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T helper 2 and T follicular helper cells: Regulation and Function of Interleukin-4

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Abstract

Type 2 immunity is characterized by expression of the cytokines interleukin (IL)-4, IL-5, IL-9 and IL-13, which can function in mediating protective immunity in the host or possess a pathogenic role. T helper (Th) 2 cells have emerged to play a beneficial role in mediating anti-parasitic immunity and are also known to be key players in mediating allergic diseases. In addition to the Th2 cells, recent studies have identified T follicular helper (Tfh) cells as an alternative source of IL-4 to regulate type 2 humoral immune responses, indicating that Th2 and Tfh cells exhibit overlapping phenotypical and functional characteristics. Th2 and Tfh cells appear to utilize unique mechanisms for regulation of IL-4 expression; however unlike Th2 cells, the regulation and function of Tfh-derived IL-4 is not yet fully understood. Understanding of the molecular mechanisms for IL-4 expression and function in both cell subsets will lead to development of pharmacological approaches to regulate the function of IL-4 in different disease settings.

Keywords

Th2; Tfh; Interleukin-4; transcription; type-2 immunity

1. Introduction

Type 2 immune responses are typically characterized by the differentiation of CD4⁺ T helper (Th)2 cells and the production of classical type 2 signature cytokines including IL-4, IL-5, IL-9 and IL-13 [1]. The major effector responses in type 2 immunity include B cell mediated humoral responses such as IgG1 and IgE antibody class switching and recruitment of inflammatory effector cells such as eosinophils, basophils and mast cells [2, 3]. Type 2 immunity is beneficial in mediating anti-parasitic immunity, wound-repair, suppressing

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Conflict of Interest

The authors declare no conflict of interest.

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autoimmune-type disease and maintaining tissue homeostasis [2, 4]. However on the other hand, type 2 immune responses are also known to be responsible for the development of asthma and other allergic inflammatory diseases [5].

In human and mice, Th2 cells have critical functions in immune responses against extracellular parasites and are involved in asthma and other allergic diseases [2, 6, 7]. Together with T cell receptor (TCR) ligation, IL-4-mediated signaling promotes Th2 differentiation. IL-4 activates the transcription factor STAT6 (signal transduction and activator of transcription 6) leading to expression and activation of GATA3, the Th2 master regulator and consequently to production of signature Th2 cytokines including IL-4, IL-5, and IL-13. In addition, Nuclear factor of activated T cells (NFAT), AP-1, Jun B, c-Maf and Interferon regulatory factor 4 (IRF4) are important for IL-4 production [8, 9]. Th2-derived IL-4 acts in an autocrine feedback loop to promote further Th2 differentiation.

Recent findings have led to identification of a new Th subset called T follicular helper (Tfh) cells because of their location in B cell follicles [10, 11]. Tfh cells are known to provide help for formation and maintenance of germinal center B cell reactions. Some of the distinct features of Tfh cells are expression of CXCR5, PD-1, ICOS, IL-21 and the requirement of the transcription factor Bcl6 [11, 12]. Of importance is the recent data using mouse models of helminth parasite infection as well as asthma model demonstrating that Tfh cells produce the Th2 signature cytokine IL-4 that regulates isotype class switching of B cells toward IgG1 and IgE [13–16], indicating that Th2 and Tfh cells, although distinct subset, yet display similar phenotypical and functional properties; however, the regulation and function of Tfh-derived IL-4 is not fully understood. In contrast to Th2 cells, regulation of IL-4 expression in Tfh cells exclusively depends on the 3' conserved non coding sequence (CNS)2 region in the IL-4 locus [17, 18]. SLAMFAP pathway and Notch and RBP-J signaling are potential candidates for regulation of IL-4 expression in Tfh cells through the CNS2 enhancer [19]. Furthermore, our recent study identifies a crucial role of the transcription factor basic leucine zipper transcription factor ATF-like (Batf) in controlling IL-4 expression in Tfh cells through direct binding and controlling access of the chromatin machinery in the CNS2 region [16]. Further understanding of the molecular details of IL-4 gene regulation in Tfh cells may facilitate the development of novel approaches to control IL-4 production in different disease settings.

In this review, we discuss the molecular mechanisms driving IL-4 production in Th2 and Tfh cells. Further, we elaborate on the current understanding of the physiological role of IL-4 derived from both subsets in shaping the type 2 immune responses and the current implications of IL-4 in a disease setting.

2. Factors controlling Th2 differentiation and IL-4 expression

Activation and differentiation of CD4⁺ T cells into effector Th2 cells require the collaboration between multiple factors such as costimulatory molecules, cytokine signaling and transcription factors. TCR signaling and costimulation by CD28 and ICOS activate downstream transcription factors that regulate early IL-4 production which in an autocrine feedback loop promote further Th2 differentiation (Fig 1). In the section below, we discuss

in detail the various signaling mechanisms and the cooperation between TCR, stimulatory molecules, cytokines signaling, transcription factors and epigenetic elements that are responsible for IL-4 production in Th2 cells.

