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The differential expression of IL-4 and IL-13 and its impact on type-2 Immunity

Katherine Bao¹ and R. Lee Reinhardt¹

¹Department of Immunology, Duke University Medical Center, Durham, NC 27710

Abstract

Allergic disease represents a significant global health burden, and disease incidence continues to rise in urban areas of the world. As such, a better understanding of the basic immune mechanisms underlying disease pathology are likely the key to developing therapeutic interventions to both prevent disease onset as well as to ameliorate disease morbidity in those individuals already suffering from a disorder linked to type-2 inflammation. Two factors central to type-2 immunity are interleukin(IL)-4 and IL-13. These two cytokines have been linked to virtually all of the major disease hallmarks associated with type-2 inflammation. Therefore, IL-4 and IL-13 and their regulatory pathways represent ideal targets to suppress disease. However, despite sharing many common regulatory pathways and receptors, these cytokines perform very distinct functions during a type-2 immune response. This review summarizes the literature surrounding the function and expression of IL-4 and IL-13 in CD4⁺ T cells and innate immune cells. It highlights recent *in vivo* findings regarding the differential expression and non-canonical regulation of IL-4 and IL-13 in various immune cells, which likely play an underappreciated and important role in type-2 allergic immunity.

Introduction

Type-2 immunity encompasses a spectrum of disorders ranging from asthma and allergy to parasitic helminth infection. Each of these ailments gives rise to a similar course of inflammation and pathology. It is estimated that more than 3 billion people worldwide are afflicted with diseases resulting from type-2 inflammation [1, 2] [3]. In developing nations, type-2 inflammation often results from repeated or chronic exposure to parasitic worms, whereas in developed nations, type-2 immunity commonly presents as asthma and allergy. Despite a growing understanding of disease pathology, the incidence of type-2 inflammatory diseases continues to rise with an expected 100 million new cases of asthma alone expected in the united states by the year 2025 [2]. Why allergic disease incidence continues to rise in urban areas of the world remains unclear, but evidence suggests that environmental factors are likely involved [4–6]. Thus, allergic disease resulting from type-2 inflammation represents a significant global health concern for the foreseeable future. As such, great interest lies in identifying factors that can be therapeutically targeted to minimize allergic hallmarks or reduce disease susceptibility.

Interleukin-4 (IL-4) and IL-13 are two cytokines central to type-2 inflammation, and represent targetable candidates for the amelioration of allergic disease [7]. IL-4 and IL-13 are required to drive most of the key hallmarks associated with type-2 inflammation including immunoglobulin E (IgE) production, smooth muscle contractility, mucus production, and innate cell recruitment to sites of inflammation [8–10]. Given the key role of IL-4 and IL-13 in type-2 inflammation, a significant amount of research has been performed to better understand the cellular and molecular mechanisms regulating IL-4 and IL-13 production. Based on their shared usage of lineage-determining factors STAT6 and GATA3, it has been commonly held that IL-4 and IL-13 are coordinately expressed within immune cells. Similarly, their use of common receptors initially was taken to suggest that these cytokines signaled via common pathways, and likely served redundant functions *in vivo*. However, several findings now provide evidence that this common “text book” view of IL-4 and IL-13 expression and function has been overly simplified. In fact, recent studies exploring type-2 immunity *in vivo* show that type-2 cytokine expression is much more dynamic than previously appreciated. This review summarizes the recent literature surrounding both coordinate and non-coordinate expression of IL-4 and IL-13 in innate and adaptive immune cells. Further, we explore the biological implications of non-coordinate type-2 cytokine expression in an effort to provide an explanation for the divergent functions associated with IL-4 and IL-13 in the context of type-2 inflammation *in vivo*.

Coordinate expression of IL-4 and IL-13: The textbook view of Th2 cytokine expression in CD4+ T cells

Il4 and *il13* are found adjacent to one another on chromosome 5 in humans and chromosome 11 in mice [11]. IL-4 and IL-13 share many cis- and trans-regulatory elements, and likely arose from a gene duplication event. Coordinate expression of these cytokines is common among CD4+ T cells isolated from allergic tissues. Indeed, at the population level, CD4+ T-helper type 2 (Th2) cells express both IL-4 and IL-13. Even at the single cell level, co-expression of IL-4 and IL-13 (or IL-5 which largely tracks with IL-13) is the predominant expression pattern among highly polarized Th2 clones, and linked expression has also been shown in primary CD4+ T cells [12–15]. However, non-coordinate expression also occurs as a significant number of Th2 clones produce either IL-4 or IL-13 (or IL-5) individually. This has been observed using a number of different methods to assay type-2 cytokine potential in individual T-helper cells [16–20]. Thus, although Th2 cells are defined by their ability to produce all three canonical type-2 cytokines, analyses of individual Th2 clones has revealed a more restricted expression profile. The precise relevance of stochastic cytokine expression among individual T cell clones is unclear, and represents an intriguing area of future study.

The Th2 locus is a 140 kilobase stretch that encompasses the genes for *il-4*, *il-13*, *rad50* and *il-5*. *Rad50* is a component of the DNA damage response, and is constitutively expressed. While unrelated to Th2 cytokines, *rad50* contains multiple regulatory DNase hypersensitive sites (Rad50 DNase hypersensitive sites RHS1-7) that regulate expression of the Th2 cytokines [21, 22]. Importantly, the efficacy of Th2 cytokine gene expression is directly regulated by the locus control region (LCR), composed of RHS4-7 located at the 3′ end of the *rad50* gene. During Th2 cell differentiation, the Th2 locus undergoes dynamic epigenetic

changes [23, 24]. Initially, naïve CD4⁺ T cells form a “poised” Th2 chromosomal conformation in which intrachromosomal interactions between the LCR and Th2 cytokine promoters are made. In Th2 conditions *in vitro*, or type-2 inflammatory contexts *in vivo*, cells differentiate along the Th2 program, and existing interactions between the LCR and cytokine promoters are further stabilized. At this time, Th2-specific transcription factors like GATA3 and STAT6 promote the formation of additional interactions within the Th2 locus to facilitate processive transcription. Together this structure acts as an “active” chromatin hub critical for coordinate expression of IL-4, IL-5, and IL-13 production by CD4⁺ T cells [22, 25–28].

Although Rad50 DNase hypersensitivity sites (RHS1-7) are located throughout the Th2 locus and have varying degrees of importance for cytokine regulation, we concentrate here on RHS4-7, as RHS5, RHS6, and RHS7 appear most critical for type-2 cytokine expression. RHS7 is required for the “poised” Th2 chromatin conformation [25]. Deletion of RHS7 results in reduced interactions from the *il4* promoter to the LCR as well as the *il5* and *il13* promoters. However only a partial defect on cytokine expression was observed in its absence indicating that other sites are required for optimal cytokine production. Indeed, RHS5 and RHS7 double deficiency further impaired IL-4, IL-5, and IL-13 expression [26].

Interestingly, deletion of RHS6 alone had the most significant impact on type-2 cytokine production, implying that this site is essential for LCR-mediated control of Th2 cytokine expression [27]. Thus far, four factors STAT6, GATA3, SATB1 and Ying Yang 1 (YY1) have been identified to bind to the LCR at RHS6 and/or RHS7 and mediate chromatin remodeling at the Th2 locus [21, 28–31]. While this provides a strong foundation with which to understand LCR functionality, the characterization of factors required for the dynamic regulation at the Th2 locus remains incomplete. Importantly, the current models for LCR-mediated type-2 cytokine regulation center around the idea of coordinate expression of Th2 cytokines. However, whether recruitment of differential factors to the “active” chromatin hub can mediate non-coordinate expression and under which conditions this might occur remains largely unexplored. How these events might shape the eventual cytokine expression profile of a given T cell and more importantly impact the overall chromatin landscape is still of great interest.

The canonical regulation of IL-4 and IL-13 in CD4⁺ Th2 cells

The prevailing model for Th2 cell subset differentiation suggests that the cytokine milieu in the environment at the time of T cell receptor engagement plays a dominant role in CD4⁺ T cell fate choice [32]. In this classical differentiation pathway, Th2 differentiation is initiated by IL-4 or IL-13 binding to IL-4 receptors, which induces the phosphorylation STAT6. Phosphorylated STAT6 dimerizes and translocates to the nucleus and induces GATA3 expression. GATA-3, also known as the Th2 lineage determining factor, promotes type-2 cytokine expression, and additionally possesses the ability to auto-activate its own transcription thereby stabilizing its expression [33]. Thus, signaling through the IL-4 receptor creates a positive feedback loop to initiate STAT6 and GATA-3 and maintain IL-4 and IL-13 production in Th2 cells (Figure 1). In support, deletion of STAT6 substantially impairs the ability of CD4⁺ T cells to respond to IL-4 and IL-13, limiting their production of type-2 cytokines [34–36]. Conversely, STAT6 overexpression in CD4⁺ T cells cultured

under Th1 conditions leads to IL-4 production [37]. Similarly, genetic and functional manipulation of GATA-3 expression revealed its importance in Th2 differentiation and maintenance [38–42]. As seen with STAT6, overexpression of GATA-3 in early Th1 cells was sufficient to drive both IL-4 and IL-13 expression [43]. GATA-3 is additionally implicated in the remodeling of the Th2 locus [40, 44, 45]. The role of these epigenetic modifications in promoting somatic cell “memory” of the Th2 expression program are nicely reviewed elsewhere [23, 24, 46].

Non-canonical pathways of IL-4 and IL-13 in CD4⁺ T cells

Although it is clear that STAT6 plays an important role in Th2 differentiation, many publications have shown that that STAT6 is not absolutely required to achieve IL-4 production. Studies both *in vitro* and *in vivo* have demonstrate that STAT6-deficient T cells are capable of producing IL-4 early during the differentiation process, but likely require IL-4 receptor-mediated signaling and STAT6 to maintain a committed Th2 fate [33, 47–49]. Further evidence that the canonical pathway is not absolutely required for IL-4 production comes from studies using IL-4 receptor-alpha (IL4R α)-deficient mice. Deletion of the IL4R α subunit prevents canonical IL-4-receptor and IL-13-mediated signaling, as this subunit is required to bind both IL-4 and IL-13. Although the relative impact of IL4R α -deficiency on IL-4 production appears more severe at later stages of Th2 commitment, similar to that seen in STAT6-deficient mice, it is clear that early IL-4 production does not depend on this classical pathway *in vivo* [50, 51]. Together, these findings suggest that IL-4 and IL-13 are not required for early type-2 cytokine production by T cells. This theory was confirmed using mice deficient in both IL-4 and IL-13 [52, 53]. Thus, non-canonical pathways must exist to induce IL-4 and IL-13 expression, which are independent of the classical pathway factors IL-4, IL-4R α receptor, and STAT6.

A number of non-canonical pathways have been described to facilitate IL-4 and IL-13 production in CD4⁺ T cells (Figure 1). IL-2 can drive IL-4 transcription in an IL-4R-independent manner through the phosphorylation of STAT5 [54, 55] [56, 57]. STAT5 in this case refers to two highly related proteins, STAT5A and STAT5B, often considered to be redundant in their contribution to type-2 immunity. However, STAT5A-deficient mice show a more pronounced deficiency in cytokine production, which suggests that STAT5A is likely dominant in initiating transcription of IL-4 within CD4⁺ T cells [57–59]. In support of a role for STAT5 in type-2 cytokine expression, the added effect of STAT5A deficiency in STAT6-deficient mice further impaired the Th2 defects initially observed in STAT6-deficient animals [60]. Conversely, constitutive expression of STAT5A promoted IL-4 expression in the absence of STAT6 and IL-4R α [56]. STAT5-mediated IL-4 expression also appears independent of GATA-3 [61, 62]. Thus, STAT5 can induce IL-4 expression independently of classical Th2 transcription factors STAT6 and GATA-3.

