, Fu YX. Lymphotoxin Controls the IL-22 Protection Pathway in Gut Innate Lymphoid Cells during Mucosal Pathogen Challenge. *Cell Host Microbe*. 2011; 10:44–53. [PubMed: 21767811]
94. Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD, Browning JL, Lipp M, Cyster JG. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature*. 2000; 406:309–14. [PubMed: 10917533]
95. Finke D, Acha-Orbea H, Mattis A, Lipp M, Kraehenbuhl J. CD4+CD3- cells induce Peyer's patch development: role of alpha4beta1 integrin activation by CXCR5. *Immunity*. 2002; 17:363–73. [PubMed: 12354388]
96. Scandella E, Bolinger B, Lattmann E, Miller S, Favre S, Littman DR, Finke D, Luther SA, Junt T, Ludewig B. Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. *Nat Immunol*. 2008; 9:667–75. [PubMed: 18425132]

97. Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG, Eberl G. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature*. 2008; 456:507–10. [PubMed: 18987631]
98. Luger A, Ross M, Sieker M, Heidemann J, Williams IR, Domschke W, Kucharzik T. CCR6 identifies lymphoid tissue inducer cells within cryptopatches. *Clin Exp Immunol*. 2010; 160:440–9. [PubMed: 20148914]
99. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity*. 2004; 21:241–54. [PubMed: 15308104]
100. Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, Hooper LV. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science*. 2011; 334:255–8. [PubMed: 21998396]
101. Stange J, Hepworth MR, Rausch S, Zajic L, Kuhl AA, Uyttenhove C, Renauld JC, Hartmann S, Lucius R. IL-22 mediates host defense against an intestinal intracellular parasite in the absence of IFN-gamma at the cost of Th17-driven immunopathology. *J Immunol*. 2012; 188:2410–8. [PubMed: 22266282]
102. Pogonka T, Schelzke K, Stange J, Papadakis K, Steinfelder S, Liesenfeld O, Lucius R. CD8+ cells protect mice against reinfection with the intestinal parasite *Eimeria falciformis*. *Microbes Infect*. 2010; 12:218–26. [PubMed: 20034589]
103. De Luca A, Zelante T, D'Angelo C, Zagarella S, Fallarino F, Spreca A, Iannitti RG, Bonifazi P, Renauld JC, Bistoni F, Puccetti P, Romani L. IL-22 defines a novel immune pathway of antifungal resistance. *Mucosal Immunol*. 2010; 3:361–73. [PubMed: 20445503]
104. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A*. 2008; 105:15064–9. [PubMed: 18806221]
105. Hanash AM, Dudakov JA, Hua G, O'Connor MH, Young LF, Singer NV, West ML, Jenq RR, Holland AM, Kappel LW, Ghosh A, Tsai JJ, Rao UK, Yim NL, Smith OM, Velardi E, Hawryluk EB, Murphy GF, Liu C, Fouser LA, Kolesnick R, Blazar BR, van den Brink MR. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity*. 2012; 37:339–50. [PubMed: 22921121]
106. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity*. 2008; 29:947–57. [PubMed: 19100701]
107. Pickert G, Neufert C, Leppkes M, Zheng Y, Wittkopf N, Warntjen M, Lehr HA, Hirth S, Weigmann B, Wirtz S, Ouyang W, Neurath MF, Becker C. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J Exp Med*. 2009; 206:1465–72. [PubMed: 19564350]
108. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, Eberl G. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. *J Exp Med*. 2011; 208:125–34. [PubMed: 21173107]
109. Buonocore S, Ahern PP, Uhlig HH, Ivanov, Littman DR, Maloy KJ, Powrie F. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*. 2010; 464:1371–5. [PubMed: 20393462]
110. Geremia A, Arancibia-Carcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, Travis SP, Powrie F. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med*. 2011
111. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, Glimcher LH. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell*. 2007; 131:33–45. [PubMed: 17923086]
112. Nguyen LP, Bradfield CA. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol*. 2008; 21:102–16. [PubMed: 18076143]
113. Takamura T, Harama D, Matsuoka S, Shimokawa N, Nakamura Y, Okumura K, Ogawa H, Kitamura M, Nakao A. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. *Immunol Cell Biol*. 2010; 88:685–9. [PubMed: 20231854]

