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Transcriptional regulation of Th2 cell differentiation

Jinfang Zhu
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Abstract

CD4 T helper 2 (Th2) cells have critical functions in immune responses against extracellular parasites and are involved in asthma and other allergic diseases. The differentiation of naïve CD4 T cells into Th2 cells is initiated from T-cell receptor and cytokine-mediated signaling followed by upregulation of GATA3 and activation of signal transducer and activator of transcription 5 (STAT5), two indispensable events for this differentiation process. In this review, regulation of GATA3 expression and STAT5 activation and functions of these two transcription factors in inducing the expression of Th2 cytokines, cytokine receptors as well as epigenetic modification at Th2 cytokine locus are summarized. Furthermore, I present positive and negative regulatory networks important for Th2 cell commitment, selective growth of committed Th2 cells and suppression of alternative lineage fates. Finally, the difference between *in vitro* and *in vivo* Th2 differentiation is discussed.

Keywords

T-cell differentiation; transcription factors; cytokines

CD4 T helper (Th) cells have critical functions in regulating adaptive immune responses. Mossman and Coffman[1] first recognized the existence of Th1 and Th2 cells. Although Th1 cells are critical for cell-mediated immunity, Th2 cells are involved in humoral immune responses.[2,3] Now, it is known that naïve CD4 T cells have at least four distinct fates, Th1, Th2, Th17 and induced regulatory T (iTreg) cells, to choose from when they receive signals triggered by antigens and cytokines.[4]

CD4 T helper 2 (Th2) cells are important for immune responses against extracellular parasites and involved in the development of asthma and other allergic diseases. By secreting a variety of signature cytokines, Th2 cells help B cells to make IgE[5] (through interleukin (IL)-4), induce alternative macrophage activation[6] (through IL-4/IL-13), recruit eosinophils[7] (through IL-5), activate mast cells (through IL-9[8]) and act on epithelial cells (through IL-9,[9] IL-13[10–12] and amphiregulin[13]). Th2 cells also produce IL-10, IL-21 and IL-25, which are also involved in regulating the magnitude of Th2 responses.

Cytokine milieu is an important determinant for Th cell differentiation. Together with T-cell receptor (TCR) ligation, IL-4-mediated signaling promotes Th2 differentiation.[14,15] IL-4 activates the signal transducer and activator of transcription 6 (STAT6) leading to the induction of the transcription factor GATA3.[16,17] IL-2-mediated STAT5 activation is also critical for Th2 cell differentiation.[14,18,19] In this review, I discuss how GATA3 expression

Correspondence: Dr J Zhu, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, Bldg 10, Rm 11N323, Bethesda, MD 20892, USA. jfzhu@niaid.nih.gov.

and STAT5 activation are regulated and how these two molecules, in collaboration with other transcription factors, induce fully differentiated Th2 cells.

GATA3 and STAT5 are Indispensable for Th2 Differentiation

GATA3 has important functions at multiple stages of CD4 T cell development.[20] It is also recognized as the master regulator of Th2 cells.[16,17] The expression of GATA3 is upregulated during Th2 differentiation.[17,21,22] Enforced expression of GATA3 in Th1 cells induces IL-4 and endogenous GATA3 expression.[21,23] On the other hand, a dominant-negative form of GATA3 suppresses Th2 cytokine expression and blocks induction of airway hyperreactivity.[24] *Gata3* conditional knockout studies show that Th2 differentiation, both *in vitro* and *in vivo*, completely depends on GATA3 expression.[25,26] In differentiated Th2 cells, continuous GATA3 expression is essential for the production of IL-5 and IL-13, but not IL-4.[25]

STAT5 family members include STAT5a and STAT5b, encoded by two related genes head to head in the genome.[27] They are critical for cytokine-mediated T-cell proliferation and survival.[28] IL-2 is the most potent inducer of STAT5 activation and IL-2-mediated STAT5 signaling is required for Th2 cell differentiation *in vitro.*[18,19] Although STAT5b is still expressed in STAT5a single knockout cells, such cells have profound defects in Th2 cell differentiation both *in vitro* and *in vivo* indicating there is a dose effect of STAT5 activation during Th2 differentiation.[18,19,29,30]

Enforced expression of either GATA3 or a constitutively active STAT5a in Th1 cells results in IL-4 production and co-expression of these two molecules maximizes the Th2-inducing effect.[19] On the other hand, the constitutively active STAT5a fails to induce IL-4 in GATA3-deficient cells[25] and anti-IL-2 blocks the ability of GATA3 to promote IL-4 expression.[18] Therefore, both GATA3 expression and STAT5 activation are necessary for Th2 cell differentiation *in vitro*[31] and possibly *in vivo*.

Regulation of GATA3 Expression and STAT5 Activation

STAT6 is the major signal transducer in IL-4-mediated Th2 cell differentiation.[32–34] IL-4 can be either provided exogenously or produced by naïve CD4 T cells in response to low-dose peptide stimulation.[35] CD4 T cells deficient in STAT6 fail to develop into IL-4-producing cells *in vitro* and STAT6 activation is necessary and sufficient for inducing high expression levels of GATA3.[36,37] STAT6 may also be involved in chromatin remodeling at the *Il4/Il13* locus control region (LCR).[38] However, some *in vivo* Th2 responses can be obtained in the absence of STAT6[39–41] but such Th2 differentiation still requires GATA3 expression,[25,26] suggesting that either GATA3 can be induced by IL-4/STAT6-independent pathway or *in vivo* Th2 differentiation in some cases only requires basal levels of GATA3 expression found in activated CD4 T cells.