Another non-canonical pathway described to be involved in type-2 cytokine expression is the Notch pathway. The Notch pathway works independently of STAT6 and IL-4R α . Notch receptor ligation by classical Notch ligands Jagged 1, Jagged 2 or Delta-like ligands 1–4 results in the enzymatic cleavage of Notch intracellular domain (NICD) by gamma secretase [63]. Cleaved NICD translocates to the nucleus to form a complex with recombination-

signal-binding protein for Immunoglobulin-kappa J region (RBPJ) and co-activators like Mastermind-like to initiate gene expression. Inhibition of Notch signaling results in significant impairments in Th1-, Th2- and Th17- associated cytokine expression [64–70]. In particular, deletion of RBPJ or Notch1 and Notch2 in T cells resulted in a significant impairment in Th2 differentiation [65, 71, 72]. In further support of a role of Notch in non-canonical IL-4 production, over-expression of NICD in T cells increased IL-4 production in a STAT6-independent manner [72]. Notch-mediated signaling likely influences Th2 cytokine expression by regulating both *il4* and *gata3* gene transcription [65, 66]. It was originally suggested that distinct Notch receptor and ligand pairs could differentially instruct T-helper cell fates [72, 73]. More recently a model emerged suggesting that Notch signals augment the expression of various cytokines in differentiating CD4+ T cells [74, 75]. Thus, Notch may be required to maintain cytokine commitment in various T-helper cell subsets. Certainly, signaling through Notch is important for STAT6-independent activation of GATA-3, and likely plays an important role Th2 cytokine expression [33].

Like Notch, Mammalian target of rapamycin signaling (mTOR) is believed to influence both Th1 and Th2 cell differentiation. Whether mTOR promotes Th1 or Th2 differentiation relies on the co-activators present. mTOR and rapamycin insensitive companion of mTOR (RICTOR) form mTOR signaling complex 2 (mTORC2) which initiates the Th2 differentiation program. Deletion of RICTOR in T cells led to a substantial reduction in IL-4 production and Th2 differentiation [76, 77]. RICTOR-deficient T cells displayed impaired GATA3 expression, providing a likely explanation for the resulting phenotype.

c-Maf binds directly to the IL-4 promoter to promote IL-4 expression in Th2 cells [78, 79]. IL-4 production and Th2 differentiation are severely blunted in c-Maf-deficient T cells [80]. To induce IL-4 expression, c-Maf works in concert with NFATc2, IRF4, and the AP1 factor JunB [79, 81, 82]. Cooperation with NFAT and JunB suggests that c-Maf functions early in Th2 differentiation by pairing with factors downstream of T cell receptor signaling. Early c-Maf activation in T cells is independent of IL-4 and STAT6 [83]. Instead, c-Maf is induced within hours of TCR activation and requires IL-6-mediated STAT3 to initiate expression. Additionally, inducible costimulator (ICOS) and Vav-1 appear to play a role in c-Maf-mediated IL-4 expression [84, 85]. These findings suggest that c-Maf-mediated IL-4 production may be independent of canonical IL-4Ra signaling. However, c-Maf-mediated, non-canonical IL-4 production is likely important only under conditions when exogenous IL-4 is limited, as providing recombinant IL-4 to c-Maf-deficient mice induced equivalent levels of type-2 cytokines as wild-type cells [80]. Taken together, the data indicate that c-Maf likely acts to amplify signals derived from the canonical IL-4 signaling pathway, but, in the absence of canonical IL-4 signaling, c-Maf works with antigen-driven TCR signaling and IL-6 to promote IL-4 expression. Although canonical and non-canonical functions of c-Maf in T cells have been reported [78], the biological significance of c-Maf-mediated, non-canonical IL-4 production remains an interesting area for further exploration.

Innate cells have dichotomous expression patterns of IL-4 and IL-13 during type-2 inflammation

As discussed previously, both coordinate and non-coordinate expression of IL-4 and IL-13 in CD4⁺T cells are likely context-dependent throughout a type-2 immune response. This raises the question of how other immune cells capable of producing IL-4 and IL-13 regulate type-2 cytokines. Innate immune cells represent a substantial pool of type-2 cytokine competent cells. Basophils, eosinophils, and mast cells constitutively express both IL-4 and IL-13 mRNA transcripts and represent important early sources of type-2 cytokines during allergic immunity [86, 87]. Based on these findings, the vast majority of literature supports a model of IL-4 and IL-13 being co-expressed by type-2 innate cells. However, recent findings have described a divergence from this widely held model. For example, basophils are primed for rapid cytokine production likely due to the presence of pre-formed cytokine protein and represent a major source of IL-4 during early stages of helminth infection in the lung [88–90]. Human basophils have also been described as a source of IL-13 [89, 91, 92]. Eosinophils represent the largest IL-4-competent population in allergic tissues at steady state and in settings of type-2 inflammation [93–95]. In addition, IL-4 production by eosinophils has recently been elucidated to be important in the regulation of multiple metabolic pathways. For example, the generation of beige fat, a population of cells that serve as a line of defense against cold and obesity, was recently demonstrated to depend on eosinophil-derived IL-4 [96]. Furthermore, eosinophils maintain the homeostasis of alternatively activated macrophages (AAMs) in adipose tissue, and are important for glucose tolerance in mice fed a high fat diet [93]. In addition, increased eosinophilia as a result of parasitic helminth infection also improved glucose tolerance [93]. Like basophils, eosinophils contain pre-formed IL-4, which explains their immediate effector function [97]. Thus, basophils and eosinophils appear to exert their effector function through the release of IL-4 rather than IL-13. That said, eosinophils have been described to constitutively produce IL-13 mRNA, and like IL-4 human eosinophils can contain pre-formed IL-13 [86, 98]. However, whether these cells represent a major source of IL-13 during type-2 inflammation remains unclear. Mast cells also show evidence of constitutive IL-4 and IL-13 mRNA expression [86], and can rapidly produce IL-4 and IL-13 after IgE-mediated crosslinking or exposure to IL-33 [99–101]. This is again likely due to preformed IL-4 and IL-13 in secretory granules [102].

Although the contribution by invariant natural killer T (iNKT) cells in the pathology of allergic disease is an area of continued interest [103, 104], iNKT cells are implicated as a major source of IL-4 and IL-13 during allergic inflammation [105–109]. Initial studies showed that iNKT cells develop a poised effector state during development in the thymus to constitutively express IL-4 mRNA [110, 111]. However, like T-helper CD4⁺ T cells, IL-4 and IL-13 protein production is limited to a distinct subset of iNKT cells. Classified by their cytokine expression and distinct lineage-determining transcription factors, the subset termed iNKT2 cells are reported to produce significant amounts of IL-4 and IL-13 in the lung during chronic allergic inflammation [108, 112, 113][114]. Whether iNKT cells constitutively express IL-13 mRNA similar to what has been found for IL-4, and whether iNKT subsets arise from distinct precursors or share a common linear differentiation pathway are still interesting areas of investigation.

Another important innate cell subset important in allergic inflammation is the newly described group two innate lymphoid cell (ILC2). ILC2 cells represent an additional source of type-2 cytokine production [115–117]. Although these cells were originally thought to produce IL-4, IL-5 and IL-13 [118, 119], more careful examination suggests that ILC2s largely are restricted to producing IL-5 and IL-13 upon activation in settings of type-2 inflammation [20, 115, 120–122]. Unlike Th2 cells and iNKT cells, ILC2 cells do not express antigen receptors. Instead, ILC2 produce cytokines in response to “alarmins” IL-25, IL-33, and likely TSLP secreted by epithelial cells in damaged tissues [118, 119, 123–130]. In addition to driving type-2 pathology through the release of IL-5 and IL-13, ILC2 cells can promote cytokine production by lung resident Th2 cells [131]. ILC2 cells can also induce dendritic cell migration from the lung to the draining lymph nodes to induce Th2 differentiation [131]. Taken together innate immune cells represent important contributors of IL-4 and IL-13 during the course of type-2 inflammation. The current cytokine production potential of these various innate subsets during type-2 inflammation is summarized in figure 2.

However, it is still unclear what contribution specific innate immune subsets make with respect to these cytokines during the course of a type-2 immune response. Although this question has been addressed by *ex vivo* restimulation of innate cells isolated after the induction of type-2 inflammation, this method does not accurately reflect *in vivo* cytokine production. This point may be best illustrated by studies using cytokine reporter mice that do not require *ex vivo* manipulation to assess cytokine competency or protein production. In this regard, both IL-4 and IL-13 protein reporter strains suggest that the presence of mRNA expression of IL-4 and IL-13 within innate subsets does not reflect the true nature of cytokine protein found in allergic tissues [20, 115]. Thus, a more thorough investigation of *in vivo* IL-4 and IL-13 production by innate cells is needed to better understand the principles of type-2 inflammation.

IL-4 and IL-13 serve distinct functions during type-2 immunity

IL-4 and IL-13 are critical for immunity to various intestinal nematode infections [132, 133]. The requirement for one or both of these cytokines for protective anti-helminth immunity is perhaps most evident in studies with mice deficient in either IL-4R α or STAT6 [134, 135]. Because the receptors for IL-4 and IL-13 both contain IL-4R α and signal through STAT6, it was commonly accepted that IL-4 and IL-13 might serve redundant functions in response to intestinal helminths because these cytokines share receptors [136]. Evolutionarily, redundancy of function could minimize the detrimental effects caused by a loss of one cytokine. Redundancy of IL-4 and IL-13 during instances of coordinate expression could also be advantageous if amplification of the response and cytokines led to beneficial outcomes. Indeed, studies with recombinant IL-4 delivered as immune complexes, was sufficient to clear chronic *Trichuris muris*, and *Heligmosomoides polygyrus* infections in wild-type mice, as well as *Nippostrongylus brasiliensis* in immunocompromised mice suggesting that IL-4 could likely compensate for IL-13 [137, 138].

Interestingly, helminth infections of IL-4-deficient and IL-13-deficient mice have revealed non-overlapping functions of both cytokines (Figure 2). Both IL-4-deficient and IL-13-

deficient mice impacted *T. muris* expulsion compared to wild-type mice [139]. However, the responses in IL-4-deficient mice did not phenocopy the response observed in IL-13-deficient mice. This suggested that IL-4 and IL-13 could serve different roles during helminth infection. Differential roles for these cytokines became even more evident in the context of *Nippostrongylus brasiliensis* infections. After *N. brasiliensis* infection, wild-type and IL-4-deficient mice cleared worms while worm clearance in IL-13-deficient mice was significantly impaired. Additionally, IL-4-deficient mice exhibited no IgE production, while wild-type and IL-13-deficient mice produced similar levels of IgE in response to *N. brasiliensis* infection [134, 140, 141]. Thus, in the context of helminth infections, IL-4 and IL-13 mediate distinct allergic hallmarks [142]. IL-4 was essential for IgE production and mast cell activation, while IL-13 was critical for mucus production, goblet cell hyperplasia, and worm expulsion [134, 140, 142, 143]. Although these studies show that IL-13 plays a more dominant role in the clearance of *N. brasiliensis*, IL-13-deficient animals cleared worms more rapidly than mice that lacked both IL-4 and IL-13, showing that IL-4 can eventually compensate in the absence of IL-13 [53]. Indeed, at high concentrations, IL-4 can act in a redundant fashion to IL-13, which explains why recombinant IL-4 alone could induce worm clearance [142]. In sum, IL-4 and IL-13 are collectively responsible for many allergic hallmarks associated with type-2 inflammation. However, *in vivo* data supports a model whereby these cytokines are ultimately responsible for distinct type-2 inflammatory hallmarks (Figure 2). Thus, under physiologic conditions IL-4 and IL-13 often play non-redundant roles in response to intestinal nematodes.

