

Epigenetic regulation of T-helper cell differentiation, memory, and plasticity in allergic asthma

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Summary

An estimated 300 million people currently suffer from asthma, which causes approximately 250 000 deaths a year. Allergen-specific T-helper (Th) cells produce cytokines that induce many of the hallmark features of asthma including airways hyperreactivity, eosinophilic and neutrophilic inflammation, mucus hypersecretion, and airway remodeling. Cytokine-producing Th subsets including Th1 (IFN- γ), Th2 (IL-4, IL-5, IL-13), Th9 (IL-9), Th17 (IL-17), Th22 (IL-22), and T regulatory (IL-10) cells have all been suggested to play a role in the development of asthma. Th differentiation involves genetic regulation of gene expression through the concerted action of cytokines, transcription factors, and epigenetic regulators. We describe how Th differentiation and plasticity is regulated by epigenetic histone and DNA modifications, with a focus on the regulation of histone methylation by members of the polycomb and trithorax complexes. In addition, we outline environmental influences that could influence epigenetic regulation of Th cells and discuss the potential to regulate Th plasticity and function through drugs targeting the epigenetic machinery. It is also becoming apparent that epigenetic regulation of allergen-specific memory Th cells may be important in the development and persistence of chronic allergies. Finally, we describe how epigenetic modifiers regulate cytokine memory in Th cells and describe recently identified hybrid, plastic, and pathogenic memory Th subsets the context of allergic asthma.

KEYWORDS

allergy, epigenetics, inflammation, memory, plasticity, T cells

1 | CYTOKINE PRODUCTION BY CD4⁺ T-HELPER CELLS

The classical type 2 cytokines are interleukin (IL)-4, IL-5, and IL-13, and elevated levels of each of these cytokines have been found in the lungs of asthmatics compared with healthy controls.^{1,2} T-helper (Th) lymphocytes isolated from the peripheral blood of atopic individuals produce higher levels of type 2 cytokines than those from non-atopics, and bronchoalveolar lavage (BAL) cytokine profiles in asthmatics show

preferential production of IL-5 and decreased interferon- γ (IFN- γ) compared with healthy controls.² Th2 cells produce IL-4, IL-5, and IL-13, and IL-4 is central to allergic asthma through its ability to also drive Th2 cell development.³ IL-4 and IL-13 signal through the IL-4 receptor α -chain, and both contribute to most parameters of asthma pathology to varying degrees. These include airway hyperresponsiveness (AHR), mucus hyperproduction, airway remodeling, and immunoglobulin E (IgE) isotype switching and B-cell proliferation,⁴ and an anti-IL-4 receptor alpha blocking monoclonal antibody was recently found to significantly improve lung function in patients with moderate to severe asthma.⁵ Allergic asthmatics often have elevated numbers of eosinophils in the airways. IL-5 controls accumulation of eosinophils in

the lungs in mouse models of asthma and promotes eosinophil recruitment and survival.^{6–9} Eosinophils can secrete inflammatory cytokines and chemokines and are a potent source of the chemical mediators leukotriene C4 and platelet-activating factor.¹⁰ While the exact role of eosinophils in asthma remains incompletely defined, the IL-5 monoclonal antibody Mepolizumab has been shown to reduce exacerbations in severe eosinophilic asthma patients.¹¹ Tumor growth factor (TGF) is also secreted by eosinophils and is thought to drive airway fibrosis, a characteristic feature of chronic airway inflammation.¹²

In the majority of cases, inhaled steroids provide control of asthma symptoms. However, in approximately 10% of cases, symptoms are refractive to treatment.¹³ In these individuals, high numbers of neutrophils, not eosinophils, are often found in the lungs¹³ and increased levels of IL-17 have been found in BAL samples and in lung biopsies of patients with asthma.¹⁴ IL-17 can induce neutrophil accumulation in the lungs and promote development of AHR, increased mucus production due to goblet cell hyperplasia and airway remodeling.¹⁵ IL-22 is produced primarily by Th17 cells¹⁶; however, discrete populations of IL-22-producing cells have also been identified and named Th22 cells.^{17–19} The IL-22 receptor is expressed on and is important for immune barrier function at mucosal surfaces.²⁰ IL-22 is capable of driving lung inflammation in the presence of IL-17, but appears to be protective in the absence of IL-17,²¹ and IL-22 has been proposed to suppress IFN- γ -mediated lung inflammation in asthmatic patients.²² IL-22 can also attenuate IL-25 production by lung epithelial cells.²³ Because IL-25 can enhance both Th2 and group 2 innate lymphoid cell (ILC2) responses, it is probable that IL-22 attenuates airway inflammation by this pathway in some circumstances. IL-26 is also produced by T cells and monocytes, but the exact Th subsets that produce IL-26 and the involvement of IL-26 in asthma is poorly understood. It was recently reported, however, that increased IL-26 concentrations were found in sputum from pediatric patients with non-eosinophilic uncontrolled asthma, and sputum IL-26 concentrations were shown to correlate with those of blood neutrophils.²⁴ Granulocyte macrophage colony stimulating factor (GM-CSF) has also been implicated in development of asthma. Th cells that produce high levels of GM-CSF and low levels of IL-4 and IFN- γ have been described and the name ThGM suggested.²⁵ However, other cells can also produce GM-CSF, and the predominant source of GM-CSF in the asthmatic lung is not completely known. GM-CSF has been shown to drive allergic airway inflammation in mice by inducing IL-33 production from dendritic cells (DC).²⁶ As described below, IL-33 has several overlapping functions with IL-25 in Th2 cell differentiation.