Low-dose peptide stimulation of naïve CD4 T cells induces IL-4/ STAT6-independent early GATA3 expression to a certain level.[35] Such GATA3 induction is not observed when cells are stimulated with high-dose peptide, possibly because a strong Erk activation blocks the induction. The detail mechanism through which TCR-mediated signaling induces GATA3 is unknown. NF-κB1 has been shown to have an important function in regulating GATA3 expression.[42] Bcl-3, as the partner of NF-κB1, directly binds to the promoter of the *Gata3*.[43] The role of NF-κB1 in low-dose peptide-induced GATA3 expression remains to be determined.

Notch signaling is important for Th2 differentiation.[44,45] It has been shown that Jag1/Notch interaction induces Th2 differentiation, whereas Dll1/Notch interaction results in Th1

polarization.[44] A Notch/CSL-binding site was identified in the alternative distal promoter of *Gata3*, which is located ~10kb upstream of the regular *Gata3* promoter,[46] suggesting Notch signaling directly regulates GATA3 expression. A recent report shows that TCF-1/β-catenin may have an important function in regulating IL-4-independent early GATA3 expression in some settings but the dominant transcription starting site of *Gata3* is downstream of the proximal promoter.[47] Most recently, transcription factor Dec2 has been shown to have an important function in Th2 differentiation through forming a positive regulatory feedback loop with GATA3.[48] GATA3 induces Dec2 expression and in turn Dec2 upregulates GATA3. Dec2 directly binds to the *Gata3* promoter. In Dec2-deficient cells, GATA3 induction is impaired; when GATA3 is deleted from Th2 cells, Dec2 expression gradually decreases. The initial signaling responsible for early Dec2 upregulation, just as for early GATA3 induction, has not been determined.

GATA3 induces its own expression.[23] In fact, our unpublished ChIPseq data showed that GATA3 strongly binds to multiple sites at *Gata3* locus extending up to 1 Mb 3′ of *Gata3*, suggesting the regulatory elements for GATA3 expression can be far apart.

TCR-mediated signaling transiently inhibits cytokine signaling including IL-2-mediated STAT5 and IL-4-mediated STAT6 activation.[49] Indeed, STAT5 activation by IL-2 is evident after 24h of T-cell activation when low strength of TCR signaling is provided, but IL-2 signaling remains suppressed at this time point if T cells receive strong TCR stimulation.[35] Therefore, low strength of T-cell activation is critical for the initiation of Th2 cell differentiation through both GATA3 upregualtion and STAT5 activation.

Many cytokines including IL-2, IL-7 and TSLP can induce STAT5 activation. IL-2 is mainly produced by CD4 T cells after activation, whereas IL-7 is constitutively expressed by stromal cells. TSLP can be produced by epithelial cells, mast cells and basophils during the initiation of *in vivo* Th2 responses.[50–52] These STAT5 activators can be potential initiators for Th2 responses *in vivo* as GATA3 is induced by T-cell activation and only limited amounts of GATA3 may be required for IL-4 production.[19] Interestingly, both Notch pathway and NF-κB pathway, which are important for inducing GATA3, have also been reported to regulate the expression of IL-2 and CD25,[53,54] and thus IL-2-mediated STAT5 activation.

Other Transcription Factors Involved in Th2 Differentiation

Besides GATA3 and STAT5, many other transcription factors are also involved in regulating IL-4 production and Th2 differentiation. Growth factor independent 1 (Gfi-1) is a STAT6-dependent immediate early gene induced by IL-4.[55] TCR activation also induces Gfi-1, but IL-4 substantially prolongs its expression. Gfi-1 is important for cytokine-mediated growth of Th2 cells but has a minimal effect on the growth of other Th cells. Thus, Gfi-1 selects $GATA3^{hi}$ cells to grow. It seems that Gfi-1 regulates molecules both upstream and downstream of STAT5 activation.[55,56]

Many transcription factors directly act on *Il4* promoter. IL-4 production by Th2 cells requires TCR-mediated Ca^{2+} signaling. Indeed, NFAT1 has been shown to bind to the *Il4* promoter.[57] C-Maf is selectively upregulated in Th2 cells and in its absence, the production of IL-4 but not other Th2 cytokines is diminished.[58] JunB, expressed at high levels in Th2 cells, collaborates with c-Maf to induce IL-4 production by directly acting on the *Il4* promoter.[59] The expression of JunB may depend on a Th2-specific transcription factor Dec2.[48] IRF-4 is also required for Th2 cell differentiation.[57,60] It has been shown that IRF-4 functions both upstream and downstream of GATA3 and the latter is through its direct binding to the *Il4* promoter.

Notch signaling regulates GATA3 expression as discussed earlier, but Notch/CSL-binding site has also been found in the DNase I hyper-sensitivity site (HS) V of the *Il4* gene[44] suggesting it may regulate IL-4 production directly. Ikaros is important for Th2 cell differentiation as Ikaros-deficient cells fail to produce IL-4.[61]

Cross-Regulation between Transcription Factors of Different Lineages

T-bet is the Th1 master regulator.[62] T-bet expression is induced by IFNγ suggesting IFNγ/T-bet forms a powerful amplification loop for Th1 differentiation.[63] IL-12-mediated STAT4 activation is also important for Th1 responses.[64,65] Together with IL-18, IL-12 is able to induce IFNγ production in a TCR-independent manner.[66,67] Runx3 expression is upregulated in Th1 cells and it binds to the *Ifng* promoter.[68,69] Eomesodermin (Eomes),[70] another T-box family member, is responsible for IFNγ production in T-bet-deficient CD8 T cells. Therefore, transcription factors T-bet, STAT4, Runx3 and Eomes are involved in IFNγ production.