Similarly, *in vivo* studies show that IL-4 and IL-13 likely act in a redundant and non-redundant fashion during allergic disease. Both IL-4 and IL-13 when delivered to naïve mice are sufficient to induce airway hyperreactivity in models of allergic asthma [8, 10]. Similarly, overexpression of IL-4 in the lung induced peripheral type-2 hallmarks such as innate cell recruitment and airway inflammation [9]. IL-4 blockade and IL-4-deficient mice also demonstrate that IL-4 has a role in airway inflammation [144, 145]. However, IL-4 expression during type-2 inflammation is largely associated with humoral immunity in more physiologic models of allergic lung inflammation. Both IgE and IgG1 production are dependent on IL-4. In contrast, IL-13 appears dominant in driving allergic pathology in the lung. IL-13 was both sufficient and necessary to induce robust airway hyperreactivity (AHR) and many pathological hallmarks associated with allergic airway disease [8, 10]. IL-13 is critical for driving goblet cell hyperplasia, smooth muscle contraction, and mucus production [8, 10]. IL-13 also has a role in the recruitment of eosinophils from the blood into the lung parenchyma [146]. Thus, although IL-5 is critical for eosinophil mobilization from the bone marrow, IL-13 and possibly IL-4 are important for entry of eosinophils into peripheral sites of type-2 inflammation [94, 147]. Although T cells are a major source of type-2 cytokines and aid in mediating innate cell recruitment to the lung, T cell derived cytokines are not required for this process to occur. Mice with CD4⁺ T cells deficient in IL-4 and IL-13, generated robust lung eosinophilia during allergic inflammation [148]. Likewise, basophil recruitment does not require IL-4 or IL-13 from CD4⁺ T cells [94, 149].

Mechanisms that drive divergent functions of IL-4 and IL-13 during allergic immunity: Mechanism I: The differential expression and function of the Type-I and Type-II IL-4 receptors

Given the distinct allergic hallmarks attributed to IL-4 and IL-13 during allergic immunity, understanding how such closely related cytokines, mediated such distinct functions during type-2 inflammation is an area of great interest. Identifying the unique molecular pathways behind the differential hallmarks associated with IL-4 and IL-13 are seminal to the design of therapeutics seeking to target singular pathologic outcomes associated with a type-2 response. One likely mechanism that allows for the distinct allergic hallmarks associated with IL-4 and IL-13 revolves around the differential usage and expression of the type-I and type-II IL-4 receptors on target cells [150–153]. IL-4R α is paired with the common gamma chain to form the type-I receptor or with IL-13R α for the type-II receptor. IL-4 directly binds to IL-4R α . Therefore, IL-4 can bind to both type-I and type-II receptors. IL-13 binds to IL-13R α I, and as a result IL-13 is only able to signal through the type-II receptor.

Distinct cellular expression patterns of the type-I and type-II receptors also dictate patterning of cytokine function and the unique functions ascribed to these cytokines during type-2 inflammation. The type-I receptor is found primarily on hematopoietic cells, and is often the only IL-4 receptor expressed on T cells, basophils, mast cells, and mouse B cells [153]. As such, based on the restrictive bias of cytokine signaling via the type-I receptor, IL-4 is expected to have a greater impact on these cells than IL-13. Accordingly, IL-4, and not IL-13, promotes humoral aspects of allergic responses (Figure 2). Importantly, the type-I IL-4 receptor can also activate Insulin receptor substrate (IRS)-1 and IRS2 by recruiting them to Janus Kinase (JAK)-mediated phosphorylated moieties on the IL-4R α chain after IL-4 engagement [154].

The type-II receptor is largely confined to non-hematopoietic cells. These cells express little or no common gamma chain, and therefore IL-4 and IL-13 signal predominantly through the type-II receptor in these cells. Restricted type-II receptor expression in the airways allows IL-13 to have a greater impact on epithelial cell function, smooth muscle contractions, airway resistance, goblet cell hyperplasia, and mucus production [152]. However, IL-4 can also bind the type-II receptor, and compared to IL-13, IL-4 actually possesses greater affinity for the type-II receptor [154, 155]. Thus, the stoichiometry of IL-4 to IL-13 in the tissue is important in the preferential signaling and outcomes associated with these cytokines. Importantly, despite IL-4 having greater affinity for the type-II receptor, a mechanism exists to allow IL-13 to outcompete IL-4 for the type-II receptor. This mechanism relies on the relative efficiency of type-II receptor assembly downstream of ligand engagement [155]. Based on crystal structures of the type-II receptor, IL-13 binds to and promotes the assembly of the type-II receptor more efficiently than IL-4. Furthermore, because IL-13 binds to IL-13R α I, IL-13 signaling is dominant when the expression of IL-13R α I is enriched, or IL-4 is limiting. Thus, IL-13R α I expression and the relative abundance of IL-13 and IL-4 in the tissue are likely important facets associated with the differential allergic hallmarks centered around IL-4 and IL-13 [156]. Indeed, increased IL-13 relative to IL-4 is likely to influence type-2 pathology at peripheral sites of infection in mice

infected with *N. brasiliensis* or *Aspergillus* [20, 156]. These model systems induce more IL-13 than IL-4, but the predominance of IL-13 in the allergic lung is not always the case as certain models of allergic airway inflammation show that IL-4 can be predominant in some cases. One example of this is during allergic sensitization with house dust mite antigen, where IL-4 predominates and is sufficient to promote peripheral allergic hallmarks such as infiltration of eosinophils into the lung [156]. Thus, although IL-13 plays a more dominant role in peripheral aspects of allergic inflammation, much of this effect is predicated on the preferential expression of the type-II receptor in this environment (smooth muscle and goblet cells), and the ability of IL-13 to engage the type-II receptor in a way that favors signaling over IL-4 ligation.

However, some cells, such as macrophages and dendritic cells, express both the type-I and type-II IL-4 receptors. Thus, how and if IL-4 and IL-13 differentially regulate the cellular function in these cell types is an intriguing question. In the case of alternatively activated macrophages (AAM), these cells appear to respond more robustly to IL-4 than IL-13 [154]. Indeed the importance for IL-4 in alternative macrophage activation is supported by studies showing that AAM activation occurs normally in IL-13R α 1-deficient mice [150, 152]. This finding implies that the type-I receptor and IL-4 are sufficient for AAM activation in macrophages that do not express the type-II receptor. Additionally, the type-I and type-II receptors can modulate different cellular functions in cells expressing both receptors by activating distinct signaling cascades. IL-4 bound to the type-I receptor induces the phosphorylation of the common gamma chain and independently of STAT6 signaling, efficiently induces the phosphorylation of IRS1 and IRS2 [157, 158]. However, IL-4 bound to the type-II receptor weakly activates the same IRS pathway. Intriguingly, differential activation is due to the extracellular domains of the IL-4 receptors, and is not the result of disparate pairing of the common gamma- or IL-13R α 1- chains. This was shown in studies with chimeric IL-4 receptors, which replaced the extracellular IL-13R α 1 of the type-II receptors with the common gamma chain [159]. Thus, the induction of IRS1 and IRS2 signaling by type-I and type-II receptors provides an additional mechanism by which IL-4 and IL-13 can mediate distinct type-2 hallmarks. In sum, it is clear that the context of receptor engagement, the location of IL-4 receptor expression, the receptor subtype present in the tissue, the relative abundance of each cytokine, and the presence of unique signaling pathways downstream of the type-I and type-II receptors all play an important role in the differential impact of IL-4 and IL-13 in allergic tissues.

Mechanism II: Differential expression of IL-4 and IL-13 in Tfh and Th2 cells

Although differential receptor expression provides one probable mechanism to explain the divergent functions of IL-4 and IL-13 during type-2 immunity, other mechanisms likely contribute to this dichotomy as well. One additional mechanism to explain how these cytokines can evoke different allergic hallmarks would be through the differential expression of IL-4 and IL-13. Given that IL-4 is largely responsible for humoral aspects of allergic immunity, it might be expected that IL-4-mediated signaling would predominate over IL-13 in the B cell follicles. Conversely, given the dependence on IL-13 to drive peripheral hallmarks associated with type-2 inflammation, it might be expected that the expression of IL-13 would predominate over IL-4 in non-lymphoid tissues and at mucosal barriers. Indeed,

in many settings of allergic type-2 lung inflammation, IL-13 expression has been described to be increased relative to IL-4 [150, 152]. However, if differential expression of IL-4 and IL-13 is a mechanism that contributes to the differential role that these cytokines play in type-2 inflammation, it would imply that non-coordinate expression of IL-4 and IL-13 from various immune cells must occur. Such a model of differential type-2 cytokine production to explain the dichotomous effect that each cytokine has on the divergent hallmarks of type-2 immunity would challenge the existing dogma in the field which largely emphasizes coordinate type-2 cytokine expression. However, recent *in vivo* studies suggest that coordinate expression may indeed be the exception rather than the rule.

Most studies demonstrating coordinate production of IL-4 and IL-13 in CD4⁺ T cells have relied on *in vitro* culture or *ex vivo* restimulation assays to assess cytokine production. Although such systems have been invaluable for revealing the molecular mechanisms responsible for the regulation of IL-4 and IL-13, the artificial manipulation associated with such *ex vivo* or *in vitro* techniques leave open questions surrounding the relevance of these findings *in vivo*. The necessity for such techniques in the past was largely due to the inherent difficulty in detecting IL-4 and IL-13 *in situ*. Thus, cellular restimulation has been a necessity to evaluate *in vivo* and *in vitro* cytokine production. To circumvent these caveats, cytokine reporter mice have proved valuable. These mice not only increase the sensitivity of detection for these cytokines *in vivo*, but they also bypass the need for *ex vivo* restimulation [20, 47, 99, 115, 116, 122, 160, 161]. Indeed, studies using cytokine reporter mice revealed that IL-4 and IL-13 production *in vivo* may be more complicated than previously appreciated. IL-4 reporter models show CD4⁺ T cells and innate immune cells begin to express IL-4 mRNA (or are competent for IL-4 expression) during development or shortly after activation with antigen [47, 149]. CD4⁺ T cells acquire IL-4 mRNA competency in the lymph node paracortex presumably after interacting with antigen-bearing dendritic cells (figure 2) [162]. Remarkably, the majority of IL-4 mRNA positive CD4⁺ T cells in the paracortex were negative for protein production [161, 162]. In fact, protein production appeared restricted both temporally and spatially from the development of mRNA competency and expression. Studies show that CD4⁺ T cell-mediated IL-4-protein production is mainly derived from follicular helper CD4⁺ T cells (Tfh) found in the B cell follicles and germinal centers. IL-4 protein production was also found at the border between the B and T cell zones albeit to a lesser degree [162]. This preferential production of IL-4 protein by Tfh cells in an infected lymph node has also been confirmed in models of parasitic helminth and viral infections [163–165].

The studies outlined above suggest that cytokine competency and production by CD4⁺ T cells are separated both spatially and temporally in secondary lymphoid tissues. Importantly, CD4⁺ Tfh cells residing in B cell follicles do not express IL-13 [20]. The ability to express IL-4 but not IL-13 suggests that Tfh and Th2 cells are likely distinct subsets of CD4⁺ T cells. This theory is further supported by the observed differential expression of BCL-6 and GATA3 in Tfh and Th2 cells, respectively [20, 162]. IL-4-producing Tfh cells in the secondary lymphoid organs during *Leishmania major*, helminth, and LCMV infection express high levels of BCL6 [20, 162, 163]. BCL6 is considered a “master regulator” of Tfh cell fate and function [166–168]. BCL6 expression spikes upon activation of naïve CD4⁺ T cells, however its persistence and level of expression appears most important later in the

response where it appears critical for the eventual commitment of CD4⁺ T cells to the Tfh cell fate [169–171]. Interestingly, such BCL6-expressing, IL-4-producing Tfh cells do not express high levels of GATA-3 the “master regulator” of canonical Th2 cells [20, 163]. The few IL-13-producing CD4⁺ T cells found in lymph nodes resemble canonical Th2 cells found in the inflamed lung and express little if any BCL6 and high levels of GATA-3 [20]. In support of GATA-3 being more important for cytokine production in Th2 as opposed to Tfh cells, preferential deletion of GATA-3 in differentiated Th2 cells showed that GATA-3, was essential for IL-5 and IL-13 expression in Th2 cells, but had less of an impact on IL-4 expression [172]. Thus, increased GATA-3 expression appears essential for IL-5 and IL-13 production by Th2 cells, whereas IL-4 appears to be less dependent on GATA-3 in both Th2 and Tfh cells [20, 172–174].