It has recently been proposed that Th9 cells are a discrete subset of Th cells.²⁷ Th9 cells have the ability to produce IL-9, but not the Th2 cytokines IL-4, IL-5, and IL-13. Asthma patients have an increased number of Th9 cells, and allergen challenge of mice with house dust mite causes differentiation of Th9 cells.²⁸ IL-9-deficient mice do not develop goblet cell hyperplasia and are deficient in mastocytosis,²⁹ and in transgenic mice, over-expression of IL-9 causes spontaneous lung inflammation that is dependent on further stimulation by the Th2 cytokines.³⁰ IL-9 can also induce eosinophil maturation and accumulation³¹ and contributes to thymic stromal lymphopoietin (TSLP)-induced allergic inflammation in vivo.³²

Th cells have also been shown to negatively influence allergic asthma symptoms. Th1 cells can act as negative regulators of allergic lung inflammation, mostly via inhibition of Th2 responses (see below), and BAL samples from asthmatics have decreased IFN- γ compared with controls.² IFN- γ may also have direct regulatory effects on airway inflammation. For example, aerosolized IFN- γ inhibits eosinophil infiltration into the trachea of sensitized BALB/c mice.³³ Regulatory T cells (Treg) are a subset of CD4⁺ T cells that are able to negatively regulate immune responses. Tregs exert suppressive effects via production of IL-10 and expression of surface molecules that negatively regulate immune responses such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).³⁴ Treg function has been shown to differ between atopic and non-atopic children.³⁵

Several different cytokines and Th subsets, therefore, function simultaneously during an allergic immune response (Figure 1). These influences form a balance between proinflammatory and anti-inflammatory pathways, with some cytokines, such as IL-22 acting in either pro- or anti-inflammatory ways depending on the specific cytokine microenvironment in an individual tissue. The development of asthma is, therefore, complex and effective development of therapeutics requires a thorough understanding of the many pathways involved.

2 | TH CELL DIFFERENTIATION

Naive CD4⁺ T cells circulate the body scanning for specific antigen presented in the context of major histocompatibility complex class II expressed by antigen presenting cells, such as DC. The antigenic specificities of individual T cells are determined by T-cell receptor gene rearrangement that occurs within the thymus. As described above, naive

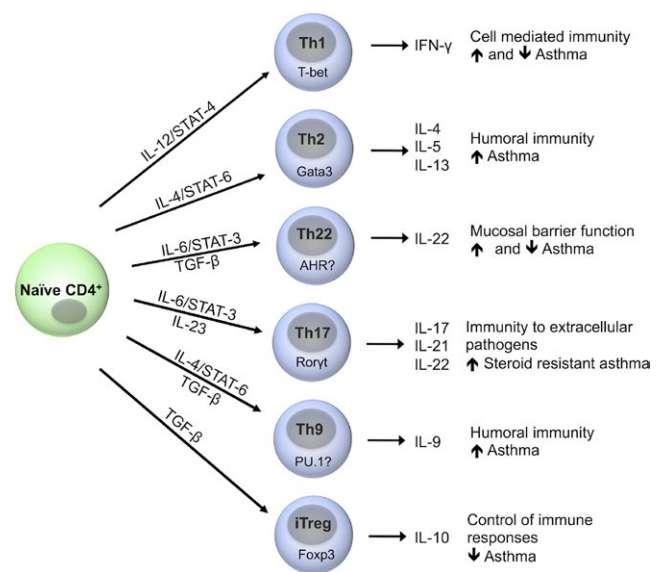


FIGURE 1 Th subset differentiation and function. Naive CD4⁺ T cells differentiate into effector Th cells after encountering antigen, and in response to signals in their microenvironment. Th subsets are initially defined based on their ability to produce a specific cytokine profile and the existence of a distinct transcriptional program that is induced in response to a lineage defining transcription factor(s)