Gata3 deletion in Th2 cells results in elevated IFNγ production[25] and such IFNγ induction is due to the activation of Runx3-Eomes pathway independent of IL-12/STAT4 and IFNγ/T-bet (our unpublished data). In addition, GATA3 deficiency in Th2 cells reverses the suppression of STAT4 expression by IL-4, consistent with an earlier report that GATA3 downregulates STAT4 when it is over-expressed in Th1 cells.[71]

On the other hand, Th1 transcription factors suppress Th2 differentiation. T-bet is able to suppress GATA3 expression.[22] In addition, T-bet inhibits GATA3-mediated IL-5 production by directly interacting with GATA3 protein.[72] Runx3 suppresses IL-4 production in Th1 cells through its binding to HSIV site of the *Il4* locus.[68]

Strong STAT5 activation is critical for Th2 differentiation as discussed earlier. STAT5a-deficient cells have been shown to be hyperresponsive to IL-12, which results in Th1 differentiation.[73] Consistent with this, T-bet expression is suppressed when a constitu-tively active form of STAT5a is introduced into Th1 cells.[19] STAT5 also inhibits the expression of Th17-related molecules, including RORγt and IL-17.[74]

Gfi-1, a positive regulator of Th2 cell expansion, suppresses IFNγ production in Th1 cells[56] and IL-17 production in Th17 cells.[75] Blimp-1 is induced in Th2 cells and it inhibits the expression of IFNγ and IL-2.[76–78] Ikaros-deficient 'Th2' cells express increased levels of both T-bet and IFNγ suggesting a main function of Ikaros during Th2 differentiation is to suppress Th1-related genes.[61]

Amplification of Th2 Responses Through Multiple Positive Feedback Loops

As discussed above, IL-4/IL-4R/STAT6/GATA3 and IL-2/STAT5 pathways are two important pathways for Th2 differentiation. *In vivo*, IL-4 can be produced by basophils,[40] NKT cells,[79] memory Th2 cells and some undefined accessory cells,[80] whereas, IL-2 is produced by naïve CD4 T cells shortly after T-cell activation. IL-7 and TSLP may substitute IL-2 as STAT5 activators *in vivo*. STAT5 has been shown to bind to the HSII and HSIII sites in the intron 2 of *Il4*[18,19] and GATA3 binds to HSVa.[81] Thus, both GATA3 and STAT5 directly act on the *Il4* gene to promote its expression. IL-4 produced by CD4 T cells further enhances IL-4Rα expression and upregulates GATA3. Therefore, the induction of IL-4 expression in CD4 T cells through IL-4/IL-4R/STAT6/GATA3 pathway provides a powerful positive feedback loop for Th2 differentiation.

IL-33Rα (also known as T1/ST2 or IL-1R-like 1), an IL-1R super-family member, is preferentially expressed in differentiated Th2 cells.[82,83] Blocking IL-33 signaling reduces

eosinophilic airway inflammation[84] and IL-33Ra-deficient mice show decreased responses to *Schistosoma mansoni* egg antigen.[85] IL-33 stimulation of IL-33Rα^{high} Th2 cells results in IL-13 but not IL-4 production in a TCR-independent manner.[86] Thus, IL-33 has an important function in amplifying Th2 responses. Interestingly, continuous IL-33Rα expression requires both GATA3 expression and STAT5 activation; GATA3 and STAT5 directly bind to the *Il1rl1* gene, which encodes IL-33Rα.[86]

Besides directly acting on the *Il4* and *Il1rl1* genes, GATA3 and STAT5 pathway cross-regulate each other at multiple levels. Th2 cells express higher levels of CD25 (IL-2Rα) than Th1 cells and such expression may partly depend on c-Maf,[82] which is a potential target of GATA3. GATA3 also directly binds to intron 1 of *Il2ra* in Th2 cells suggesting GATA3 may directly regulate CD25 expression (our unpublished data). Thus, GATA3 regulates IL-2/STAT5 signaling by modulating IL-2R expression.

On the other hand, STAT5 regulates IL-4Rα expression during T-cell activation.[87] In addition, STAT5 has an important function in maintaining GATA3 expression in differentiated Th2 cells.[86] Furthermore, GATA3 has also been reported to regulate its own expression[23] and STAT5 has a critical function in maintaining CD25 expression.[88] Therefore, the IL-2/STAT5 pathway and the IL-4/STAT6/GATA3 pathway crosstalk at different levels and the collaboration between STAT5 and GATA3 resulting in full Th2 differentiation (Figure 1).

Epigenetic Modification at the *IL4/IL13* Locus in Th2 Cells

Il4 and its congener *Il13* genes, flanked by *Rad50* and *Kif3a*, are closely linked on human chromosome 5q31 and the syntenic region on mouse chromosome 11. The LCR for *Il4-Il13* lies in a 25 kb region 3′ of *Rad50*, which is ~20 kb 5′ of *Il13*.[89] *Il5* is on the other side of *Rad50*, however, its expression may not be controlled by the LCR in *Rad50*. GATA3-binding sites are found in the promoters of the *Il5*[90] and *Il13*[91,92] suggesting GATA3 serves as a transcription activator for IL-5 and IL-13 transcription. Indeed, when GATA3 is removed from Th2 cells, IL-5 and IL-13 expression are completely abolished.[25]

Transcriptional regulation of signature cytokine expression depends not only on expression or activation of specific transcription factors, but also on chromatin epigenetic modification and accessibility of cytokine genes. During Th2 differentiation, chromatin remodeling and epigenetic modification occur at the *Il4/Il13* locus. Across the *Il4/Il13* locus, a series of Th2-specific DNase I HS sites have been identified. Among these sites, conserved non-coding sequence 1[93,94] located at *Il4-Il13* intergenic region, HSII in the intron 2 of *Il4*[95] and HSV and HSVa[96] (also known as conserved non-coding sequence 2) at 3′ of the *Il4* coding region are particularly important. HS IV is accessible in both Th1 and Th2 cells and this element seems to be the target site for silencing IL-4 in Th1 cells.[97] Runx3 binding to this site offers a potential mechanism for IL-4 repression.[68] One particular site within the LCR, RHS7, becomes hypersensitive to DNase I and is demethylated at the initiation of Th2 but not Th1 differentiation.[89,98] Deletion of RHS7 diminishes but does not abolish the production of IL-4 and IL-13 in Th2 cells.[99]