2.1 TCR and costimulation

Naïve CD4⁺ T cells are capable of producing IL-4 upon TCR activation and thereby endogenously produced IL-4 is sufficient for Th2 differentiation. Such early IL-4 production by naïve CD4⁺ T cells is caused by low-dose antigen stimulation leading to weak and transient extracellular signal-regulated kinase (ERK) activation, GATA3 induction and IL-2 production that in turn activates STAT5. GATA3 cooperates with STAT5 to induce TCR-dependent, IL-4/STAT6-independent early IL-4 transcription [20, 21]. The endogenously produced IL-4 in collaboration with continued STAT5 activation amplifies production of IL-4 and leads to completion of Th2 differentiation [21].

Triggering of Th2 differentiation via low strength TCR stimulation can bypass requirement for exogenous IL-4 but requires a second signal via costimulatory receptors [7, 22]. Of particular importance for Th2 development are CD28, inducible costimulator (ICOS) and the tumor necrosis factor receptor superfamily members (OX-40, GITR and CD30) [9]. CD28, the most important co-stimulatory receptor, binds to B7.1 (CD80) and B7.2 (CD86) expressed on activated APCs and plays a major role in early phase of T cell activation, including expression of IL-2 and bcl-xL upregulation [6]. CD28 ligation favors Th2 differentiation by enhancing NFATc1 nuclear localization and inducing GATA3 expression [6]; CD28 deficiency in T cells results in reduced proliferative responses and low production of IL-2 and IL-4. Another member of the CD28 superfamily, inducible costimulator (ICOS) is expressed in higher level on Th2 cells and its engagement is particularly important for IL-4 and IL-10 expression [23]. ICOS or ICOS ligand deficient mice possess deficiencies in T cell activation, Th2 cytokine production, immunoglobulin isotype switching (IgG1 and IgE), and germinal center formation [23, 24]. ICOS costimulation potentiates early IL-4 expression by enhancing NFATc1 expression and hence effector IL-4 production by regulating c-Maf expression in the effector phase [6, 23]. In addition to the CD28 superfamily, the tumor necrosis factor receptor family members, OX40, CD30 and glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) play crucial roles in Th2 survival and expansion and thus for promoting Th2 mediated allergic responses [25, 26].

2.2 Cytokines

Although the TCR and costimulatory molecules dramatically influence the Th cell fate, cytokines are the major determinants of differentiation of CD4⁺ T cells. Many cytokines including IL-4, IL-2, IL-6, IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) are involved in controlling Th2 differentiation [6, 7, 27]. IL-4-induced activation of STAT6 leads to expression of Th2 master regulator GATA3 which triggers transcription of *Il4* gene and other Th2-associated cytokines [27]. In addition, IL-2 produced as a result of TCR triggering leads to STAT5-induced IL-4 production; Stat5deficient cells show diminished IL-4 production [8, 28]. Although IL-4 and IL-2 are required for Th2 cell development *in vitro*, normal *in vivo* GATA3-dependent Th2 responses were observed in IL-4- and STAT6-

deficient mice, suggesting that Th2 cells can differentiate independent of IL-4-STAT6 signaling [8, 29–31]. The cytokine IL-6 is also known to induce early IL-4 production through NFAT activation [32, 33]. TSLP is sufficient to directly facilitate Th2 priming and expansion independent of IL-4 through Stat5-dependent remodeling of IL-4 locus [9]. IL-25 regulates early IL-4 expression during Th2 priming through direct activation of NFATc1 and JunB; in memory Th2 cells, IL-25 signaling helps to maintain GATA3, JunB and c-Maf expression [34]. IL-33 in cooperation with STAT5 activators including IL-2, IL-7 and TSLP induces and maintains GATA3 expression which in turn increases IL-33R expression on resting Th2 cells. IL-33R-expressing Th2 cells produce Th2 cytokines (IL-13 and IL-5) in response IL-33 plus a STAT5 activators in a TCR-independent, NF- κ B- and MAPKs-dependent manner [35].

2.3 Transcription factors

STAT6 is the major signal transducer in IL-4 mediated Th2 differentiation [36, 37]. The IL-4R/STAT6 pathway is a positive feedback loop for Th2 development and STAT6 has been shown to be sufficient to induce GATA3 and c-Maf expression. While STAT6 is clearly important for maximal Th2 development [30], STAT6 deficient CD4⁺ T cells are still capable of minimal Th2 cytokine production which is dependent on the Th2 specific transcriptional factor GATA3 [8, 29]. GATA3 is recognized as the master regulator of Th2 cells and is associated with transcriptional activation of Th2-related genes, interaction with other transcriptional factors and epigenetic modifications. [20]. While Gata3 directly binds to the IL-5 and IL-13 promoters and transactivates these genes in cooperation with STAT5, GATA3 binds to hypersensitive site II (HSII)/IE enhancer of *il4* gene and promotes *il4* gene expression [28, 31]. Besides directly acting on the *il4* gene, GATA3 and STAT5 cross-regulate each other [38]; therefore crosstalk and collaboration between IL-4/STAT6/GATA3 and IL-2-STAT5 pathways results in complete Th2 differentiation. Furthermore, GATA3 has been reported to regulate its own expression [39]. GATA3 also requires cooperation with STAT6 for its binding to target sites in Th2 cells [40]. In addition GATA3 is known to inhibit Stat4 and IL-12R β expression and interacts with T-bet and Runx3 to inhibit Th1 differentiation [20]. Recently, STAT3 was shown to be important for STAT6 interaction with relevant gene loci in the developing Th2 cells [41]. Another transcription factor IFN regulatory factor-4 (IRF4) also is involved in the Th2 differentiation by upregulating GATA3 and by cooperating with NFATc2 to activate IL-4 expression [42, 43]. c-Maf which was identified as a Th2-specific transcription factor [44] in synergy with NFAT and activating protein (AP)-1 protein JunB, selectively induces IL-4 expression but not IL-5 and IL-13 [9]. Various other factors like Dec2, T cell factor-1 (TCF1) and Growth factor independent-1(gfi-1) have been also identified to influence the Th2 effector gene expression [20].