CD4⁺ T cells can develop into several subsets following recognition of their specific antigen. Which subset depends on signals additional to those transmitted via the TCR, such as cytokines, accessory molecules, and pattern recognition receptors. Exposure to inhaled environmental allergens including pollen, animal dander, and house dust mites is thought to be centrally involved in the development of allergic asthma.^{36–38} The type of antigen encountered may also determine the cytokine production profiles of T cells. For example, mutagenesis of Derf-2, a house dust mite antigen results in a shift toward a Th1 response in human T cells.³⁹ The functional properties of allergens may also play a role in the development of the T-cell response. Proteases, lyases, lipid-transfer proteins, calcium-binding proteins, trypsin inhibitors, and actin-binding proteins appear to be particularly strong drivers of allergies.⁴⁰ More than 80 defined allergens are proteases,⁴⁰ with cysteine and serine protease activity linked to allergens including Derp1 and papain. The inactive form of Derp1 does not induce Th2 responses.⁴¹ Interestingly, in models of papain-induced asthma, ILCs have been shown to be essential for development of primary⁴² and memory⁴³ Th2 responses through licensing of DC via production of IL-13 by ILC2s. Th2 cells express the receptor for IL-33 (IL-33R); however, IL-33 does not appear to induce Th2 differentiation directly.⁴⁴ Instead, IL-33 appears to prime memory Th2 cells for high expression of IL-5.⁴⁵ TSLP is also thought to play a role in Th2 differentiation. TSLP is expressed by epithelial cells, and secretion can be enhanced in response to allergen exposure.⁴⁶ TSLP signaling in human DC results in a Th2-inducing phenotype through induction of OX40L expression.⁴⁷

For Th2 cells, the transcription factor Gata-binding protein 3 (Gata3) is both necessary and sufficient for differentiation.^{48–52} Th2 cells produce large amounts of IL-4, which induces Gata3 expression and further Th2 polarization,⁵³ and Gata3 has also been reported to positively regulate its own expression.⁵⁴ Expression of a dominant negative form of Gata3 results in reduced Th2 cytokine production and airway inflammation in a mouse model of asthma.⁵⁵ Recently, a novel DNA enzyme (SB010) that can cleave GATA3 mRNA was investigated in a clinical trial. SB010 attenuated both early- and late-phase responses of asthma after allergen provocation in asthmatics.⁵⁶ In the context of these results, mice deficient in signal transducer and activator of transcription (STAT)6, that transmits IL-4 signals within the cell, also show decreased eosinophil infiltration, mucus production, and AHR in a mouse model of asthma.⁵⁷

Differentiation into IFN- γ producing Th1 cells is programmed by the transcription factor T-bet (encoded by *Tbx21*),^{58,59} which is induced by IL-12 that signals through STAT4. In addition, Th1 cells produce IFN- γ , resulting in upregulation of T-bet and promotion Th1 differentiation.^{60,61} Both IFN- γ and T-bet have been associated with the suppression of eosinophilic lung infiltration and AHR, indicating that Th1 cells play a role in control of asthmatic inflammation and pathology. Suppression of Th2 cells is likely the main way that Th1 cells regulate allergic asthma; T-bet can directly interact with Gata3, and can inhibit Th2 differentiation by binding to Gata3 target DNA sequences.⁶²

Th17 cell differentiation is induced by RAR-related orphan receptor gamma (ROR γ), which is encoded by the *Rorc* gene.⁶³ STAT3 signals in response to engagement of the receptors for IL-6 and IL-23, which consequently induces the expression of ROR γ .⁶⁴ IL-21 is produced by

Th17 cells and can also promote pro-inflammatory Th17 cell differentiation via STAT3 signals.^{65,66} However, a role for IL-21 in neutrophilic asthma has not been defined. Interestingly, the TLR-3 ligand poly I:C can induce Th17 cells even under normally Th2-polarizing conditions.⁶⁷ TLR-3 recognizes double-stranded viral RNA, thus supporting the hypothesis that recurrent viral infections could play a role in Th17-driven asthma. The related subset of Th22 cells differentiate in response to tumor necrosis factor (TNF)- β and IL-6, and IL-22 production depends on the expression of the transcription factors aryl hydrocarbon receptor and ROR γ .^{17,19}

Th9 differentiation occurs when naive T cells are activated in the presence of TGF- β and IL-4. This combination of cytokines promotes upregulation of IL-9 expression without inducing Gata3 and the Th2 cytokines IL-4, IL-5, and IL-13.²⁷ We reported that TGF- β directly suppresses Gata3 through the transcription factor Sox4.⁶⁸ A Th9-specific transcription factor has not been clearly identified; however, B cell activating transcription factor (BATF), interferon regulatory factor 4 (IRF4), and PU.1 appear important for Th9 differentiation.⁶⁹

Like Gata3 for Th2 cells, the transcription factor forkhead box P3 (Foxp3) is essential for both differentiation and function of Tregs.⁷⁰ Peripheral Treg (pTreg) differentiation is driven primarily by combined signals from TGF- β and IL-2 signaling through STAT5.^{71,72}

The pathways that regulate Th differentiation in allergic asthma, and in general during immune responses, are, therefore, beginning to be better understood (Figure 1). This knowledge has now identified several opportunities for intervention in the development of Th-driven immune responses including regulation of cytokine signaling and transcription factor function.