It remains unclear whether IL-4-producing Tfh cells arise from Th2 cells and share a common IL-4-competent precursor, or whether Th2 cells represent a distinct developmental subset from other T-helper subsets based on affinity for antigen [162, 164, 175–179]. Furthermore, despite evidence for and the persistence of memory Tfh cells, IL-4-producing Tfh cells that arise upon secondary antigen encounter do not appear to require long-term resident Tfh cells [180]. As such a better understanding of the relationship between IL-4-producing Tfh cells and canonical Th2 cells and whether IL-4-producing Tfh cells are maintained in the absence of type-2 inflammation are critical to our basic understanding of allergic immunity (figure 2). Certainly, IL-4-producing Tfh cells do not require high levels of GATA-3, STAT6, or IL-4 to generate type-2 cytokines *in vivo*, indicating that Tfh cells likely do not require the classical IL-4 receptor/STAT6/GATA-3 pathway for IL-4 production. Indeed, as mentioned above STAT6 signaling appears to play more of a role in stabilizing the IL-4-producing phenotype in Th2 and Tfh cells, rather than being important required in the initial differentiation or early cytokine production by these subsets [20, 47]. Furthermore, Tfh and Th2 lineage-determining factors tend to antagonize the function of each other. For example, BCL6 (Tfh factor) and STAT6 (Th2 factor) recognize and compete for binding at the same DNA consensus sites, thus it was initially proposed that BCL6 inhibited Th2 cytokine production by competing for STAT6 binding sites at the Th2 cytokine locus [181]. In support of BCL6 playing an important role in inhibiting Th2 function and differentiation, BCL6-deficient mice develop robust eosinophilia, Th2 cytokine production, and disease reminiscent of allergic pathology [181–184]. However, the hyper-allergic phenotype attributed to BCL6-deficiency also occurs in the absence of STAT6, which suggests enhanced STAT6 binding at the Th2 cytokine locus is not the cause of disease in this case [183]. More recent studies suggest that BCL6 likely regulates Th2 cytokine levels indirectly by modulating GATA-3 protein expression and T regulatory function [185, 186]. The precise mechanism has yet to be determined, but GATA-3 levels are significantly increased in BCL6-deficient mice and BCL6-deficient T regulatory cells. In sum, the data suggest that the absence of BCL6 may promote the development of the Th2 program through increased GATA-3 expression.

The mechanism of Tfh-mediated IL-4 production has yet to be fully defined. STAT5 activation of IL-4 appears unlikely to be a major contributor given that IL-2 driven STAT5 activity negatively regulates BCL6 and Tfh cell differentiation [187–189]. Another molecule implicated in the production of IL-4 by Tfh cells is SLAM Associated Protein (SAP) [163].

The major role of SAP with respect to CD4⁺ T cells is to promoting germinal center formation and B cell function [190, 191]. However, SAP-mediated regulation of humoral immunity appears independent of its role in IL-4 production, and works mainly by promoting more stable contact with germinal center B cells [192, 193]. SAP can modulate IL-4 expression in CD4⁺ T cells *in vitro* through activation of PKC- θ [194]. *In vivo*, SAP-deficient CD4⁺ T cells show only a slight reduction in IL-4 when exposed to schistosome egg antigens, which stimulate robust Th2 responses [190]. Thus, the precise mechanism of SAP-mediated regulation of IL-4 in Tfh cells appears more complicated *in vivo* [190, 194]. A plausible model to explain the decrease in IL-4 production by SAP-deficient Tfh cells *in vivo* is that SAP-deficient T cells are not well maintained in germinal centers. As a result, SAP-deficient Tfh cells do not receive the necessary signals to produce IL-4 protein at high levels. This fits with the observations that most of the IL-4 production found in the B cell follicles is restricted to Tfh cells residing in mature germinal centers [162, 163].

Potential Pathways for non-classical IL-4 production in Tfh cells

As mentioned above, IL-4 protein production is centralized to germinal center reactions within B cell follicles in lymphoid tissues. Disruption of follicular migration into the B cell follicles and germinal centers inhibits the formation of bona fide Tfh cells, and accordingly prevents the production of IL-4 protein as well. T cells that lack the B cell homing chemokine receptor CXCR5 show a significant reduction in IL-4 among cells expressing a Tfh-like phenotype [195]. The transcription factor achaete-scute homolog 2 (ASCL2) regulates CXCR5 expression, and ASCL2 overexpression in T cells led to increased IL-4 production [196]. Intriguingly, ASCL2 overexpression led to downregulated GATA-3 expression, increased IL-4, and decreased IL-13 production, a signature more closely associated with Tfh cells rather than canonical Th2 cells. ASCL2 does not bind to the IL-4 promoter, suggesting that ASCL2 indirectly regulates IL-4 expression, possibly through facilitating the localization of Tfh cells to the B cell follicles. Another factor that modulates Tfh cell entry into B cell follicles and germinal centers is Inducible Costimulator (ICOS). ICOS has long been considered an important factor in humoral immunity (IgE and IgG1), germinal center function, and type-2 inflammation [197–202]. Originally, ICOS was thought to directly regulate Th2 cytokine production [84, 199]. However, later studies showed early IL-4 mRNA competency among CD4⁺ T cells was unaffected in mice where ICOS signaling was absent [162]. Remarkably, despite the large number of CD4⁺ T cells capable of making IL-4 in the lymph node paracortex, follicular migration was severely inhibited. As such, ICOS likely influences Tfh cell cytokine production by modulating Tfh cell entry into the B cell follicles, a mechanism confirmed by a recent study [203]. In addition, ICOS deficiency prevents PI3K-mediated BCL6 expression, dampening Tfh development and maintenance [204–206]. Thus, ICOS is critical to the development of Tfh cells and migration of CD4⁺ T cells into the B cell follicle where it ultimately is poised to produce IL-4.

ICOS may directly influence IL-4 production through its regulation of c-Maf expression [207]. As discussed above, c-Maf was shown to influence IL-4 production in Th2 cells, and remains an intriguing candidate for Tfh cell mediated production of IL-4. However, it is likely that c-Maf plays a more important role in IL-4 production in Tfh cells due to the

decreased expression of GATA-3 observed in IL-4-producing Tfh cells. In support of such a scenario, c-Maf has been characterized as an important player in Tfh cell differentiation [208, 209]. c-Maf can cooperate with the AP-1 factor BATF for optimal Tfh cell development [208]. BATF is critical for Th17 differentiation and IL-17 production through interactions with IRF4 [210–214]. Additionally, recent studies suggest that BATF may play a larger role in Th2 cytokine production than previously appreciated [215–217]. Thus, more thorough investigation of cooperating interactions between c-Maf and BATF in Th2 and Tfh cytokine production may reveal novel pathways involved in non-classical IL-4 production (figure 1).

As discussed above, Notch receptors have been implicated in Th2 cell cytokine expression. As such, it would seem plausible that Notch signaling might also influence Tfh cell IL-4 production. Interestingly, Notch binds to the CNS2 enhancer at the *il4* locus to potentiate *il-4* transcription. Studies in which the CNS2 enhancer was deleted provided the first line of evidence that Notch may indeed play a specific role in IL-4 production in Tfh cells. In these studies, CNS2-deficient mice were able to mount productive Th2 responses in peripheral tissues, but failed to generate IgE [218, 219]. As IL-4 from Tfh cells is critical for IgE production, the deletion of the CNS2 enhancer revealed that it is likely important for IL-4 production in Tfh cells. Indeed, IL-4, but not IL-13 production, was restricted in CNS2-deficient CD4⁺ T cells, again suggesting that Tfh cell cytokine production but not Th2 function was impaired. Importantly, CNS2 is known to contain multiple binding sites for the Notch regulator, recombination signal binding protein-J (RBPJ) [220]. In addition to Notch and RBPJ, SAP may be important in CNS2-regulated IL-4 production [220]. Taken together, these studies suggest that Notch might represent a significant regulator in non-canonical IL-4 production in Tfh cells. However, this has been a difficult question to address at present given that Notch is required for Tfh cell differentiation [221].

Mechanism III: Differential expression of IL-4 and IL-13 by innate immune subsets during allergic inflammation

Innate immune cells elicited during type-2 immunity are believed to produce both IL-4 and IL-13. This is highlighted by studies showing that IL-4 and IL-13 mRNA are constitutively expressed by basophils, eosinophils, and mast cells [86]. However, as discussed above, mRNA expression does not necessarily correlate with cytokine protein production *in vivo*. Thus, studies utilizing *ex vivo* restimulation may mask the true nature of cytokine production. Indeed, using IL-4 and IL-13 reporter mice to track *in vivo* cytokine production, it is clear that innate immune cells may also exhibit differential IL-4 and IL-13 production. *In vivo* models of type-2 inflammation with helminth infection revealed that basophils, eosinophils, and Th2 cells found in the lung produced IL-4 protein however basophils and eosinophils did not contribute IL-13 *in vivo*. Among the IL-13-producing cells, Th2 and ILC2 cells represented the predominant cell subsets. Strikingly, mature ILC2 cells did not show evidence of IL-4 production during type-2 inflammatory conditions.

The regulation of non-coordinate type-2 cytokine expression in innate immune cells matches nicely with what was observed among Tfh and Th2 cells. Basophils (like Tfh cells) make

IL-4 and not IL-13 and express little GATA-3 protein. Conversely, ILC2 cells, which make IL-13 and not IL-4, express high levels of GATA-3. This implies that levels of GATA-3 can influence IL-13 production *in vivo*. Intriguingly, studies using conditional GATA-3 deletion or overexpression showed that a distinct threshold of GATA-3 was required for ILC2 development and subsequent IL-5 and IL-13 expression, and the threshold of GATA-3 likely impacts ILC3 differentiation as well [128, 222, 223]. Whether IL-13 expression is dependent matching this high threshold of GATA-3 in cells other than ILC2 and Th2 cells remains unclear. However, our laboratory has shown that GATA-3 levels can vary in iNKT cells, and those that do not reach a similar level of GATA-3 as that expressed in Th2 cells preferentially make IL-4, while those with high GATA-3 are able to produce both IL-4 and IL-13 (Personal communication RLR). With the gradual elucidation that innate immune subsets preferentially express different Th2 cytokines, one potential ramification is that different cell types may be recruited to specific niches within inflamed tissues, and as such each cytokine can exert a distinct effect based on where cells are found in the local tissues. Understanding the specific location and orchestration of these cells during type-2 inflammation will better define their specific roles in driving distinct type-2 allergic hallmarks.

Two distinct arms of type-2 inflammation and their therapeutic implications

We can now roughly group IL-4 and IL-13 into two functionally distinct arms of type-2 inflammation. One arm consisting of IL-4-producing follicular T cells driving humoral aspects of type-2 immunity, and the other arm orchestrated by IL-13-producing Th2 cells and mediating peripheral aspects of type-2 inflammation (figure 2). The divergent roles of these two arms during type-2 immune responses provide an opportunity to develop therapeutics that control specific allergic hallmarks linked to either IL-4 or IL-13. For example, IgE-driven allergies may be most effectively treated by dampening IL-4 production and Tfh cells, while it may be more beneficial to target IL-13, Th2 cells, and ILC2 cells to limit mucus production and airway hyperactivity to reduce asthma. Similarly, therapeutics specifically targeting the IL-13-Th2 arm may be better able to enhance Th2-mediated worm expulsion or increase the effectiveness of helminth mediated therapies [224].