2.4 Epigenetic regulation

Extensive studies at the molecular and cellular level and *in vivo* animal models have identified the crucial DNA elements involved in IL-4 regulation which are usually conserved within species (conserved non coding sequences, CNS) and are accessible to DNAase1 (hypersensitive sites, HS) [20, 27]. The IL-4 and IL-13 intergenic region encompasses the hypersensitive sites HSS0-HSS3 (CNS1) which is a well-known GATA responsive element

(CGRE) which exerts its effects through the GATA3 transcription factor [20, 45, 46]. The HSI, HSII and HSIII regions are included within the IL-4 gene locus while the HSIV, HSV (CNS2) and HSV_A regions are located in the IL-4/Kif3a intergenic region [20, 27]. Apart from the above regions, a Th2 locus control region (LCR), comprising the RHS4, RHS5, RHS6 and RHS7 regions, crucial for the production of all signature Th2 cytokines including IL-4, IL-5 and IL-13 is located in the unrelated rad50 gene [20, 27]. The transcription factors GATA-3 and STAT5 bind to the HSII region and increase the accessibility of the IL4 locus and transcription of IL-4 gene [28, 31]. HS V/V_A binds to the GATA-3, NFAT1 (NFATc2) and Notch transcription factors and is crucial for IL-4 expression in Th2 and Tfh cells [17, 18, 29, 47]. HSIV is a well-known IL-4 silencer locus and its deletion leads to increase in IL-4 expression in naïve, Th1, Th2 and in macrophages as well. HSIV defective mice show aberrant expression of IFN- γ in Th2 cells [48]. The transcription factor Runx3 binds at the HSIV region and inhibits IL-4 expression in Th1 cells and GATA3 interacts with Runx3 to prevent its function in Th2 cells [49, 50]. Apart from the above regions, deletion of the entire Th2 LCR at the 3' end of the unrelated RAD50 locus caused almost complete reduction of all three Th2 cytokines [20]. Deletion of RHS6 region caused a major defect in all three Th2 cytokines while deletion of RHS7 in mice showed defect in IL-4 and IL-13 but not as much in IL-5 [51]. The transcription factor YY1 is known to bind to RHS7 and recruit GATA3 to control Th2 cytokine expression [20, 52]. The role of chromatin remodeling and epigenetic processes like DNA methylation, posttranslational histone modifications (acetylation, methylation, phosphorylation, etc) and cis regulatory elements in regulating IL-4 gene expression during differentiation of naïve T cells to Th2 cells has been extensively investigated and reviewed [53–55]. Upon initiation of Th2 differentiation from naïve T cells, the active histone modifications are increased and stabilized throughout the IL-4 locus in Th2 cells while repressive modifications like H3K27 trimethylation with the association of the H3K27 methyltransferase EZH2 is particularly enriched in the HSS3 and HSIV locus in Th1 cells leading to IL-4 silencing in Th1 cells [9, 27, 54]. DNA methylation is also known to play a crucial role in Th2 cytokine expression including IL-4 expression [56, 57]. Although DNA methylation is not required for initial IL-4 transcription; it is required for its maintenance in polarized Th2 cells [56, 58]. Under Th2 polarizing conditions, TCR signaling along with CD28 costimulation leads to progressive demethylation of the IL-4 promoter and the Th2 cell specific RHS7 locus resulting in IL-4 transcription [55, 59, 60]. The demethylation observed is independent of the Th2 master regulator GATA3. Cells that lack DNMT1 and MBD2 have increased IL-4 production [57, 61].

3. T follicular helper cells as an alternative source of IL-4 production

Apart from Th2 cells, Tfh cells have emerged as a distinct subset of IL-4-secreting T helper cells that are localized in germinal centers (GCs) and provide help to B cells [12, 15]. Tfh cells were identified by their expression of chemokine CXCR5, which allow these cells to migrate to the B cell follicles within GCs [62]. In addition to CXCR5, additional surface Tfh cell markers including ICOS, CD40L, PD-1, and BTLA have been reported [11, 62]. Tfh cell differentiation is regulated by IL-6 and IL-21, possibly via STAT3 factor and B cell lymphoma 6 (Bcl6) which is specifically expressed in Tfh cells and is required for their lineage commitment [63, 64]. While initially it has been acknowledged that Tfh cells only

secrete IL-21, that are critical for Tfh development and function [64, 65], recent data suggest that Tfh cells also produce the Th2 signature cytokine IL-4 [15]; however, the regulation and function of Tfh-derived IL-4 is not yet fully understood.