GATA3 is responsible for chromatin remodeling at *Il4/Il13* locus by inducing DNase I HSs.[23,100,101] STAT5 induces accessibility at HSII site of the *Il4* locus.[19] IL-2/STAT5 signaling is also partially responsible for demethylation of RHS7 in LCR during Th2 differentiation.[98] Our unpublished data suggest GATA3 strongly binds to the HSII and three different sites within LCR in addition to HSVa indicating that GATA3 and STAT5, through their direct binding to two critical regulatory elements HSII and LCR, collaborate in chromatin remodeling of Th2 cytokine locus.

GATA3 may also be involved in regulating DNA CpG methylation. Dnmt-1, a DNA methyltrasferase, has an important function in silencing *Il4* locus and Dnmt-1-deficient cells aberrantly express IL-4 without upregulaing GATA3.[102] Likewise, methyl CpG-binding domain protein-2-deficient Th1 cells produce IL-4 even when GATA3 expression level is low.[103] Indeed, GATA3 is able to block the ability of methyl CpG-binding domain protein-2 to bind to methyl CpG, suggesting GATA3 upregulation may reverse inhibition of IL-4 production caused by CpG methylation at *Il4* locus.

Histone modifications are also critical for gene regulation. Trimethylation at histone 3 lysine 4 (H3K4me3) is associated with active gene loci and H3K27me3 with silenced gene loci. At the *Il4/Il13* locus, H3K4me3 modification was found in Th2 cells but H3K27me3 in Th1 cells.[104] Histone H3K4 methyltransferase MLL is required for maintaining H3K4 modification at *Il4/Il13* locus in Th2 cells[105] whereas H3K27 methyltransferase EZH2 is responsible for suppressive H3K27me3 modification at *Il4/Il13* locus in Th1 cells.[106] The functions of GATA3 and STAT5 in regulating histone modifications are not known.

Although Th2 cells are capable of producing both IL-4 and IL-13, at single cell level, the expression of each cytokine from one of two alleles may be different, resulting in heterogenous cytokine expression pattern and monoallelic expression.[107] The actual induction of cytokine expression from a given allele seems to be stochastic and probabilistic,[108] and differential state of chromatin structure and gene accessibility at different alleles may determine the probability of gene expression.[109] Therefore, IL-4 non-producers can still be Th2 cells because they may be expressing IL-5 or IL-13 and/or they may express IL-4 at a different time point.

IL-4/STAT6-Dependent and -Independent Th2 Differentiation *In Vivo*

IL-4/STAT6 signaling is critical for Th2 differentiation *in vitro*. Although some *in vivo* Th2 responses including that to *Trichuris muris* infection[110] and to some allergens[111] are IL-4/STAT6 dependent, other responses, such as to *Nippostrongylus brasiliensis* and *S. mansoni* infection, are IL-4 independent.[39–41,112,113] However, STAT6 may still be required for the migration of Th2 cells to the lung tissue[112] as well as for the generation of Th2 memory cells[41] and IL-4/STAT6 remains critical for inducing IgE production in B cells.

GATA3 is critical for *in vivo* Th2 responses even in the model in which IL-4 is dispensable,[25] suggesting that either there is an IL-4-independent pathway for GATA3 induction or the basal expression of GATA3 is sufficient for *in vivo* Th2 cell differentiation. Notch[44] and β-catenin[47] signaling pathways may be important for *in vivo* Th2 responses and GATA3 upregualtion, however, whether these pathways are responsible for IL-4-independent *in vivo* Th2 responses in general has not been determined.

Low-dose peptide stimulation of naïve CD4 T cells *in vitro* results in IL-4-independent GATA3 upregulation as discussed above.[35] Recently, it has been reported that *S. mansoni* egg product, Omega-1, is the key factor for inducing Th2 responses and this product downmodulates dendritic cell (DC) functions and suppresses IL-12 production by DCs.[114,115] Basophils are also reported to be critical for many *in vivo* Th2 responses by directly presenting antigens to CD4 T cells.[111,116,117] Interestingly, basophils express lower levels of MHCII than DCs consistent with the idea that low strength of TCR signaling preferentially induces Th2 responses.

In addition to presenting antigens, basophils produce many Th2-prone cytokines, including IL-4 and TSLP. STAT5 can be activated by TSLP suggesting TSLP/STAT5 pathway may serve as an initiation step of Th2 differentiation under certain conditions. However, just as both IL-4-dependent and -independent Th2 responses exist *in vivo*, TSLP may be required

for some but not all *in vivo* Th2 responses. Whether *in vivo* Th2 responses require either IL-4 or TSLP needs further investigation.

Concluding Remarks

GATA3 expression and STAT5 activation are two major elements for Th2 differentiation definitely *in vitro* and possibly *in vivo*. Many other transcription factors are also involved in regulating or fine-tuning Th2 responses. Full Th2 cell differentiation is achieved through three mechanisms: Th2 lineage commitment, selective growth of differentiating Th2 cells and suppression of alternative lineage fates.[31] GATA3, STAT5, NFAT1, IRF-4, c-Maf, JunB and Dec2 can directly act on the *Il4* and/or *Gata3* gene to control their expression (Th2 lineage commitment); GATA3, STAT5, c-Maf and Gfi-1 regulate either the expression of CD25 (IL-2Rα), IL-4Rα and IL-33Rα or responsiveness of Th2 cells to cytokines (selective growth of differentiating Th2 cells); GATA3, STAT5, Ikaros, Blimp-1 and Gfi-1 suppress the expression of molecules of other lineages, including IFNγ, STAT4, T-bet, Eomes, RORγt and IL-17, many of which can block Th2 differentiation (suppression of alternative lineage fates). These three mechanisms constitute powerful positive feedback loops, which promote terminal differentiation of Th2 cells.