Defining the transcription factors involved in Tfh vs Th2 cell function may also provide another layer of specificity that could be exploited to enhance or limit these two distinct arms. For example, high GATA-3 expression is likely required for IL-13 production. Thus, treatments designed to down-modulate GATA-3 activity in combination with anti-IL-13 therapy may more effectively limit pathology associated with IL-13-driven allergic asthma. Although the significance of different pathways used by Th2 and Tfh cells to produce IL-13 and IL-4 is only now being realized, future discoveries are likely to provide opportunities to better ameliorate a broad spectrum of diseases linked to type-2 inflammation.

Outside of type-2 inflammation, IL-4 and Tfh cells also represent key factors in normal germinal center function and high-affinity antibody production. Therefore identifying pathways responsible for IL-4 production by Tfh cells is also likely to benefit vaccine design and efficacy. Because IL-4 represents a B cell survival factor in the germinal center, the regulation of IL-4 expression may also be important in our understanding of B and T cell

lymphoma development. Indeed, most B cell lymphomas arise from germinal center B cells, and Tfh cells are believed to play an important role in follicular lymphoma biology as tumor cells proliferate near Tfh-like CD4⁺ T cells [225, 226]. In line with these findings, enhanced expression of IL-4 is found in Tfh cells isolated from follicular lymphoma biopsies suggestive of its significance in follicular lymphoma development [227–229]. Interestingly, many of the factors that play a role in non-classical IL-4 production by Tfh cells have also been implicated in B cell lymphoma generation, progression, or maintenance. For example, alterations in Notch signaling are associated with leukemia as well germinal center B cell lymphomas, and c-Maf is also associated with Tfh cells and T cell lymphoma development [230–235]. Thus, understanding the role of Tfh cells and IL-4 in B and T cell lymphoma generation, maintenance, and progression is likely to reveal novel pathways to treat such malignancies.

In summary, a better understanding of the cellular and molecular mechanisms that regulate IL-4 and IL-13 is important not only to advance our basic understanding of type-2 inflammatory diseases, but also paves the way to uncover novel pathways involved in a spectrum of disorders where disease outcome is influenced by the presence or absence of these two cytokines. Future studies in this arena are sure to uncover new therapeutics designed for more individualized care among a wide spectrum allergic diseases, as well have the potential to positively influence the way we think about infectious disease and cancer.

References

1. Pulendran B, Artis D. New paradigms in type 2 immunity. *Science*. 2012; 337:431–5. [PubMed: 22837519]
2. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004; 59:469–78. [PubMed: 15080825]
3. WHO fact sheet on Soil-transmitted helminth infections
4. Rook GA. Hygiene hypothesis and autoimmune diseases. *Clin Rev Allergy Immunol*. 2012; 42:5–15. [PubMed: 22090147]
5. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nature reviews Immunology*. 2001; 1:69–75.
6. Strachan DP. Hay fever, hygiene, and household size. *Bmj*. 1989; 299:1259–60. [PubMed: 2513902]
7. Catley MC, Coote J, Bari M, Tomlinson KL. Monoclonal antibodies for the treatment of asthma. *Pharmacol Ther*. 2011; 132:333–51. [PubMed: 21944943]
8. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*. 1998; 282:2261–3. [PubMed: 9856950]
9. Rankin JA, Picarella DE, Geba GP, Temann UA, Prasad B, DiCosmo B, et al. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci U S A*. 1996; 93:7821–5. [PubMed: 8755560]
10. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science*. 1998; 282:2258–61. [PubMed: 9856949]
11. McKenzie AN, Li X, Largaespada DA, Sato A, Kaneda A, Zurawski SM, et al. Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *Journal of immunology*. 1993; 150:5436–44.
12. Dolganov G, Bort S, Lovett M, Burr J, Schubert L, Short D, et al. Coexpression of the interleukin-13 and interleukin-4 genes correlates with their physical linkage in the cytokine gene cluster on human chromosome 5q23-31. *Blood*. 1996; 87:3316–26. [PubMed: 8605348]

13. Loots GG, Locksley RM, Blankespoor CM, Wang ZE, Miller W, Rubin EM, et al. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science*. 2000; 288:136–40. [PubMed: 10753117]
14. Kelly BL, Locksley RM. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. *Journal of immunology*. 2000; 165:2982–6.
15. Bucy RP, Karr L, Huang GQ, Li J, Carter D, Honjo K, et al. Single cell analysis of cytokine gene coexpression during CD4+ T-cell phenotype development. *Proc Natl Acad Sci U S A*. 1995; 92:7565–9. [PubMed: 7638231]
16. Bucy RP, Panoskaltis-Mortari A, Huang GQ, Li J, Karr L, Ross M, et al. Heterogeneity of single cell cytokine gene expression in clonal T cell populations. *J Exp Med*. 1994; 180:1251–62. [PubMed: 7523568]
17. Guo L, Hu-Li J, Paul WE. Probabilistic regulation in TH2 cells accounts for monoallelic expression of IL-4 and IL-13. *Immunity*. 2005; 23:89–99. [PubMed: 16039582]
18. Assenmacher M, Schmitz J, Radbruch A. Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. *Eur J Immunol*. 1994; 24:1097–101. [PubMed: 7910138]
19. Kelso A, Groves P, Ramm L, Doyle AG. Single-cell analysis by RT-PCR reveals differential expression of multiple type 1 and 2 cytokine genes among cells within polarized CD4+ T cell populations. *Int Immunol*. 1999; 11:617–21. [PubMed: 10323215]
20. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nature immunology*. 2012; 13:58–66.
21. Lee DU, Rao A. Molecular analysis of a locus control region in the T helper 2 cytokine gene cluster: a target for STAT6 but not GATA3. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:16010–5. [PubMed: 15507491]
22. Lee GR, Fields PE, Griffin TJ, Flavell RA. Regulation of the Th2 cytokine locus by a locus control region. *Immunity*. 2003; 19:145–53. [PubMed: 12871646]
23. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annual review of immunology*. 2006; 24:607–56.
24. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. *Immunity*. 2006; 24:369–79. [PubMed: 16618596]
25. Lee GR, Spilianakis CG, Flavell RA. Hypersensitive site 7 of the TH2 locus control region is essential for expressing TH2 cytokine genes and for long-range intrachromosomal interactions. *Nature immunology*. 2005; 6:42–8. [PubMed: 15608641]
26. Koh BH, Hwang SS, Kim JY, Lee W, Kang MJ, Lee CG, et al. Th2 LCR is essential for regulation of Th2 cytokine genes and for pathogenesis of allergic asthma. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:10614–9. [PubMed: 20483988]
27. Williams A, Lee GR, Spilianakis CG, Hwang SS, Eisenbarth SC, Flavell RA. Hypersensitive site 6 of the Th2 locus control region is essential for Th2 cytokine expression. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:6955–60. [PubMed: 23569250]
28. Spilianakis CG, Flavell RA. Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nature immunology*. 2004; 5:1017–27. [PubMed: 15378057]
29. Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA. Th2-specific chromatin remodeling and enhancer activity in the Th2 cytokine locus control region. *Immunity*. 2004; 21:865–76. [PubMed: 15589174]
30. Hwang SS, Kim YU, Lee S, Jang SW, Kim MK, Koh BH, et al. Transcription factor YY1 is essential for regulation of the Th2 cytokine locus and for Th2 cell differentiation. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:276–81. [PubMed: 23248301]
31. Cai S, Lee CC, Kohwi-Shigematsu T. SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. *Nat Genet*. 2006; 38:1278–88. [PubMed: 17057718]

32. Seder RA, Paul WE, Ben-Sasson SZ, LeGros GS, Kagey-Sobotka A, Finkelman FD, et al. Production of interleukin-4 and other cytokines following stimulation of mast cell lines and in vivo mast cells/basophils. *Int Arch Allergy Appl Immunol*. 1991; 94:137–40. [PubMed: 1834578]
33. Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity*. 2000; 12:27–37. [PubMed: 10661403]
34. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity*. 1996; 4:313–9. [PubMed: 8624821]
35. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. *Nature*. 1996; 380:627–30. [PubMed: 8602263]
36. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature*. 1996; 380:630–3. [PubMed: 8602264]
37. Kurata H, Lee HJ, O'Garra A, Arai N. Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. *Immunity*. 1999; 11:677–88. [PubMed: 10626890]
38. Tamauchi H, Terashima M, Ito M, Maruyama H, Ikewaki N, Inoue M, et al. Evidence of GATA-3-dependent Th2 commitment during the in vivo immune response. *Int Immunol*. 2004; 16:179–87. [PubMed: 14688073]
39. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:1993–8. [PubMed: 14769923]
40. Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. *The Journal of experimental medicine*. 2000; 192:105–15. [PubMed: 10880531]
41. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell*. 1997; 89:587–96. [PubMed: 9160750]
42. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *The Journal of biological chemistry*. 1997; 272:21597–603. [PubMed: 9261181]
43. Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity*. 1998; 9:745–55. [PubMed: 9846495]
44. Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nature immunology*. 2002; 3:643–51. [PubMed: 12055628]
45. Yamashita M, Ukai-Tadenuma M, Miyamoto T, Sugaya K, Hosokawa H, Hasegawa A, et al. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. *The Journal of biological chemistry*. 2004; 279:26983–90. [PubMed: 15087456]
46. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol*. 2009; 9:91–105. [PubMed: 19151746]
47. Mohrs M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity*. 2001; 15:303–11. [PubMed: 11520464]
48. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4+ lymphocytes with a Th2 cytokine profile. *Journal of immunology*. 2000; 164:3047–55.
49. van Panhuys N, Tang SC, Prout M, Camberis M, Scarlett D, Roberts J, et al. In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc Natl Acad Sci U S A*. 2008; 105:12423–8. [PubMed: 18719110]
50. Cunningham AF, Serre K, Toellner KM, Khan M, Alexander J, Brombacher F, et al. Pinpointing IL-4-independent acquisition and IL-4-influenced maintenance of Th2 activity by CD4 T cells. *Eur J Immunol*. 2004; 34:686–94. [PubMed: 14991598]