Recently, several groups have analyzed the relationship between IL-4 producing CD4⁺ T cells and Tfh cells during parasitic infection (*Leishmania major*, *Nippostrongylus brasiliensis*, *Heligmosomoids polygyrus* and *Schistosoma mansoni*) using IL-4 dual reporter mice (4get/KN2), in which cells that expressed IL-4 are marked by GFP (4get) and IL-4-producing subsets additionally displayed surface huCD2 (KN2) [13, 15, 66]. Analysis of Th differentiation during infection of 4get/KN2 mice confirmed previous reports showing that Tfh cells can express IL-4 [15] and show that most IL-4 producing subset of cells (huCD2+CD4+) express high levels of Tfh markers such as CXCR5, ICOS, PD-1, BTLA, Bcl6 and IL-21 is highly enriched in B cell follicles and germinal centers of the reactive lymph nodes [15]. Within non-lymphoid effector site, huCD2+ IL-4 producing cells do not express the Tfh markers [13], suggesting IL-4 producing cells in the lymph nodes are phenotypically different from peripheral Th2 cells and have canonical characteristics of Tfh cells. Moreover, adoptive transfer of lung and lymph node huCD2+CD4+ T cells from *N. brasiliensis* infected 4get/KN2 mice into mice deficient in both IL-4 and IL-13 following *N. brasiliensis* infection shows three-fold higher recruitment of eosinophils to the lung of mice that received lung huCD2⁺ cells compared to mice that received huCD2⁺ cells from lymph nodes [67]. Collectively, these studies have shown that IL-4 secreting Tfh cells and Th2 cells are distinct Th subsets with respect to their phenotypical characteristics, localizations and effector functions.

Interestingly, while analysis of μ MT/4get mice (B-cell deficient mice) immunized with serum schistosome egg antigen (SEA) revealed the absence of Tfh cells compared to B-cell sufficient 4get mice, the overall percentage of IL-4-competent (IL-4/GFP) CD4⁺ T cells were similar in both mice, suggesting that Tfh population could be derived from the Th2 lineage in the presence of B cells [13]. Moreover, the finding that transcript level of GATA3, master regulator of Th2 differentiation, was comparable between Tfh cells found in B cell follicles and IL-4-competent cells (PD1-IL-4/GFP+CD4+) from outside the B cells follicles from SEA immunized mice [13], further indicated that Th2 cells localized within lymph nodes have potential to become either Tfh cells within GCs of lymph nodes or migrate to peripheral tissues to perform Th2 effector functions. The relationship between Tfh and Th2 cells was confirmed when Tfh cells developed from CXCR5⁺PD1⁺IL4/GFP⁺CD4⁺ T cells in a germinal center-dependent manner upon transfer into recipient naïve mice and antigen challenge *in vivo* [13]. In the view of T cells plasticity, it is not clear whether Th2 to Tfh conversion is a final step in the lineage pathway. It is possible that different signals might regulate the reversible transition between the two Th lineages.

Although generation and accumulation of Tfh cells can accrue in the absence of IL-4, still IL-4 is critical for mature B cell responses [15, 67]. B-T cell conjugates within GCs of lymph nodes from *L. major* infected mice showed IgG1 switch and germinal transcript by B cells made contact with IL-4-expressing T cells, whereas IgG2a-producing B cells made contact with IFN γ -producing T cells [66], indicating that specific cytokines produced by Tfh cells regulates isotype class switching in conjugated B cells.

Coproduction of IL-4 and IL-21 is coupled in Tfh cells and required for optimal antibody responses [66, 68]. Interestingly, while Tfh cells could persist for up to 60 days after primary antigen activation, memory CD4⁺ T cells, rather than persistent Tfh cells, are required for Tfh population expansion upon secondary immunization [66]. B cells are necessary for expansion of Tfh cells following secondary immunization; moreover, memory B cells require IL-4 for efficient differentiation to plasmoblasts, indicating a pivotal role for IL-4 in the interplay between Tfh and B cells during secondary immune responses.

4. Critical elements for IL-4 regulation in Tfh cells

Over the recent past, a lot of interest has been generated in determining the factors leading to IL-4 production by Tfh cell differentiation, since in contrast to Th2 cells regulation of IL-4 expression in Tfh cells exclusively depends on the CNS2 regions in the IL-4 locus (Fig 2). Here, we elaborate on the current findings on cooperative effects of costimulatory molecules, cytokine signaling and epigenetic mechanisms that regulate IL-4 production by Tfh cells.

4.1 Costimulation

Signaling lymphocytic activation molecule (SLAM)-associated protein, SAP is expressed in CD4⁺ T cells and required for GC responses [69]. While absence of SAP had moderate defect in non-CG Tfh cell differentiation, SAP deficient mice have an absence of GC Tfh cell subset [70]. GC center Tfh cells co-expressing CXCR5, PD-1, Bcl6 and GL-7 are a functionally distinct subset of further polarized Tfh cells, with enhanced B cell help capacity and a specialized ability to produce IL-4 in Th2 independent-manner [64, 66]. SAP deficient Tfh cells are defective in IL-4 and IL-21 production. Moreover, SLAM (Slamf1, CD150), a surface receptor that utilizes SAP signaling, is specifically required for IL-4 production by GC Tfh cells [70]. These data reveal a prominent role for SLAM receptor ligation in IL-4 production by GC Tfh cells and GC Tfh differentiation. Further work is required to determine whether downstream components of SAP (Fyn and PKC θ) are required for IL-4 induction in GC Tfh cells.