Much has been learned from simplified *in vitro* Th2 differentiation models. However, the transcriptional regulation in developing Th2 cells in response to helminth infections and allergens is far from being understood. In addition, whether many epigenetic modifications observed in Th2 cells cultured *in vitro* represent modifications that would occur during *in vivo* Th2 differentiation is unknown. The fact that IL-4 is required for *in vitro* Th2 differentiation but not for many *in vivo* Th2 models suggests the complexity of *in vivo* situations. More factors such as cytokines including TSLP,[50] IL-25[80] and IL-33,[118] and many cell types including basophils, DCs, mast cells and NKT cells influence *in vivo* Th2 differentiation. Thus, it is likely that many *in vivo* Th2 responses differ from each other because of unique activation of certain Th2 components in different models. Generation of indicator mice reporting the expression or activation of key transcription factors and developing sensitive assays handling small cell numbers will greatly help investigate the transcriptional regulation of Th2 differentiation *in vivo*.

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References

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986; 136:2348–2357. [PubMed: 2419430]
2. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989; 7:145–173. [PubMed: 2523712]
3. Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell. 1994; 76:241–251. [PubMed: 7904900]
4. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. 2008; 112:1557–1569. [PubMed: 18725574]
5. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature. 1993; 362:245–248. [PubMed: 8384701]
6. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3:23–35. [PubMed: 12511873]

7. Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. Science (NY). 1989; 245:308–310.
8. Townsend JM, Fallon GP, Matthews JD, Smith P, Jolin EH, McKenzie NA. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. Immunity. 2000; 13:573–583. [PubMed: 11070175]
9. Longphre M, Li D, Gallup M, Drori E, Ordoñez CL, Redman T, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest. 1999; 104:1375–1382. [PubMed: 10562299]
10. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med. 2002; 8:885–889. [PubMed: 12091879]
11. Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003; 21:425–456. [PubMed: 12615888]
12. Urban JF Jr, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, et al. IL-13 IL-4Ralpha and Misc: Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. Immunity. 1998; 8:255–264. [PubMed: 9492006]
13. Zaiss DM, Yang L, Shah PR, Kobie JJ, Urban JF, Mosmann TR. Amphiregulin, a TH2 cytokine enhancing resistance to nematodes. Science. 2006; 314:1746. [PubMed: 17170297]
14. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. J Exp Med. 1990; 172:921–929. [PubMed: 2117636]
15. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol. 1990; 145:3796–3806. [PubMed: 2147202]
16. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997; 89:587–596. [PubMed: 9160750]
17. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997; 272:21597–21603. [PubMed: 9261181]
18. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, et al. Interleukin 2 plays a central role in Th2 differentiation. Proc Natl Acad Sci USA. 2004; 101:3880–3885. [PubMed: 15004274]
19. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. Immunity. 2003; 19:739–748. [PubMed: 14614860]
20. Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: essential functions before and after T-helper-2-cell differentiation. Nat Rev Immunol. 2009; 9:125–135. [PubMed: 19151747]
21. Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity. 1998; 9:745–755. [PubMed: 9846495]
22. Usui T, Preiss JC, Kanno Y, Yao ZJ, Bream JH, O'Shea JJ, et al. T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med. 2006; 203:755–766. [PubMed: 16520391]
23. Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity. 2000; 12:27–37. [PubMed: 10661403]
24. Zhang DH, Yang L, Cohn L, Parkyn L, Homer R, Ray P, et al. Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. Immunity. 1999; 11:473–482. [PubMed: 10549629]
25. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004; 5:1157–1165. [PubMed: 15475959]
26. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc Natl Acad Sci USA. 2004; 101:1993–1998. [PubMed: 14769923]
27. Lin JX, Leonard WJ. The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene. 2000; 19:2566–2576. [PubMed: 10851055]