51. Noben-Trauth N, Shultz LD, Brombacher F, Urban JF Jr, Gu H, Paul WE. An interleukin 4 (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94:10838–43. [PubMed: 9380721]
52. Cunningham AF, Fallon PG, Khan M, Vacheron S, Acha-Orbea H, MacLennan IC, et al. Th2 activities induced during virgin T cell priming in the absence of IL-4, IL-13, and B cells. *J Immunol*. 2002; 169:2900–6. [PubMed: 12218103]
53. McKenzie GJ, Fallon PG, Emson CL, Grecis RK, McKenzie AN. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med*. 1999; 189:1565–72. [PubMed: 10330435]
54. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J Exp Med*. 1990; 172:921–9. [PubMed: 2117636]
55. Ben-Sasson SZ, Le Gros G, Conrad DH, Finkelman FD, Paul WE. IL-4 production by T cells from naive donors. IL-2 is required for IL-4 production. *J Immunol*. 1990; 145:1127–36. [PubMed: 2143207]
56. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. *Immunity*. 2003; 19:739–48. [PubMed: 14614860]
57. Kagami S, Nakajima H, Suto A, Hirose K, Suzuki K, Morita S, et al. Stat5a regulates T helper cell differentiation by several distinct mechanisms. *Blood*. 2001; 97:2358–65. [PubMed: 11290598]
58. Kagami S, Nakajima H, Kumano K, Suzuki K, Suto A, Imada K, et al. Both stat5a and stat5b are required for antigen-induced eosinophil and T-cell recruitment into the tissue. *Blood*. 2000; 95:1370–7. [PubMed: 10666213]
59. Moriggl R, Topham DJ, Teglund S, Sexl V, McKay C, Wang D, et al. Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. *Immunity*. 1999; 10:249–59. [PubMed: 10072077]
60. Takatori H, Nakajima H, Hirose K, Kagami S, Tamachi T, Suto A, et al. Indispensable role of Stat5a in Stat6-independent Th2 cell differentiation and allergic airway inflammation. *J Immunol*. 2005; 174:3734–40. [PubMed: 15749913]
61. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. *J Exp Med*. 2005; 202:793–804. [PubMed: 16172258]
62. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, et al. Interleukin 2 plays a central role in Th2 differentiation. *Proc Natl Acad Sci U S A*. 2004; 101:3880–5. [PubMed: 15004274]
63. Radtke F, Fasnacht N, Macdonald HR. Notch signaling in the immune system. *Immunity*. 2010; 32:14–27. [PubMed: 20152168]
64. Minter LM, Turley DM, Das P, Shin HM, Joshi I, Lawlor RG, et al. Inhibitors of gamma-secretase block in vivo and in vitro T helper type 1 polarization by preventing Notch upregulation of Tbx21. *Nature immunology*. 2005; 6:680–8. [PubMed: 15991363]
65. Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, et al. Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. *Immunity*. 2007; 27:89–99. [PubMed: 17658279]
66. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity*. 2007; 27:100–10. [PubMed: 17658278]
67. Tu L, Fang TC, Artis D, Shestova O, Pross SE, Maillard I, et al. Notch signaling is an important regulator of type 2 immunity. *J Exp Med*. 2005; 202:1037–42. [PubMed: 16230473]
68. Skokos D, Nussenzweig MC. CD8– DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. *J Exp Med*. 2007; 204:1525–31. [PubMed: 17576775]
69. Mukherjee S, Schaller MA, Neupane R, Kunkel SL, Lukacs NW. Regulation of T cell activation by Notch ligand, DLL4, promotes IL-17 production and Rorc activation. *J Immunol*. 2009; 182:7381–8. [PubMed: 19494260]

70. Keerthivasan S, Suleiman R, Lawlor R, Roderick J, Bates T, Minter L, et al. Notch signaling regulates mouse and human Th17 differentiation. *J Immunol.* 2011; 187:692–701. [PubMed: 21685328]
71. Tanigaki K, Tsuji M, Yamamoto N, Han H, Tsukada J, Inoue H, et al. Regulation of alphabeta/gammadelta T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. *Immunity.* 2004; 20:611–22. [PubMed: 15142529]
72. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell.* 2004; 117:515–26. [PubMed: 15137944]
73. Maekawa Y, Tsukumo S, Chiba S, Hirai H, Hayashi Y, Okada H, et al. Delta1-Notch3 interactions bias the functional differentiation of activated CD4+ T cells. *Immunity.* 2003; 19:549–59. [PubMed: 14563319]
74. Ong CT, Sedy JR, Murphy KM, Kopan R. Notch and presenilin regulate cellular expansion and cytokine secretion but cannot instruct Th1/Th2 fate acquisition. *PloS one.* 2008; 3:e2823. [PubMed: 18665263]
75. Bailis W, Yashiro-Ohtani Y, Fang TC, Hatton RD, Weaver CT, Artis D, et al. Notch simultaneously orchestrates multiple helper T cell programs independently of cytokine signals. *Immunity.* 2013; 39:148–59. [PubMed: 23890069]
76. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nature immunology.* 2011; 12:295–303. [PubMed: 21358638]
77. Lee K, Gudapati P, Dragovic S, Spencer C, Joyce S, Killeen N, et al. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity.* 2010; 32:743–53. [PubMed: 20620941]
78. Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med.* 1998; 188:1859–66. [PubMed: 9815263]
79. Ho IC, Hodge MR, Rooney JW, Glimcher LH. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell.* 1996; 85:973–83. [PubMed: 8674125]
80. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. *Immunity.* 1999; 10:745–51. [PubMed: 10403649]
81. Rengarajan J, Mowen KA, McBride KD, Smith ED, Singh H, Glimcher LH. Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. *The Journal of experimental medicine.* 2002; 195:1003–12. [PubMed: 11956291]
82. Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *The EMBO journal.* 1999; 18:420–32. [PubMed: 9889198]
83. Yang Y, Ochando J, Yopp A, Bromberg JS, Ding Y. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. *Journal of immunology.* 2005; 174:2720–9.
84. Nurieva RI, Duong J, Kishikawa H, Dianzani U, Rojo JM, Ho I, et al. Transcriptional regulation of th2 differentiation by inducible costimulator. *Immunity.* 2003; 18:801–11. [PubMed: 12818161]
85. Tanaka Y, So T, Lebedeva S, Croft M, Altman A. Impaired IL-4 and c-Maf expression and enhanced Th1-cell development in Vav1-deficient mice. *Blood.* 2005; 106:1286–95. [PubMed: 15845902]
86. Gessner A, Mohrs K, Mohrs M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J Immunol.* 2005; 174:1063–72. [PubMed: 15634931]
87. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol.* 1997; 158:3539–44. [PubMed: 9120316]
88. Sullivan BM, Liang HE, Bando JK, Wu D, Cheng LE, McKerrow JK, et al. Genetic analysis of basophil function in vivo. *Nature immunology.* 2011; 12:527–35. [PubMed: 21552267]

89. Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *European journal of immunology*. 1996; 26:2493–8. [PubMed: 8898965]
90. van Panhuys N, Prout M, Forbes E, Min B, Paul WE, Le Gros G. Basophils are the major producers of IL-4 during primary helminth infection. *J Immunol*. 186:2719–28.
91. Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. *Journal of immunology*. 1996; 156:4833–8.
92. Phillips C, Coward WR, Pritchard DI, Hewitt CR. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *Journal of leukocyte biology*. 2003; 73:165–71. [PubMed: 12525574]
93. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science*. 2011; 332:243–7. [PubMed: 21436399]
94. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity*. 2004; 20:267–77. [PubMed: 15030771]
95. Shinkai K, Mohrs M, Locksley RM. Helper T cells regulate type-2 innate immunity in vivo. *Nature*. 2002; 420:825–9. [PubMed: 12490951]
96. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, et al. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell*. 2014; 157:1292–308. [PubMed: 24906148]
97. Spencer LA, Melo RC, Perez SA, Bafford SP, Dvorak AM, Weller PF. Cytokine receptor-mediated trafficking of preformed IL-4 in eosinophils identifies an innate immune mechanism of cytokine secretion. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:3333–8. [PubMed: 16492782]
98. Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *Journal of leukocyte biology*. 2009; 85:117–23. [PubMed: 18840671]
99. Junttila IS, Watson C, Kummola L, Chen X, Hu-Li J, Guo L, et al. Efficient cytokine-induced IL-13 production by mast cells requires both IL-33 and IL-3. *J Allergy Clin Immunol*. 2013; 132:704–12. e10. [PubMed: 23683462]
100. Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol*. 1998; 102:491–502. [PubMed: 9768593]
101. Stassen M, Muller C, Arnold M, Hultner L, Klein-Hessling S, Neudorfl C, et al. IL-9 and IL-13 production by activated mast cells is strongly enhanced in the presence of lipopolysaccharide: NF-kappa B is decisively involved in the expression of IL-9. *J Immunol*. 2001; 166:4391–8. [PubMed: 11254693]
102. Wilson SJ, Shute JK, Holgate ST, Howarth PH, Bradding P. Localization of interleukin (IL) -4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy. *Clin Exp Allergy*. 2000; 30:493–500. [PubMed: 10718846]
103. Iwamura C, Nakayama T. Role of NKT cells in allergic asthma. *Current opinion in immunology*. 2010; 22:807–13. [PubMed: 21115332]
104. Thomas SY, Chyung YH, Luster AD. Natural killer T cells are not the predominant T cell in asthma and likely modulate, not cause, asthma. *J Allergy Clin Immunol*. 2010; 125:980–4. [PubMed: 20304475]
105. Lisbonne M, Diem S, de Castro Keller A, Lefort J, Araujo LM, Hachem P, et al. Cutting edge: invariant V alpha 14 NKT cells are required for allergen-induced airway inflammation and hyperactivity in an experimental asthma model. *Journal of immunology*. 2003; 171:1637–41.
106. Meyer EH, Goya S, Akbari O, Berry GJ, Savage PB, Kronenberg M, et al. Glycolipid activation of invariant T cell receptor+ NK T cells is sufficient to induce airway hyperactivity independent of conventional CD4+ T cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:2782–7. [PubMed: 16478801]

107. Akbari O, Faul JL, Hoyte EG, Berry GJ, Wahlstrom J, Kronenberg M, et al. CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma. *N Engl J Med*. 2006; 354:1117–29. [PubMed: 16540612]
108. Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, et al. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nature medicine*. 2003; 9:582–8.
109. Scanlon ST, Thomas SY, Ferreira CM, Bai L, Krausz T, Savage PB, et al. Airborne lipid antigens mobilize resident intravascular NKT cells to induce allergic airway inflammation. *The Journal of experimental medicine*. 2011; 208:2113–24. [PubMed: 21930768]
110. Matsuda JL, Gapin L, Baron JL, Sidobre S, Stetson DB, Mohrs M, et al. Mouse V alpha 14i natural killer T cells are resistant to cytokine polarization in vivo. *Proc Natl Acad Sci U S A*. 2003; 100:8395–400. [PubMed: 12829795]
111. Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, et al. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J Exp Med*. 2003; 198:1069–76. [PubMed: 14530376]
112. Lee PT, Benlagha K, Teyton L, Bendelac A. Distinct functional lineages of human V(alpha)24 natural killer T cells. *The Journal of experimental medicine*. 2002; 195:637–41. [PubMed: 11877486]
113. Wang ZY, Kusam S, Munugalavada V, Kapur R, Brutkiewicz RR, Dent AL. Regulation of Th2 cytokine expression in NKT cells: unconventional use of Stat6, GATA-3, and NFAT2. *J Immunol*. 2006; 176:880–8. [PubMed: 16393972]
114. Lee YJ, Holzapfel KL, Zhu J, Jameson SC, Hogquist KA. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nature immunology*. 2013; 14:1146–54. [PubMed: 24097110]
115. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010; 464:1367–70. [PubMed: 20200518]
116. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Easley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:11489–94. [PubMed: 20534524]
117. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature*. 2010; 463:540–4. [PubMed: 20023630]
118. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity*. 2001; 15:985–95. [PubMed: 11754819]
119. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med*. 2006; 203:1105–16. [PubMed: 16606668]
120. Klein Wolterink RG, Kleinjan A, van Nimwegen M, Bergen I, de Bruijn M, Levani Y, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. *Eur J Immunol*. 2012; 42:1106–16. [PubMed: 22539286]
121. Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature*. 2013; 502:245–8. [PubMed: 24037376]
122. Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *The Journal of allergy and clinical immunology*. 2012; 129:191–8. e1–4. [PubMed: 22079492]
123. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *The Journal of experimental medicine*. 2013; 210:2939–50. [PubMed: 24323357]
124. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway

contraction. *The Journal of allergy and clinical immunology*. 2013; 132:933–41. [PubMed: 23810766]