114. Benson JM, Shepherd DM. Aryl hydrocarbon receptor activation by TCDD reduces inflammation associated with Crohn's disease. *Toxicol Sci.* 2011; 120:68–78. [PubMed: 21131560]
115. Monteleone I, Rizzo A, Sarra M, Sica G, Sileri P, Biancone L, MacDonald TT, Pallone F, Monteleone G. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology.* 2011; 141:237–48. 48e1. [PubMed: 21600206]
116. Lehmann GM, Xi X, Kulkarni AA, Olsen KC, Pollock SJ, Bagloli CJ, Gupta S, Casey AE, Huxlin KR, Sime PJ, Feldon SE, Phipps RP. The aryl hydrocarbon receptor ligand ITE inhibits TGFbeta1-induced human myofibroblast differentiation. *Am J Pathol.* 2011; 178:1556–67. [PubMed: 21406171]
117. Fukunaga BN, Hankinson O. Identification of a novel domain in the aryl hydrocarbon receptor required for DNA binding. *J Biol Chem.* 1996; 271:3743–9. [PubMed: 8631989]
118. Gu YZ, Hogenesch JB, Bradfield CA. The PAS superfamily: sensors of environmental and developmental signals. *Annu Rev Pharmacol Toxicol.* 2000; 40:519–61. [PubMed: 10836146]
119. Schrenk D. Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. *Biochem Pharmacol.* 1998; 55:1155–62. [PubMed: 9719469]
120. Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. *Drug Metab Dispos.* 1998; 26:1194–8. [PubMed: 9860927]
121. Negishi T, Kato Y, Ooneda O, Mimura J, Takada T, Mochizuki H, Yamamoto M, Fujii-Kuriyama Y, Furusako S. Effects of aryl hydrocarbon receptor signaling on the modulation of TH1/TH2 balance. *J Immunol.* 2005; 175:7348–56. [PubMed: 16301641]
122. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature.* 2008; 453:106–9. [PubMed: 18362914]
123. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature.* 2008; 453:65–71. [PubMed: 18362915]
124. Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL, Quintana FJ. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. *Nat Immunol.* 2010; 11:846–53. [PubMed: 20676092]
125. Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, Kuchroo VK. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol.* 2010; 11:854–61. [PubMed: 20676095]
126. Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med.* 2009; 206:2027–35. [PubMed: 19703987]
127. Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A.* 2010; 107:19961–6. [PubMed: 21041655]
128. Furumatsu K, Nishiumi S, Kawano Y, Ooi M, Yoshie T, Shiomi Y, Kutsumi H, Ashida H, Fujii-Kuriyama Y, Azuma T, Yoshida M. A role of the aryl hydrocarbon receptor in attenuation of colitis. *Dig Dis Sci.* 2011; 56:2532–44. [PubMed: 21374063]
129. Singh NP, Singh UP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Activation of aryl hydrocarbon receptor (AhR) leads to reciprocal epigenetic regulation of FoxP3 and IL-17 expression and amelioration of experimental colitis. *PLoS One.* 2011; 6:e23522. [PubMed: 21858153]
130. Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta.* 2003; 1619:263–8. [PubMed: 12573486]
131. Veldhoen M, Brucklacher-Waldert V. Dietary influences on intestinal immunity. *Nat Rev Immunol.* 2012; 12:696–708. [PubMed: 23007570]
132. Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, Agarwal A, Huang W, Parkurst CN, Muratet M, Newberry KM, Meadows S, Greenfield A, Yang Y, Jain P, Kirigin FK, Birchmeier