28. Moriggl R, Topham DJ, Teglund S, Sexl V, McKay C, Wang D, et al. Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. Immunity. 1999; 10:249–259. [PubMed: 10072077]
29. Kagami S, Nakajima H, Suto A, Hirose K, Suzuki K, Morita S, et al. Stat5a regulates T helper cell differentiation by several distinct mechanisms. Blood. 2001; 97:2358–2365. [PubMed: 11290598]
30. Kagami S, Nakajima H, Kumano K, Suzuki K, Suto A, Imada K, et al. Both stat5a and stat5b are required for antigen-induced eosinophil and T-cell recruitment into the tissue. Blood. 2000; 95:1370–1377. [PubMed: 10666213]
31. Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. Cell Res. 2006; 16:3–10. [PubMed: 16467870]
32. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity. 1996; 4:313–319. [PubMed: 8624821]
33. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature. 1996; 380:630–633. [PubMed: 8602264]
34. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. Nature. 1996; 380:627–630. [PubMed: 8602263]
35. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. J Exp Med. 2005; 202:793–804. [PubMed: 16172258]
36. Kurata H, Lee HJ, O'Garra A, Arai N. Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity. 1999; 11:677–688. [PubMed: 10626890]
37. Zhu J, Guo L, Watson CJ, Hu-Li J, Paul WE. Stat6 is necessary and sufficient for IL-4′ s role in Th2 differentiation and cell expansion. J Immunol. 2001; 166:7276–7281. [PubMed: 11390477]
38. Lee DU, Rao A. Molecular analysis of a locus control region in the T helper 2 cytokine gene cluster: a target for STAT6 but not GATA3. Proc Natl Acad Sci USA. 2004; 101:16010–16015. [PubMed: 15507491]
39. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the *in vivo* or *in vitro* development of CD4+ lymphocytes with a Th2 cytokine profile. J Immunol. 2000; 164:3047–3055. [PubMed: 10706693]
40. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, et al. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. J Exp Med. 2004; 200:507–517. [PubMed: 15314076]
41. Finkelman FD, Morris SC, Orekhova T, Mori M, Donaldson D, Reiner SL, et al. Stat6 regulation of *in vivo* IL-4 responses. J Immunol. 2000; 164:2303–2310. [PubMed: 10679064]
42. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. Nat Immunol. 2001; 2:45–50. [PubMed: 11135577]
43. Corn RA, Hunter C, Liou HC, Siebenlist U, Boothby MR. Opposing roles for RelB and Bcl-3 in regulation of T-box expressed in T cells, GATA-3, and Th effector differentiation. J Immunol. 2005; 175:2102–2110. [PubMed: 16081776]
44. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. Cell. 2004; 117:515–526. [PubMed: 15137944]
45. Tanigaki K, Tsuji M, Yamamoto N, Han H, Tsukada J, Inoue H, et al. Regulation of alphabeta/gammadelta T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. Immunity. 2004; 20:611–622. [PubMed: 15142529]
46. Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, et al. Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. Immunity. 2007; 27:89–99. [PubMed: 17658279]

47. Yu Q, Sharma A, Oh SY, Moon HG, Hossain MZ, Salay TM, et al. T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-gamma. Nat Immunol. 2009; 10:992–999. [PubMed: 19648923]
48. Yang XO, Angkasekwinai P, Zhu J, Peng J, Liu Z, Nurieva R, et al. Requirement for the basic helix-loop-helix transcription factor Dec2 in initial TH2 lineage commitment. Nat Immunol. 2009; 10:1260–1266. [PubMed: 19881507]
49. Zhu J, Huang H, Guo L, Stonehouse T, Watson CJ, Hu-Li J, et al. Transient inhibition of interleukin 4 signaling by T cell receptor ligation. J Exp Med. 2000; 192:1125–1134. [PubMed: 11034602]
50. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. Nat Immunol. 2005; 6:1047–1053. [PubMed: 16142237]
51. Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. J Exp Med. 2006; 203:269–273. [PubMed: 16432252]
52. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol. 2008; 9:310–318. [PubMed: 18300366]
53. Adler SH, Chiffoleau E, Xu L, Dalton NM, Burg JM, Wells AD, et al. Notch signaling augments T cell responsiveness by enhancing CD25 expression. J Immunol. 2003; 171:2896–2903. [PubMed: 12960312]
54. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 1998; 16:225–260. [PubMed: 9597130]
55. Zhu J, Guo L, Min B, Watson CJ, Hu-Li J, Young HA, et al. Growth factor independent-1 induced by IL-4 regulates Th2 cell proliferation. Immunity. 2002; 16:733–744. [PubMed: 12049724]
56. Zhu J, Jankovic D, Grinberg A, Guo L, Paul WE. Gfi-1 plays an important role in IL-2-mediated Th2 cell expansion. Proc Natl Acad Sci USA. 2006; 103:18214–18219. [PubMed: 17116877]
57. Rengarajan J, Mowen KA, McBride KD, Smith ED, Singh H, Glimcher LH. Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. J Exp Med. 2002; 195:1003–1012. [PubMed: 11956291]
58. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity. 1999; 10:745–751. [PubMed: 10403649]
59. Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. EMBO J. 1999; 18:420–432. [PubMed: 9889198]
60. Lohoff M, Mittrucker HW, Prechtl S, Bischof S, Sommer F, Kock S, et al. Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4. Proc Natl Acad Sci USA. 2002; 99:11808–11812. [PubMed: 12189207]
61. Quirion MR, Gregory GD, Umetsu SE, Winandy S, Brown MA. Cutting edge: Ikaros is a regulator of Th2 cell differentiation. J Immunol. 2009; 182:741–745. [PubMed: 19124715]
62. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000; 100:655–669. [PubMed: 10761931]
63. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci USA. 2001; 98:15137–15142. [PubMed: 11752460]
64. Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature. 1996; 382:174–177. [PubMed: 8700209]
65. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature. 1996; 382:171–174. [PubMed: 8700208]
66. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, et al. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity. 1997; 7:571–581. [PubMed: 9354477]
67. Yang J, Zhu H, Murphy TL, Ouyang W, Murphy KM. IL-18-stimulated GADD45 beta required in cytokine-induced, but not TCR-induced, IFN-gamma production. Nat Immunol. 2001; 2:157–164. [PubMed: 11175814]