125. Camelo A, Barlow JL, Drynan LF, Neill DR, Ballantyne SJ, Wong SH, et al. Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13. *J Gastroenterol*. 2012
126. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *The Journal of allergy and clinical immunology*. 2013; 131:187–200. e1–8. [PubMed: 23006545]
127. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med*. 2013; 5:170ra16.
128. Mjosberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, Blom B, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity*. 2012; 37:649–59. [PubMed: 23063330]
129. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J Immunol*. 2012; 188:1503–13. [PubMed: 22198948]
130. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity*. 2012; 36:451–63. [PubMed: 22425247]
131. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*. 2014; 40:425–35. [PubMed: 24613091]
132. Finkelman FD, Shea-Donohue T, Morris SC, Gildea L, Strait R, Madden KB, et al. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol Rev*. 2004; 201:139–55. [PubMed: 15361238]
133. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, et al. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol*. 1997; 15:505–33. [PubMed: 9143698]
134. Urban JF Jr, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, et al. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity*. 1998; 8:255–64. [PubMed: 9492006]
135. Urban JF Jr, Katona IM, Paul WE, Finkelman FD. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proc Natl Acad Sci U S A*. 1991; 88:5513–7. [PubMed: 2062833]
136. Zurawski SM, Vega F Jr, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *The EMBO journal*. 1993; 12:2663–70. [PubMed: 8101483]
137. Mandel TE, Phipps RP, Abbot AP, Tew JG. Long-term antigen retention by dendritic cells in the popliteal lymph node of immunized mice. *Immunology*. 1981; 43:353–62. [PubMed: 7251058]
138. Urban JF Jr, Maliszewski CR, Madden KB, Katona IM, Finkelman FD. IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J Immunol*. 1995; 154:4675–84. [PubMed: 7722320]
139. Bancroft AJ, McKenzie AN, Grecis RK. A critical role for IL-13 in resistance to intestinal nematode infection. *J Immunol*. 1998; 160:3453–61. [PubMed: 9531306]
140. McKenzie GJ, Bancroft A, Grecis RK, McKenzie AN. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Curr Biol*. 1998; 8:339–42. [PubMed: 9512421]
141. Lawrence RA, Gray CA, Osborne J, Maizels RM. *Nippostrongylus brasiliensis*: cytokine responses and nematode expulsion in normal and IL-4-deficient mice. *Exp Parasitol*. 1996; 84:65–73. [PubMed: 8888733]
142. Fallon PG, Jolin HE, Smith P, Emson CL, Townsend MJ, Fallon R, et al. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity*. 2002; 17:7–17. [PubMed: 12150887]

143. Madden KB, Whitman L, Sullivan C, Gause WC, Urban JF Jr, Katona IM, et al. Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. *Journal of immunology*. 2002; 169:4417–22.
144. Brusselle GG, Kips JC, Tavernier JH, van der Heyden JG, Cuvelier CA, Pauwels RA, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin Exp Allergy*. 1994; 24:73–80. [PubMed: 8156448]
145. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med*. 1996; 183:109–17. [PubMed: 8551213]
146. Castilow EM, Meyerholz DK, Varga SM. IL-13 is required for eosinophil entry into the lung during respiratory syncytial virus vaccine-enhanced disease. *J Immunol*. 2008; 180:2376–84. [PubMed: 18250447]
147. Heller NM, Gwinn WM, Donnelly RP, Constant SL, Keegan AD. IL-4 engagement of the type I IL-4 receptor complex enhances mouse eosinophil migration to eotaxin-1 in vitro. *PLoS One*. 2012; 7:e39673. [PubMed: 22761864]
148. Voehringer D, Reese TA, Huang X, Shinkai K, Locksley RM. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J Exp Med*. 2006; 203:1435–46. [PubMed: 16702603]
149. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, et al. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J Exp Med*. 2004; 200:507–17. [PubMed: 15314076]
150. Munitz A, Brandt EB, Mingler M, Finkelman FD, Rothenberg ME. Distinct roles for IL-13 and IL-4 via IL-13 receptor alpha1 and the type II IL-4 receptor in asthma pathogenesis. *Proc Natl Acad Sci U S A*. 2008; 105:7240–5. [PubMed: 18480254]
151. Junttila IS, Mizukami K, Dickensheets H, Meier-Schellersheim M, Yamane H, Donnelly RP, et al. Tuning sensitivity to IL-4 and IL-13: differential expression of IL-4Ralpha, IL-13Ralpha1, and gammaC regulates relative cytokine sensitivity. *The Journal of experimental medicine*. 2008; 205:2595–608. [PubMed: 18852293]
152. Ramalingam TR, Pesce JT, Sheikh F, Cheever AW, Mentink-Kane MM, Wilson MS, et al. Unique functions of the type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha1 chain. *Nat Immunol*. 2008; 9:25–33. [PubMed: 18066066]
153. Murata T, Obiri NI, Puri RK. Structure of and signal transduction through interleukin-4 and interleukin-13 receptors (review). *Int J Mol Med*. 1998; 1:551–7. [PubMed: 9852261]
154. Heller NM, Qi X, Junttila IS, Shirey KA, Vogel SN, Paul WE, et al. Type I IL-4Rs selectively activate IRS-2 to induce target gene expression in macrophages. *Sci Signal*. 2008; 1:ra17. [PubMed: 19109239]
155. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, et al. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell*. 2008; 132:259–72. [PubMed: 18243101]
156. Rothenberg ME, Wen T, Shik D, Cole ET, Mingler MM, Munitz A. IL-13 receptor alpha1 differentially regulates aeroallergen-induced lung responses. *J Immunol*. 2011; 187:4873–80. [PubMed: 21957151]
157. Keegan AD, Nelms K, White M, Wang LM, Pierce JH, Paul WE. An IL-4 receptor region containing an insulin receptor motif is important for IL-4-mediated IRS-1 phosphorylation and cell growth. *Cell*. 1994; 76:811–20. [PubMed: 8124718]
158. Wang HY, Zamorano J, Yoerkie JL, Paul WE, Keegan AD. The IL-4-induced tyrosine phosphorylation of the insulin receptor substrate is dependent on JAK1 expression in human fibrosarcoma cells. *J Immunol*. 1997; 158:1037–40. [PubMed: 9013940]
159. Heller NM, Qi X, Gesbert F, Keegan AD. The extracellular and transmembrane domains of the gammaC and interleukin (IL)-13 receptor alpha1 chains, not their cytoplasmic domains, dictate the nature of signaling responses to IL-4 and IL-13. *The Journal of biological chemistry*. 2012; 287:31948–61. [PubMed: 22829596]

160. Hu-Li J, Pannetier C, Guo L, Lohning M, Gu H, Watson C, et al. Regulation of expression of IL-4 alleles: analysis using a chimeric GFP/IL-4 gene. *Immunity*. 2001; 14:1–11. [PubMed: 11163225]
161. Mohrs K, Wakil AE, Killeen N, Locksley RM, Mohrs M. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity*. 2005; 23:419–29. [PubMed: 16226507]
162. Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat Immunol*. 2009; 10:385–93. [PubMed: 19252490]
163. Yusuf I, Kageyama R, Monticelli L, Johnston RJ, Ditoro D, Hansen K, et al. Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). *Journal of immunology*. 2010; 185:190–202.
164. Zaretsky AG, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J Exp Med*. 2009; 206:991–9. [PubMed: 19380637]
165. King IL, Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J Exp Med*. 2009; 206:1001–7. [PubMed: 19380638]
166. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science*. 2009; 325:1006–10. [PubMed: 19608860]
167. Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity*. 2009; 31:457–68. [PubMed: 19631565]
168. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, et al. Bcl6 mediates the development of T follicular helper cells. *Science*. 2009; 325:1001–5. [PubMed: 19628815]
169. Kitano M, Moriyama S, Ando Y, Hikida M, Mori Y, Kurosaki T, et al. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity*. 2011; 34:961–72. [PubMed: 21636294]
170. Baumjohann D, Okada T, Ansel KM. Cutting Edge: Distinct waves of BCL6 expression during T follicular helper cell development. *Journal of immunology*. 2011; 187:2089–92.
171. Poholek AC, Hansen K, Hernandez SG, Eto D, Chande A, Weinstein JS, et al. In vivo regulation of Bcl6 and T follicular helper cell development. *Journal of immunology*. 2010; 185:313–26.
172. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat Immunol*. 2004; 5:1157–65. [PubMed: 15475959]
173. Kishikawa H, Sun J, Choi A, Miaw SC, Ho IC. The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. *J Immunol*. 2001; 167:4414–20. [PubMed: 11591766]
174. Zhang DH, Yang L, Ray A. Differential responsiveness of the IL-5 and IL-4 genes to transcription factor GATA-3. *J Immunol*. 1998; 161:3817–21. [PubMed: 9780145]
175. Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B, et al. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J Immunol*. 2013; 190:4014–26. [PubMed: 23487426]
176. Liu X, Yan X, Zhong B, Nurieva RI, Wang A, Wang X, et al. Bcl6 expression specifies the T follicular helper cell program in vivo. *The Journal of experimental medicine*. 2012; 209:1841–52. [PubMed: 22987803]
177. Tubo NJ, Pagan AJ, Taylor JJ, Nelson RW, Linehan JL, Ertelt JM, et al. Single Naive CD4(+) T Cells from a Diverse Repertoire Produce Different Effector Cell Types during Infection. *Cell*. 2013; 153:785–96. [PubMed: 23663778]
178. Hale JS, Youngblood B, Latner DR, Mohammed AU, Ye L, Akondy RS, et al. Distinct Memory CD4(+) T Cells with Commitment to T Follicular Helper- and T Helper 1-Cell Lineages Are Generated after Acute Viral Infection. *Immunity*. 2013; 38:805–17. [PubMed: 23583644]
179. Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. *Nat Immunol*. 2009; 10:375–84. [PubMed: 19252493]

180. Fairfax KC, Everts B, Amiel E, Smith AM, Schramm G, Haas H, et al. IL-4-Secreting Secondary T Follicular Helper (Tfh) Cells Arise from Memory T Cells, Not Persisting Tfh Cells, through a B Cell-Dependent Mechanism. *J Immunol.* 2015; 194:2999–3010. [PubMed: 25712216]
181. Dent AL, Doherty TM, Paul WE, Sher A, Staudt LM. BCL-6-deficient mice reveal an IL-4-independent, STAT6-dependent pathway that controls susceptibility to infection by *Leishmania major*. *J Immunol.* 1999; 163:2098–103. [PubMed: 10438949]
182. Dent AL, Shaffer AL, Yu X, Allman D, Staudt LM. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science.* 1997; 276:589–92. [PubMed: 9110977]
183. Dent AL, Hu-Li J, Paul WE, Staudt LM. T helper type 2 inflammatory disease in the absence of interleukin 4 and transcription factor STAT6. *Proc Natl Acad Sci U S A.* 1998; 95:13823–8. [PubMed: 9811885]
184. Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nat Genet.* 1997; 16:161–70. [PubMed: 9171827]
185. Kusam S, Toney LM, Sato H, Dent AL. Inhibition of Th2 differentiation and GATA-3 expression by BCL-6. *J Immunol.* 2003; 170:2435–41. [PubMed: 12594267]
186. Sawant DV, Sehra S, Nguyen ET, Jadhav R, Englert K, Shinnakasu R, et al. Bcl6 controls the Th2 inflammatory activity of regulatory T cells by repressing Gata3 function. *Journal of immunology.* 2012; 189:4759–69.
187. Pepper M, Pagan AJ, Igyarto BZ, Taylor JJ, Jenkins MK. Opposing signals from the bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. *Immunity.* 2011; 35:583–95. [PubMed: 22018468]
188. Nurieva RI, Podd A, Chen Y, Alekseev AM, Yu M, Qi X, et al. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. *The Journal of biological chemistry.* 2012; 287:11234–9. [PubMed: 22318729]
189. Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S. STAT5 is a potent negative regulator of TFH cell differentiation. *The Journal of experimental medicine.* 2012; 209:243–50. [PubMed: 22271576]
190. Cannons JL, Yu LJ, Jankovic D, Crotty S, Horai R, Kirby M, et al. SAP regulates T cell-mediated help for humoral immunity by a mechanism distinct from cytokine regulation. *J Exp Med.* 2006; 203:1551–65. [PubMed: 16754717]
191. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R. SAP is required for generating long-term humoral immunity. *Nature.* 2003; 421:282–7. [PubMed: 12529646]
192. Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, et al. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity.* 2010; 32:253–65. [PubMed: 20153220]
193. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature.* 2008; 455:764–9. [PubMed: 18843362]
194. Cannons JL, Yu LJ, Hill B, Mijares LA, Dombroski D, Nichols KE, et al. SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity.* 2004; 21:693–706. [PubMed: 15539155]
195. Haynes NM, Allen CD, Lesley R, Ansel KM, Killeen N, Cyster JG. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1-high germinal center-associated subpopulation. *J Immunol.* 2007; 179:5099–108. [PubMed: 17911595]
196. Liu X, Chen X, Zhong B, Wang A, Wang X, Chu F, et al. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. *Nature.* 2014; 507:513–8. [PubMed: 24463518]
197. Greenwald RJ, McAdam AJ, Van der Woude D, Satoskar AR, Sharpe AH. Cutting edge: inducible costimulator protein regulates both Th1 and Th2 responses to cutaneous leishmaniasis. *J Immunol.* 2002; 168:991–5. [PubMed: 11801630]
198. Tesciuba AG, Subudhi S, Rother RP, Faas SJ, Frantz AM, Elliot D, et al. Inducible costimulator regulates Th2-mediated inflammation, but not Th2 differentiation, in a model of allergic airway disease. *Journal of immunology.* 2001; 167:1996–2003.