- C, Wagner EF, Murphy KM, Myers RM, Bonneau R, Littman DR. A validated regulatory network for Th17 cell specification. *Cell*. 2012; 151:289–303. [PubMed: 23021777]
133. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011; 474:307–17. [PubMed: 21677747]
134. Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, Diepolder H, Marquardt A, Jagla W, Popp A, Leclair S, Herrmann K, Seiderer J, Ochsenkuhn T, Goke B, Auernhammer CJ, Dambacher J. IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. *Am J Physiol Gastrointest Liver Physiol*. 2006; 290:G827–38. [PubMed: 16537974]
135. Andoh A, Zhang Z, Inatomi O, Fujino S, Deguchi Y, Araki Y, Tsujikawa T, Kitoh K, Kim-Mitsuyama S, Takayanagi A, Shimizu N, Fujiyama Y. Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. *Gastroenterology*. 2005; 129:969–84. [PubMed: 16143135]
136. Schmechel S, Konrad A, Diegelmann J, Glas J, Wetzke M, Paschos E, Lohse P, Goke B, Brand S. Linking genetic susceptibility to Crohn's disease with Th17 cell function: IL-22 serum levels are increased in Crohn's disease and correlate with disease activity and IL23R genotype status. *Inflamm Bowel Dis*. 2008; 14:204–12. [PubMed: 18022867]
137. Wolk K, Witte E, Hoffmann U, Doecke WD, Endesfelder S, Asadullah K, Sterry W, Volk HD, Wittig BM, Sabat R. IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. *J Immunol*. 2007; 178:5973–81. [PubMed: 17442982]
138. Sonnenberg GF, Monticelli LA, Alenghat T, Fung TC, Hutnick NA, Kunisawa J, Shibata N, Grunberg S, Sinha R, Zahm AM, Tardif MR, Sathaliyawala T, Kubota M, Farber DL, Collman RG, Shaked A, Fouser LA, Weiner DB, Tessier PA, Friedman JR, Kiyono H, Bushman FD, Chang KM, Artis D. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science*. 2012; 336:1321–5. [PubMed: 22674331]

Figure 1. Ahr-mediated transcriptional control of ROR γ t⁺ ILCs and the immune function of ROR γ t⁺ ILCs in the gut

Th17/Th22 cells, T cells, and ROR γ t⁺ ILCs are major cell populations that co-express transcription factors Ahr and ROR γ t in the gut. Ahr ligands can be generated from diet (e.g., food containing Tryptophan), microflora (e.g., commensal or pathogenic bacteria), and/or host cells. In the gut, Ahr is important for the development of intraepithelial T cells, which have immune defensive function. Ahr is essential for the development and function of intestinal ROR γ t⁺ ILCs. Ahr also modulates IL-7/IL-7R expression, which confers the survival and maintenance of ROR γ t⁺ ILCs. Ahr regulates IL-22 expression by ROR γ t⁺ ILCs through cooperative interaction with ROR γ t, which directly binds at the *Il22* promoter to induce transcription. Ahr controls the transcription of *Kit* that is crucial for ROR γ t⁺ ILC development by direct binding at the promoter region of the *kit*. Notch signaling pathway is also involved in the regulation of ROR γ t⁺ ILC development by Ahr. All the ROR γ t⁺ cell populations express IL-23R. An active interplay between ROR γ t⁺ ILCs and antigen presenting cells (i.e., dendritic cells (DCs) and macrophages (M Φ)) through various cytokines (e.g., IL-23, IL-1, and TNF) is crucial for the development and function of ROR γ t⁺ ILCs. ROR γ t⁺ cells produce large amounts of IL-22 and/or IL-17. Engagement of IL-22R that is expressed by intestinal epithelial cells and gut stem cells promotes anti-microbial peptide secretion and epithelial regeneration. A potential crosstalk between ROR γ t⁺ ILCs and Th17/Th22 cells mediated by Ahr may be important for the host to fight against bacterial infections, while keeping autoimmunity at bay.