68. Djuretic IM, Levanon D, Negreanu V, Groner Y, Rao A, Ansel KM. Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. Nat Immunol. 2007; 8:145–153. [PubMed: 17195845]
69. Naoe Y, Setoguchi R, Akiyama K, Muroi S, Kuroda M, Hatam F, et al. Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf beta binding to the Il4 silencer. J Exp Med. 2007; 204:1749–1755. [PubMed: 17646405]
70. Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. Science (NY). 2003; 302:1041–1043.
71. Usui T, Nishikomori R, Kitani A, Strober W. GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. Immunity. 2003; 18:415–428. [PubMed: 12648458]
72. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science. 2005; 307:430–433. [PubMed: 15662016]
73. Takatori H, Nakajima H, Kagami S, Hirose K, Suto A, Suzuki K, et al. Stat5a inhibits IL-12-induced Th1 cell differentiation through the induction of suppressor of cytokine signaling 3 expression. J Immunol. 2005; 174:4105–4112. [PubMed: 15778369]
74. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity. 2007; 26:371–381. [PubMed: 17363300]
75. Zhu J, Davidson TS, Wei G, Jankovic D, Cui K, Schones DE, et al. Down-regulation of Gfi-1 expression by TGF-beta is important for differentiation of Th17 and CD103+ inducible regulatory T cells. J Exp Med. 2009; 206:329–341. [PubMed: 19188499]
76. Cimmino L, Martins GA, Liao J, Magnusdottir E, Grunig G, Perez RK, et al. Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. J Immunol. 2008; 181:2338–2347. [PubMed: 18684923]
77. Martins G, Calame K. Regulation and functions of Blimp-1 in T and B lymphocytes. Annu Rev Immunol. 2008; 26:133–169. [PubMed: 18370921]
78. Wang L, van Panhuys N, Hu-Li J, Kim S, Le Gros G, Min B. Blimp-1 induced by IL-4 plays a critical role in suppressing IL-2 production in activated CD4 Tcells. J Immunol. 2008; 181:5249–5256. [PubMed: 18832679]
79. Yoshimoto T, Paul WE. CD4pos, NK1 1pos T cells promptly produce interleukin 4 in response to *in vivo* challenge with anti-CD3. J Exp Med. 1994; 179:1285–1295. [PubMed: 7908323]
80. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies *in vivo*. Immunity. 2001; 15:985–995. [PubMed: 11754819]
81. Agarwal S, Avni O, Rao A. Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer *in vivo*. Immunity. 2000; 12:643–652. [PubMed: 10894164]
82. Hwang ES, White IA, Ho IC. An IL-4-independent and CD25-mediated function of c-maf in promoting the production of Th2 cytokines. Proc Natl Acad Sci USA. 2002; 99:13026–13030. [PubMed: 12271139]
83. Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita D, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. J Exp Med. 1998; 187:787–794. [PubMed: 9480988]
84. Lohning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC, et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci USA. 1998; 95:6930–6935. [PubMed: 9618516]
85. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. J Exp Med. 2000; 191:1069–1076. [PubMed: 10727469]
86. Guo L, Wei G, Zhu J, Liao W, Leonard WJ, Zhao K, et al. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. Proc Natl Acad Sci USA. 2009; 106:13463–13468. [PubMed: 19666510]

87. Liao W, Schones DE, Oh J, Cui Y, Cui K, Roh TY, et al. Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor alpha-chain expression. Nat Immunol. 2008; 9:1288–1296. [PubMed: 18820682]

88. Kim HP, Kelly J, Leonard WJ. The basis for IL-2-induced IL-2 receptor alpha chain gene regulation: importance of two widely separated IL-2 response elements. Immunity. 2001; 15:159–172. [PubMed: 11485747]

89. Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA. Th2-specific chromatin remodeling and enhancer activity in the Th2 cytokine locus control region. Immunity. 2004; 21:865–876. [PubMed: 15589174]

90. Siegel MD, Zhang DH, Ray P, Ray A. Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLE0 elements. J Biol Chem. 1995; 270:24548–24555. [PubMed: 7592673]

91. Kishikawa H, Sun J, Choi A, Miaw SC, Ho IC. The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. J Immunol. 2001; 167:4414–4420. [PubMed: 11591766]

92. Yamashita M, Ukai-Tadenuma M, Kimura M, Omori M, Inami M, Taniguchi M, et al. Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus. J Biol Chem. 2002; 277:42399–42408. [PubMed: 12205084]

93. Loots GG, Locksley RM, Blankespoor CM, Wang ZE, Miller W, Rubin EM, et al. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. Science (NY). 2000; 288:136–140.

94. Mohrs M, Blankespoor CM, Wang ZE, Loots GG, Afzal V, Hadeiba H, et al. Deletion of a coordinate regulator of type 2 cytokine expression in mice. Nat Immunol. 2001; 2:842–847. [PubMed: 11526400]

95. Henkel G, Weiss DL, McCoy R, Deloughery T, Tara D, Brown MA. A DNase I-hypersensitive site in the second intron of the murine IL-4 gene defines a mast cell-specific enhancer. J Immunol. 1992; 149:3239–3246. [PubMed: 1431102]

96. Solymar DC, Agarwal S, Bassing CH, Alt FW, Rao A. A 3′ enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. Immunity. 2002; 17:41–50. [PubMed: 12150890]

97. Ansel KM, Greenwald RJ, Agarwal S, Bassing CH, Monticelli S, Interlandi J, et al. Deletion of a conserved Il4 silencer impairs T helper type 1-mediated immunity. Nat Immunol. 2004; 5:1251–1259. [PubMed: 15516924]

98. Kim ST, Fields PE, Flavell RA. Demethylation of a specific hypersensitive site in the Th2 locus control region. Proc Natl Acad Sci USA. 2007; 104:17052–17057. [PubMed: 17940027]

99. Lee GR, Spilianakis CG, Flavell RA. Hypersensitive site 7 of the TH2 locus control region is essential for expressing TH2 cytokine genes and for long-range intrachromosomal interactions. Nat Immunol. 2005; 6:42–48. [PubMed: 15608641]

100. Seki N, Miyazaki M, Suzuki W, Hayashi K, Arima K, Myburgh E, et al. IL-4-induced GATA-3 expression is a time-restricted instruction switch for Th2 cell differentiation. J Immunol. 2004; 172:6158–6166. [PubMed: 15128803]

101. Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. J Exp Med. 2000; 192:105–115. [PubMed: 10880531]