199. Tafuri A, Shahinian A, Bladt F, Yoshinaga SK, Jordana M, Wakeham A, et al. ICOS is essential for effective T-helper-cell responses. *Nature*. 2001; 409:105–9. [PubMed: 11343123]
200. Dong C, Temann UA, Flavell RA. Cutting edge: critical role of inducible costimulator in germinal center reactions. *J Immunol*. 2001; 166:3659–62. [PubMed: 11238604]
201. Loke P, Zang X, Hsuan L, Waitz R, Locksley RM, Allen JE, et al. Inducible costimulator is required for type 2 antibody isotype switching but not T helper cell type 2 responses in chronic nematode infection. *Proc Natl Acad Sci U S A*. 2005; 102:9872–7. [PubMed: 15994233]
202. Scales HE, Ierna MX, Gutierrez-Ramos JC, Coyle AJ, Garside P, Lawrence CE. Effect of inducible costimulator blockade on the pathological and protective immune responses induced by the gastrointestinal helminth *Trichinella spiralis*. *European journal of immunology*. 2004; 34:2854–62. [PubMed: 15368302]
203. Xu H, Li X, Liu D, Li J, Zhang X, Chen X, et al. Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature*. 2013; 496:523–7. [PubMed: 23619696]
204. Choi YS, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity*. 2011; 34:932–46. [PubMed: 21636296]
205. Gigoux M, Shang J, Pak Y, Xu M, Choe J, Mak TW, et al. Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:20371–6. [PubMed: 19915142]
206. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity*. 2008; 29:138–49. [PubMed: 18599325]
207. Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nature immunology*. 2009; 10:167–75. [PubMed: 19098919]
208. Ise W, Kohyama M, Schraml BU, Zhang T, Schwer B, Basu U, et al. The transcription factor BATF controls the global regulators of class-switch recombination in both B cells and T cells. *Nature immunology*. 2011; 12:536–43. [PubMed: 21572431]
209. Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK, et al. Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. *J Immunol*. 2012; 188:3734–44. [PubMed: 22427637]
210. Betz BC, Jordan-Williams KL, Wang C, Kang SG, Liao J, Logan MR, et al. Batf coordinates multiple aspects of B and T cell function required for normal antibody responses. *The Journal of experimental medicine*. 2010; 207:933–42. [PubMed: 20421391]
211. Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM, et al. BATF-JUN is critical for IRF4-mediated transcription in T cells. *Nature*. 2012; 490:543–6. [PubMed: 22992523]
212. Glasmacher E, Agrawal S, Chang AB, Murphy TL, Zeng W, Vander Lugt B, et al. A genomic regulatory element that directs assembly and function of immune-specific AP-1-IRF complexes. *Science*. 2012; 338:975–80. [PubMed: 22983707]
213. Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, et al. A validated regulatory network for Th17 cell specification. *Cell*. 2012; 151:289–303. [PubMed: 23021777]
214. Schraml BU, Hildner K, Ise W, Lee WL, Smith WA, Solomon B, et al. The AP-1 transcription factor Batf controls T(H)17 differentiation. *Nature*. 2009; 460:405–9. [PubMed: 19578362]
215. Ubel C, Sopel N, Graser A, Hildner K, Reinhardt C, Zimmermann T, et al. The activating protein 1 transcription factor basic leucine zipper transcription factor, ATF-like (BATF), regulates lymphocyte- and mast cell-driven immune responses in the setting of allergic asthma. *The Journal of allergy and clinical immunology*. 2014; 133:198–206. e1–9. [PubMed: 24290279]
216. Jabeen R, Goswami R, Awe O, Kulkarni A, Nguyen ET, Attenasio A, et al. Th9 cell development requires a BATF-regulated transcriptional network. *The Journal of clinical investigation*. 2013; 123:4641–53. [PubMed: 24216482]
217. van den Ham HJ, de Waal L, Zaaraoui-Boutahar F, Bijl M, van Ijcken WF, Osterhaus AD, et al. Early divergence of Th1 and Th2 transcriptomes involves a small core response and sets of transiently expressed genes. *European journal of immunology*. 2013; 43:1074–84. [PubMed: 23436590]

218. Vijayanand P, Seumois G, Simpson LJ, Abdul-Wajid S, Baumjohann D, Panduro M, et al. Interleukin-4 production by follicular helper T cells requires the conserved IL4 enhancer hypersensitivity site V. *Immunity*. 2012; 36:175–87. [PubMed: 22326582]
219. Harada Y, Tanaka S, Motomura Y, Ohno S, Yanagi Y, Inoue H, et al. The 3' Enhancer CNS2 Is a Critical Regulator of Interleukin-4-Mediated Humoral Immunity in Follicular Helper T Cells. *Immunity*. 2012; 36:188–200. [PubMed: 22365664]
220. Tanaka S, Tsukada J, Suzuki W, Hayashi K, Tanigaki K, Tsuji M, et al. The interleukin-4 enhancer CNS-2 is regulated by Notch signals and controls initial expression in NKT cells and memory-type CD4 T cells. *Immunity*. 2006; 24:689–701. [PubMed: 16782026]
221. Auderset F, Schuster S, Fasnacht N, Coutaz M, Charmoy M, Koch U, et al. Notch signaling regulates follicular helper T cell differentiation. *Journal of immunology*. 2013; 191:2344–50.
222. Serafini N, Klein Wolterink RG, Satoh-Takayama N, Xu W, Vossenrich CA, Hendriks RW, et al. Gata3 drives development of RORgammat+ group 3 innate lymphoid cells. *The Journal of experimental medicine*. 2014; 211:199–208. [PubMed: 24419270]
223. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity*. 2012; 37:634–48. [PubMed: 23063333]
224. Parker W, Ollerton J. Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. *Evolution, medicine, and public health*. 2013; 2013:89–103.
225. Manconi R, Poletti A, Volpe R, Sulfaro S, Carbone A. Dendritic reticulum cell pattern as a microenvironmental indicator for a distinct origin of lymphoma of follicular mantle cells. *Br J Haematol*. 1988; 68:213–8. [PubMed: 3280004]
226. Carbone A, Gloghini A, Gruss HJ, Pinto A. CD40 ligand is constitutively expressed in a subset of T cell lymphomas and on the microenvironmental reactive T cells of follicular lymphomas and Hodgkin's disease. *The American journal of pathology*. 1995; 147:912–22. [PubMed: 7573367]
227. Ame-Thomas P, Le Priol J, Yssel H, Caron G, Pangault C, Jean R, et al. Characterization of intratumoral follicular helper T cells in follicular lymphoma: role in the survival of malignant B cells. *Leukemia*. 2011
228. Calvo KR, Dabir B, Kovach A, Devor C, Bandle R, Bond A, et al. IL-4 protein expression and basal activation of Erk in vivo in follicular lymphoma. *Blood*. 2008; 112:3818–26. [PubMed: 18682601]
229. Pangault C, Ame-Thomas P, Ruminy P, Rossille D, Caron G, Baia M, et al. Follicular lymphoma cell niche: identification of a preeminent IL-4-dependent T(FH)-B cell axis. *Leukemia*. 2010; 24:2080–9. [PubMed: 20944673]
230. Fabbri G, Rasi S, Rossi D, Trifonov V, Khiabani H, Ma J, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. *The Journal of experimental medicine*. 2011; 208:1389–401. [PubMed: 21670202]
231. Puente XS, Pinyol M, Quesada V, Conde L, Ordonez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011; 475:101–5. [PubMed: 21642962]
232. Lee SY, Kumano K, Nakazaki K, Sanada M, Matsumoto A, Yamamoto G, et al. Gain-of-function mutations and copy number increases of Notch2 in diffuse large B-cell lymphoma. *Cancer Sci*. 2009; 100:920–6. [PubMed: 19445024]
233. Morito N, Yoh K, Fujioka Y, Nakano T, Shimohata H, Hashimoto Y, et al. Overexpression of c-Maf contributes to T-cell lymphoma in both mice and human. *Cancer research*. 2006; 66:812–9. [PubMed: 16424013]
234. Murakami YI, Yatabe Y, Sakaguchi T, Sasaki E, Yamashita Y, Morito N, et al. c-Maf expression in angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol*. 2007; 31:1695–702. [PubMed: 18059226]
235. Bisig B, Thielen C, Herens C, Gofflot S, Travert M, Delfau-Larue MH, et al. c-Maf expression in angioimmunoblastic T-cell lymphoma reflects follicular helper T-cell derivation rather than oncogenesis. *Histopathology*. 2012; 60:371–6. [PubMed: 22126447]

Figure 1. Classical and non-classical regulation of type-2 cytokine expression

Th2 cells classically produce IL-4 and IL-13 using the canonical pathway where IL-4 signals through the IL-4 receptor to phosphorylate of STAT6 and increase GATA-3 expression. STAT6 and GATA-3 then promote the expression of IL-4 and IL-13. Tfh cells express IL-4 but not IL-13, and they regulate IL-4 expression in a non-classical manner. Tfh cells do not utilize canonical STAT6 or GATA-3 to induce IL-4 expression. The non-canonical pathways used by Tfh cells to express IL-4 are not clearly defined, but many candidates exist. Th2 cells also can express type-2 cytokines in a STAT6/GATA-3 independent, non-canonical fashion. Green arrows represent regulators that are positively influence cytokine expression. Blue arrows represent molecules that likely influence cytokine production. Red hatches represent molecules that inhibit cytokine production. Solid arrows indicate direct regulation of IL-4 and IL-13. Dashed arrows represent indirect regulation.

Figure 2. Divergent expression and functions of IL-4 and IL-13 highlight two distinct arms of type-2 immunity

During allergic immune response a naïve CD4⁺ T cells in the paracortex of lymph nodes must make several fate choices. One of which is to become a Th2 cell or a Tfh cell. Although it is clear that committed Tfh cells and Th2 cells represent unique cellular subsets and perform distinct function, it still remains unclear whether these Tfh cells and Th2 cells share a common IL-4-competent precursor (pTh), or whether Tfh and Th2 cells arise from distinct progenitors (pTh2 and pTfh). IL-4 competent CD4⁺ T cells express IL-4 mRNA in the paracortex, but do not generate protein until the reach the B cell follicle as a Tfh cell or site of allergic inflammation as a Th2 cell. Tfh cells in the follicles and germinal centers secrete IL-4 protein and are essential for humoral immunity. Th2 cells at sights of allergic inflammation produce both IL-4 and IL-13, and contribute to the peripheral aspects of allergic immunity. The peripheral allergic hallmarks are largely dependent on IL-13 expression. Innate immune cells also contribute to the peripheral immune response. Like Tfh and Th2 precursors, innate cells constitutively express cytokine mRNA, but exhibit a distinct preference to secrete either IL-4 or IL-13. ((Light green = IL-4 mRNA expression); Dark Green = IL-4 Protein expression; Yellow = IL-13 protein production)).