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

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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 \textit{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 heath 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

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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 (IL4Rα)-deficient mice. Deletion of the IL4Rα 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 IL4Rα 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-4Rα 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-4Rα [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-4Rα. 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 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-4Rα or STAT6 \[134, 135\]. Because the receptors for IL-4 and IL-13 both contain IL-4Rα 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 *Trichurus 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 *Nippostronglus 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-4Rα is paired with the common gamma chain to form the type-I receptor or with IL-13Rα for the type-II receptor. IL-4 directly binds to IL-4Rα. Therefore, IL-4 can signal through both type-I and type-II receptors. IL-13 binds to IL-13RαI, 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-4Rα 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-13RαI, IL-13 signaling is dominant when the expression of IL-13RαI is enriched, or IL-4 is limiting. Thus, IL-13RαI 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.
infect T cells 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 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-13RαI-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-13Ra1- chains. This was shown in studies with chimeric IL-4 receptors, which replaced the extracellular IL-13Ra1 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 IL13 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 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-baring 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 contributer 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-theta [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 though 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 Th 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.

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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 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 pThf). 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 sites 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)).