ICOS is a critical regulator of the generation and function of Tfh cells [71]. ICOSL blockade in *L. major* infected 4get/KN2 reporter mice led to the reduced GC formation, to fewer hCD2⁺ IL-4-producing Tfh cells within B cell follicles and GC B cells without affecting IL-4 expressing Th2 cells [67], indicating that ICOSL is not required for Th2 cell development but is essential for IL-4 producing Tfh cells within GCs of reactive lymph nodes.

4.2 Epigenetic control

IL-4 expression is known to be governed by a set of coordinated changes occurring at the IL-4 locus including chromatin remodeling, epigenetic changes and accessibility to various transcription factors [27, 47, 54, 56]. The cell type specificity of gene expression is primarily conferred by distal *cis*-regulatory elements and the role of various regulatory elements and epigenetic changes in IL-4 regulation in Th2 cells and macrophages has been studied previously and discussed in the earlier section of this review.

The DNase I hypersensitivity site (HS)V overlaps with a highly conserved sequence 2 (CNS2) and has been recently identified as a putative distal enhancer located 3' of the IL-4 gene [27]. Analyses of either HSV or CNS2 deficient mice demonstrated non-redundant function of conserved cis-element CNS2 in Tfh-specific regulation of IL-4 gene [17, 18]. Deletion of CNS2 resulted in profound reduction in IgG1 and IgE post-immunization, and this defect was mainly due to loss of IL-4 expression by Tfh cells. CNS-GFP mouse model that allowed the visualization of Th cells expressing IL-4 gene demonstrated that GFP+ T cells exhibiting Tfh markers were preferentially localized in B cell follicles and GC areas [17]. Although GFP+ Tfh cells were found to develop normally from naïve T cells post-immunization, CNS2 is an active and specific enhancer for IL-4-producing Tfh cells and crucial for IgG1 and IgE class switching. In contrast to Tfh cells, Th2 cells, basophils and eosinophils are less dependent on HSV and CNS2 for IL-4 production.

Analysis of histone proteins in Tfh cells showed abundance of acetylated H3K9/14 at the HS2, HS5a, and CNS2 sites [17] and H3K4me2 methylation at the CNS2 locus [18]. CNS2 deficiency however did not affect these modifications in other loci except the CNS2 region, suggesting that this region is indeed a critical element that regulates *Il4* expression in Tfh cells [17]. In naïve T cells, along with enriched H3K4me2 methylation, the CNS2 region is in a highly demethylated state indicating towards its importance in early IL-4 expression during *in vivo* immune response. CNS2 deficiency also leads to retained H3K27me3 (silencing marker) recruitment particularly in Tfh cells further suggesting that IL-4 production in Tfh cells is solely CNS2 dependent [18].

CNS2 is known to exert its enhancer effects by the SLAM-SAP pathway and Notch and RBP-J signaling for IL-4 production [19, 70]. Epigenetic marks on CNS2 site were slightly affected in the absence of SLAM and were not altered in the absence of RBP-J, indicating marginal participation of SLAM but not Notch pathway for CNS2 activity in Tfh cells [72]. Our recent study identifies a crucial role of the transcription factor Batf in controlling IL-4 expression in Tfh cells through the CNS2 locus [16]. The recruitment of active histone proteins, AcH3 and H3k4 in the CNS2 region in Tfh cells was Batf-dependent indicating that Batf regulates IL-4 expression in Tfh cells through direct binding and controlling access of the chromatin machinery in the CNS2 region. These data sets collectively indicate that Th2 and Tfh cells utilize unique mechanisms for regulation of IL-4 expression, and further investigation of the molecular pathways for IL-4 expression in Tfh cells is warranted.

4.3 Transcriptional requirement

IL-4 appears to be differentially regulated in Th2 and Tfh cells [18, 67]; however, transcriptional control of IL-4 production in Tfh cells remains mainly unknown. Our recent study identifies a novel role for the transcription factor Batf in regulating IL-4 expression in Tfh cells [16]. Our *in vivo* results showed a T-cell-intrinsic defect in IL-4 production along with other effector Th2-related cytokines in Batf KO mice. However, contrary to our expectations, analysis of *in vitro*-differentiated Th2 cells from Batf KO mice did not reveal any defect in the expression of the quintessential Th2 cytokine IL-4, suggesting that Batf could contribute to IL-4 expression in helper subset distinct from Th2. Interestingly, our *in*

vivo studies revealed a novel non-redundant role of Batf in the regulation of IL-4 expression in Tfh cells compared with non Tfh cells.

We also detected that Batf directly binds to and activates CNS2 region in the IL-4 locus [16]. Since Batf lacks the transactivation domain, the transactivation observed at the CNS2 locus could be due to its interaction with endogenous factors. Batf physically interacts with IRF4 leading to transactivation of the CNS2 region in the IL-4 locus. In addition, Batf-IRF4 function towards IL-4 induction in Tfh cells is dependent on both Stat3 and Stat6. Whether Stat3 and Stat6 act alone or in a complex with Batf-IRF4 and therefore contribute to IL-4 expression in Tfh cells awaits further extensive investigation.