102. Makar KW, Perez-Melgosa M, Shnyreva M, Weaver WM, Fitzpatrick DR, Wilson CB. Active recruitment of DNA methyltransferases regulates interleukin 4 in thymocytes and T cells. Nat Immunol. 2003; 4:1183–1190. [PubMed: 14595437]

103. Hutchins AS, Mullen AC, Lee HW, Sykes KJ, High FA, Hendrich BD, et al. Gene silencing quantitatively controls the function of a developmental trans-activator. Mol Cell. 2002; 10:81–91. [PubMed: 12150909]

104. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity. 2009; 30:155–167. [PubMed: 19144320]

105. Yamashita M, Hirahara K, Shinnakasu R, Hosokawa H, Norikane S, Kimura MY, et al. Crucial role of MLL for the maintenance of memory T helper type 2 cell responses. Immunity. 2006; 24:611–622. [PubMed: 16713978]

106. Koyanagi M, Baguet A, Martens J, Margueron R, Jenuwein T, Bix M. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. J Biol Chem. 2005; 280:31470–31477. [PubMed: 16009709]

107. Guo L, Hu-Li J, Paul WE. Probabilistic regulation in TH2 cells accounts for monoallelic expression of IL-4 and IL-13. Immunity. 2005; 23:89–99. [PubMed: 16039582]

108. Guo L, Hu-Li J, Paul WE. Probabilistic regulation of IL-4 production in Th2 cells: accessibility at the Il4 locus. Immunity. 2004; 20:193–203. [PubMed: 14975241]

109. Guo L, Hu-Li J, Zhu J, Watson CJ, Difilippantonio MJ, Pannetier C, et al. In TH2 cells the Il4 gene has a series of accessibility states associated with distinctive probabilities of IL-4 production. Proc Natl Acad Sci USA. 2002; 99:10623–10628. [PubMed: 12149469]

110. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. J Exp Med. 1994; 179:347–351. [PubMed: 8270879]

111. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. Nat Immunol. 2009; 10:713–720. [PubMed: 19465907]

112. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. Immunity. 2004; 20:267–277. [PubMed: 15030771]

113. van Panhuys N, Tang SC, Prout M, Camberis M, Scarlett D, Roberts J, et al. *In vivo* studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. Proc Natl Acad Sci USA. 2008; 105:12423–12428. [PubMed: 18719110]

114. Steinfelder S, Andersen JF, Cannons JL, Feng CG, Joshi M, Dwyer D, et al. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). J Exp Med. 2009; 206:1681–1690. [PubMed: 19635859]

115. Everts B, Perona-Wright G, Smits HH, Hokke CH, van der Ham AJ, Fitzsimmons CM, et al. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. J Exp Med. 2009; 206:1673–1680. [PubMed: 19635864]

116. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, et al. Basophils contribute to T(H)2-IgE responses *in vivo* via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. Nat Immunol. 2009; 10:706–712. [PubMed: 19465908]

117. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomin PR, et al. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. Nat Immunol. 2009; 10:697–705. [PubMed: 19465906]

118. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005; 23:479–490. [PubMed: 16286016]

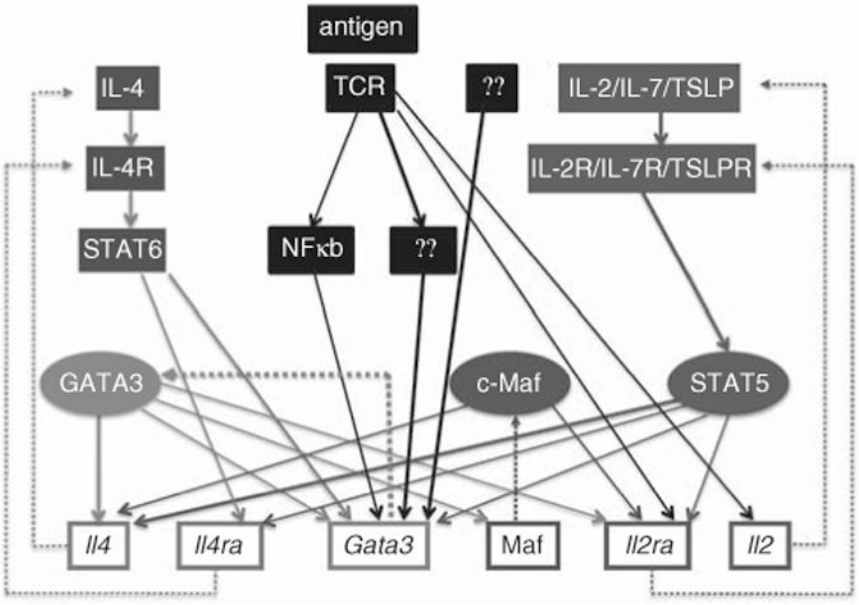

Figure 1.

Positive feedback loops for Th2 cell differentiation regulated by GATA3 and STAT5. Both IL-4 and weakly stimulated TCR-mediated signaling induce the expression of Th2 master regulator GATA3. In addition, Notch and/or other signaling pathways may regulate GATA3 expression. TCR activation also results in IL-2 production and IL-2Rα upregulation. IL-2 activates STAT5 but its function may be substituted or compensated *in vivo* by IL-7 and TSLP, the other two STAT5 activators. GATA3 collaborates with activated STAT5 to induce IL-4 production. C-Maf, possibly regulated by GATA3, further enhances the expression of IL-4. In addition, GATA3 and STAT5 regulate the expression of both IL-2R and IL-4R. Elevated IL-4 and IL-2 production together with upregulation of IL-2R and IL-4R provide powerful positive feedback loops for promoting Th2 polarization as well as selective growth of committed Th2 cells.