Apart from Batf and IRF4, we identified the positive effects of the transcription factor c-Maf in the regulation of IL-4 in Tfh cells [16]. c-Maf is known to be a target of Batf in Tfh cells [73], which is required for regulation of IL-21 expression and consequently for Tfh expansion [71]. c-Maf is also known to control IL-4 expression in cooperation with IRF4 and NFAT in Th2 cells [42]. In our study, we also observed increased binding of c-Maf to the CNS2 locus in Tfh cells as well as c-Maf-induced transactivation of the CNS2 region indicating that c-Maf aids in IL-4 transcription in Tfh cells [16]. Since Batf is essential for c-Maf expression in Tfh cells, Batf-to-c-Maf signaling could additionally contribute to the decreased IL-4 expression in Tfh cells.

We detected high Batf expression only in Tfh and Th2 cells among all T helper subsets suggesting that both subsets could share the similar mechanism for Batf expression [16]. The IL-4/Stat6 pathway is important for Batf expression and hence for IL-4 production in Tfh cells, similar to Th2 cells. Batf expression in Tfh cells is known to be also induced by IL-6 signaling via the transcription factor Stat3. Thus, Batf induction in Tfh cells by IL-6 or IL-4 through Stat3 or Stat6, respectively, helps in the generation of IL-4-expressing Tfh cells. However, the exact kinetics of IL-4/Stat6 and IL-6/Stat3 contribution in Batf-mediated IL-4 expression in Tfh cells needs further extensive investigation.

5. IL-4 function in disease settings

Type 2 immune responses are mainly driven by IL-4, IL-5, IL-9 and IL-13 and are crucial in inducing host protective immunity predominantly during helminth infection, type1 inflammation, wound repair and tissue regeneration following infection or injury [2, 3]. On the other side, type 2 responses can also drive inflammatory responses characterized by eosinophils, mast cells, basophils, IL-4- and/or IL-13-conditioned macrophages and Th2 cells, involved in the pathogenesis of many allergic and fibrotic disorders [2]. During type 2 immune response, IL-4 is mainly secreted by Th2 cells, however various other cell types such as NKT cells, eosinophils, basophils and mast cells also produce IL-4 [74]. In the recent years, Tfh cells have been acknowledged as a major alternative source of IL-4 that is essential during type 2 humoral immunity [12–15]. In the below section, we discuss the current understanding on the biological roles of IL-4 produced by Th2 and Tfh cells (Table 1).

5.1 Pathogenic role of IL-4

Hyperreactive and dysregulated type 2 immune responses lead to overproduction of type 2 cytokines including IL-4 thus causing various pathogenic disorders such as allergic asthma, allergic rhinitis, atopic dermatitis, eosinophilic oesophagitis and anaphylaxis, as well as allergies to drugs, toxins and food [2, 75]. It is also instrumental in suppressing the development of protective type 1 immunity to various pathogens thus facilitating uncontrolled or persistent infection. Role of IL-4 is also known to promote tumorigenesis through alteration of the tumor suppressing activity of monocytes or macrophages [2, 76, 77].

Over the last 15 years, Th2 cells have emerged as the key players in asthma pathogenesis. Asthma is a chronic inflammatory disease of the airways and is characterized by chronic airway inflammation, increased mucus production, reversible airway obstruction, remodeling of the airways, and airways hyperresponsiveness (AHR) [78]. By releasing a number of typical cytokines (IL-4, IL-5, IL-9 and IL-13), these cells orchestrate a number of inflammatory events that subsequently trigger cascades of other processes ultimately leading to the formation of the disease. These Th2 cytokines are responsible for the recruitment of eosinophils (IL-5), basophils (IL-4) and mast cells (IL-4, IL-9 and IL-13) to the airways and for the allergen-specific development of IgE (IL-4 and IL-13) [79]. The role of IL-4 in the development of allergic airway inflammation (asthma) and its contribution to the production of various inflammatory mediators is well appreciated [80]. High IL-4 levels have been detected in the serum and bronchoalveolar lavage of allergic individuals and peripheral blood mononuclear cells from atopic asthmatics [79, 81–83]. This complex phenotype is believed to arise from manifold interactions of infiltrating immune cells with structural cells of the airways. Studies using both IL-4-deficient mice as well as utilizing neutralizing anti-IL-4 antibodies in mice showed inhibition of the biological activities of IL-4 as well as IL-5 and IL-13 in CD4⁺ T cells leading to reduction of allergen-specific IgE and eosinophilic infiltration and airway inflammation [84].

Implication of Tfh-derived in allergic airway inflammation is being actively investigated upon. Recent report from our group has also implicated the function of Tfh-derived IL-4 in the pathogenicity of allergic inflammation [16]. Adoptive transfer experiments of WT (CD45.1⁺, CD4⁺, CD44⁺ CXCR5^{hi} and PD1^{hi}) Tfh cells from lung and lung lymph nodes of asthmatic mice into congenic mice showed asthmatic symptoms in the recipient mice such as eosinophil and lymphocyte infiltration and production of Th2 cytokines like IL-4, IL-5 and IL-13 in the lungs. These transferred Tfh cells maintained a robust Tfh phenotype including CXCR5 and IL-4 expression thus confirming their involvement in development of the allergic inflammatory responses. These symptoms were found to be dependent on Batf driven IL-4 expression in Tfh cells, since transfer of Batf deficient Tfh cells showed a dramatic decrease in the asthmatic symptoms [16]. Further studies will help in exactly delineating the kinetics of IL-4 expression during allergic disease progression.

5.2 Protective role of IL-4

In contrast with allergic diseases, IL-4 can act as an anti-inflammatory cytokine in various autoimmune diseases [85]. Th2 derived IL-4 along with its counterpart IL-13 play a critical

role in promoting host survival during infection with parasitic helminthes that have tissue-dwelling phases and they can mediate expulsion of intestinal helminthes [86, 87]. Importantly, Th2 responses are associated with development of strong antibody responses, particularly IgG1 and IgE, which in certain helminth infections are implicated in resistance to reinfection [13]. Interestingly, recent mouse studies employing helminth parasite infections showed that IL-4-producing CD4⁺ T cells are specifically restricted to the B cell follicles and associated with germinal centers [13–15, 66]. Consistent with localization, IL-4 producers express high levels of Tfh markers [13, 15]. Although IL-4 is dispensable for Tfh development, Tfh-derived IL-4 is critical to orchestrate multiple features of a mature B cell responses after helminth infection, particularly regulating isotype class switching in conjugated B cells toward IgG1 and IgE [15, 67]. Further studies designed to understand the origin, development and function of Tfh cells will help address their role in the prevention of the development of severe disease after infection with parasites.

In diseases, such as Rheumatoid arthritis where Th1 cells are the major players, IL-4 derived majorly from Th2 cells, mast cells or basophils can inhibit Th1 cell formation and production of proinflammatory cytokines like TNF- α , IL-1 α , IL-1 β , IL-6, IL-8, G-CSF, and macrophage inflammatory protein (MIP)-1 α , enhances monocyte apoptosis and acts as an autocrine growth factor promoting the differentiation of naive T cells to Th2 cells [85, 88, 89]. Although IL-4 levels are seldom detected in the synovial fluid, the progression of this disease is more aggressive in animals lacking IL-4 and IL-13 than in the wild type counterparts [90, 91]. IL-4 gene therapy can reduce inflammation and ameliorate joint destruction in animal models of rheumatoid arthritis [92, 93]. Mice treated with recombinant IL-4 or constitutively expressing IL-4 are also known to be protected from experimental autoimmune diabetes. Islet Ag-specific T cells displayed a Th2 phenotype and the IL-4 expression in the β -islets of pancreatic cells from transgenic non-obese diabetic (NOD) mice decreased diabetes development. Neutralization of IL-4 along with another Th2 cytokine IL-10 in this system abrogated the ability of NOD-IL-4 splenocytes to delay the onset of diabetes [94]. Other autoimmune diseases driven by Th1 cells like psoriasis can also be improved by IL-4 treatment [95]. The role of IL-4 in regulating inflammation within the CNS has also been demonstrated in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). IL-4 deficient mice exhibited more severe EAE [96, 97]. IL-4 retroviral-transduced T-cells delivered into the CNS also results in the amelioration of EAE [98]. Furthermore, IL-4 produced by the microglial cells in the central nervous system helps in promoting the alternatively activated macrophages that are important in controlling inflammation caused by EAE [99]. IL-4 is also known to play a protective role in Crohn's disease [100]. Decreased production of IL-4 in inflammatory bowel disease also contributes to disease pathogenesis. Less IL-4 production modulates lamina propria mononuclear cells (LPMC) reactivity and has defective immunosuppressive and anti-inflammatory mechanisms [100].

6. Conclusion and perspective

Type 2 immune responses have an important role in mediating protective immunity to the host against parasitic infections but also cause allergic diseases such as asthma. Th2 cells and innate immune cells that produce the signature Th2 cytokines interleukin are major

players in orchestrating the Th2 responses. Emerging evidence suggest that the Tfh cells, a CD4⁺T cell subset found in the B cell follicles and germinal centers of lymph nodes, is an alternative source of IL-4 *in vivo* and the IL-4 produced by these cells is essential for shaping Th2-cell based humoral immunity implicating that Th2 and Tfh cells exhibit overlapping phenotypical and functional characteristics as well as raise the possibility of flexibility between Tfh cells and other T helper effector populations.

In fact, elegant studies by Zaretsky *et al* show that in response to helminth parasite infections, IL-4 and Gata3 expressing Th2 cells can acquire Tfh cell characteristics including IL-21, CXCR5, and ICOS expression within B cell follicles, suggesting Th2/Tfh plasticity. It still remains unclear whether Tfh cells can generate from other effector T cell types and whether these follicular T cells are able to retain their phenotype beyond the lymph node follicles. Whether signals from the microenvironment dictate the reversible transition Tfh cells toward Th2 or other T helper subsets needs further extensive investigation. Studies designed to increase the understanding of the origin, development and function of Tfh cells should provide insight into the optimal requirements for antibody production in order to meet demands of an appropriate host response during parasitic infection.

Our recent report also shows that IL-4 producing Tfh cells contribute to promotion of allergic airway inflammation as well as retain a stable phenotype during the course of disease. However, while IL-4-producing Th2 cells from asthmatic mice upon adoptive transfer could trigger asthmatic symptoms in the recipient mice, the majority of these cells in lung and lung lymph nodes acquired Tfh phenotype (CXCR5, BTLA and Bcl6), suggesting plasticity of Th2 cells towards Tfh cells upon allergic inflammation and its contribution to asthma pathogenesis. Further studies are necessary to determine the exact nature of relationship between Th2 and Tfh cells during allergic responses and delineate the exact role of IL-4 derived from Tfh cells in type 2 immunity in physiological and disease settings. Furthermore, a complete mechanistic understanding of the molecular details of IL-4 gene regulation and production in Th2 and Tfh cells may facilitate the development of novel approaches to target IL-4 in both Th2 and Tfh cells during allergic inflammation.

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Abbreviations

Th2	T helper 2
Tfh	T follicular helper
IL	Interleukin
Ig	Immunoglobulin
STAT	signal transduction and activator of transcription

TCR	T cell receptor
NFAT	nuclear factor of activated T cells
IRF4	Interferon regulatory factor 4
ERK	extracellular signal-regulated kinase
ICOS	Inducible co-stimulator
CNS	conserved non-coding sequence
SLAM	signaling lymphocytic activation molecule
SAP	slam associated protein
GITR	glucocorticoid-induced tumor necrosis factor receptor
TSLP	thymic stromal lymphoprotein
AP	activating protein
TCF	T cell factor
Gfi	Growth factor independent
HS	hypersensitive sites
LCR	locus control region
GC	germinal center
Bcl6	B cell lymphoma 6
SEA	schistosome egg antigen
Batf	basic leucine zipper transcription factor ATF
VCAM	vascular cell adhesion molecule
MIP	macrophage inflammatory protein
NOD	non-obese diabetic

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Biographies

Anupama Sahoo Anupama received her Ph.D. in Life Sciences (Immunology and Cell biology) from Gwangju Institute of Science and Technology, Gwangju, South Korea in August, 2011. She continued her research in Immunology as a postdoctoral fellow at the

Department of Immunology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA (November, 2011 to January, 2015), prior to moving to Sanford Burnham Medical Research Institute, Orlando, FL, USA. Her research interests include exploring the differential expression of novel factors in T lymphocytes and their regulatory functions in infectious and autoimmune diseases and cancer.

Shradha Wali Shradha graduated with a Master's degree from University of Texas at San Antonio, USA. She is a Ph.D. student in the Department of Immunology at the University Of Texas Graduate School Of Biomedical Sciences, Houston, Texas, USA. Her current research is understanding transcriptional regulation in T follicular helper cell differentiation and activation.

Roza Nurieva Dr. Roza Nurieva received her Ph.D. in 2000 from the Gabrichevsky Research Institute of Epidemiology and Microbiology, Moscow, Russia. Her postdoctoral training was with Dr. Chen Dong at the University of Washington, Seattle, focusing on understanding the role of costimulatory molecules in regulating T helper cell activation, differentiation, and function. She is currently an Assistant Professor in the Immunology Departments at MD Anderson Cancer Center. Dr. Nurieva's main research goal is to understand the molecular and cellular mechanisms that control the development and progress of autoimmunity, inflammation and cancer, with particular emphasis on the signalling and transcriptional mechanisms underlying T helper cell development and T cell mediated immune responses.

Highlights

- IL-4 is produced by two distinct T helper subsets including T helper 2 and T follicular helper cells.
- Production of IL-4 is regulated by different transcriptional and epigenetic mechanisms in these T helper subsets.
- IL-4 plays beneficial role during parasitic infection or possess a pathogenic role in allergic diseases.

Th2 cell

Figure 1. Factors regulating IL-4 regulation in Th2 cells

Weak TCR signalling induces expression of GATA3 in a IL-4/STAT6-independent manner and IL-2 production that in turn activates STAT5. GATA3 cooperates with STAT5 and regulates early TCR-dependent IL-4 production. Endogenously produced IL-4 activates GATA3 expression in a STAT6-dependent manner. In addition, NFAT, IRF-4, c-Maf, Jun-B and Dec-2 contribute to IL-4 production in the effector phase.

Figure 2. Regulation of IL-4 expression in Tfh cells

IL-4/STAT6 and IL6/STAT3 signalling trigger Batf expression in Tfh cells, that in turn complex with IRF4 to trigger IL-4 production in Tfh cells by directly binding to and activation of the CNS2 region in the IL-4 gene locus. Additionally, Batf-to-c-Maf signaling is an important determinant of IL-4 expression in Tfh cells. Moreover, ICOS and SLAM signaling contributes to generation of IL-4-producing Tfh cells.

Table 1

Protective and pathogenic roles of Th2/Tfh cell derived Interleukin-4

	IL-4 in Th2 cells	IL-4 in Tfh cells
Protective Functions	Helminth infection: IgG1 and IgE responses Rheumatoid Arthritis: Inhibits proinflammatory cytokines, promotes differentiation of naive T cells to Th2 cells Autoimmune diabetes: delays onset of diabetes Psoriasis: IL-4 treatment can improve psoriatic symptoms EAE: IL-4 deficient mice display severe autoimmunity.	Helminth infection: Required for mature IgG1 and IgE responses
Pathogenic Functions	Asthma: Recruitment of eosinophils, basophils, mast cells, allergen specific IgE responses Atopic Dermatitis: High IL-4 in serum/PBMCs	Asthma: Increase cellular infiltration and Th2 cytokine expression in lung and lung lymphnodes