3 | EPIGENETIC REGULATION OF TH CELL DIFFERENTIATION

The ability to maintain specific phenotypic traits independently of changes to the primary DNA sequence is an essential process of cellular differentiation. Cellular differentiation is the result of stable changes in gene expression that maintain the phenotype of a given cell. This is accomplished by epigenetic influences that include covalent modifications to histones and DNA methylation. We will describe these mechanisms and then focus in more detail on methylation of histones by members of the polycomb and trithorax complexes. Direct methylation of DNA is an epigenetic mechanism that is associated with genetic repression. Alterations in the levels of DNA methylation have been observed during Th differentiation. For example, DNA methylation is lost at genetic enhancers in the Th2 locus during Th2 differentiation,^{52,73} and DNA methylation has been implicated in control of the Foxp3 locus.⁷⁴ In addition, DNA methyltransferase (DNMT) 3a negatively regulates IL-13 in Th2 cells and allergic airway inflammation.⁷⁵

DNA is packaged inside the nucleus wrapped around core histones. Numerous post-translational modifications to residues at the N-terminal domain (tail) of histones have been identified and shown to be involved in controlling gene transcription. These modifications

have been referred to as a histone code that can be read by the transcriptional machinery and thus dictate regions of gene expression. Numerous modifications have been identified that are capable of regulating gene expression. These include biotinylation, sumoylation, ubiquitylation, and acetylation of lysine; phosphorylation of serine, threonine, and tyrosine; and methylation of lysine and arginine.

Acetylation of histones has been implicated in the differentiation and function of CD4⁺ T cells. In contrast to the negative regulation of gene expression by DNA methylation, histone acetylation is associated with positive regulation of transcription. Increased histone acetylation has been observed at the Th2 cytokine loci in response to Th2 differentiation.^{52,76} Likewise, histone acetylation is increased at the IL-17 locus during Th17 differentiation.⁷⁷ The histone acetyltransferase complex including CREB-binding protein (Cbp) and p300 has also been detected at the Th2 cytokine loci,⁷⁸ and Gata3 has been shown to form a complex with p300 that activates transcription of Th2 cytokines.⁷⁹ Inhibition of Cbp and p300 also results in reduced Th17 responses in human T cells.⁸⁰ Active histone deacetylation has also been shown to be involved in negative regulation of gene expression, and several histone deacetylase (HDAC)s have been found to function in Th cells, for example, loss of HDAC1 results in increased Th2 differentiation.⁸¹ HDAC6 and sirtuin-1 can also control Treg function by deacetylation and reduced expression of *Foxp3*.⁸²

Histone methylation is one of the most well-defined epigenetic mechanisms regulating Th differentiation. Histone H3 lysine 4 trimethylation (H3K4me3) is associated with active promoters. Histone H3 lysine 4 monomethylation (H3K4me1) and histone H3 lysine 4 dimethylation (H3K4me2) are found at genetic enhancers. Histone H3 lysine 36 trimethylation (H3K36me3) is found in actively transcribed coding regions of genes. Histone methylation is also associated with genetic repression, with histone H3 lysine 27 trimethylation (H3K27me3) and histone H3 lysine 9 trimethylation (H3K9me3) found at the promoters of silenced genes. One way that histones regulate gene expression is through controlling chromatin structure. Histone 3 lysine 9 acetylation (H3K9ac) and H3K4me3 are found at transcriptionally active genes, and these also coincide with an open chromatin structure and increased accessibility for molecules including transcription factors and RNA polymerase II. In contrast, H3K27me3 and DNA methylation are found at transcriptionally inactive genes, and these coincide with a closed chromatin structure that decreases the accessibility of transcription regulatory molecules and prevents gene transcription. Changes in several epigenetic histone modifications have been found to correlate with gene expression in T cells.^{3,83–85} For example, inactive or repressed genes are typically associated with increased levels of H3K27me3. These genes include *Ifng* and *Tbx21* in Th2 cells and *Il4* and *Gata3* in Th1 cells.^{86–89} In addition to direct epigenetic control gene promoters, the Th2 cytokine loci are also controlled by a number of enhancer elements. Enhancers are non-coding DNA sequences that regulate expression of specific genes and are associated with areas of DNase hypersensitivity, an indicator of open chromatin. It was recently shown that H3K4me1, H3K4me2, and H3K27ac also mark enhancer elements.^{90,91}

Epigenetic histone modifications are mediated by a variety of enzyme complexes (Figure 2) including the Polycomb and Trithorax

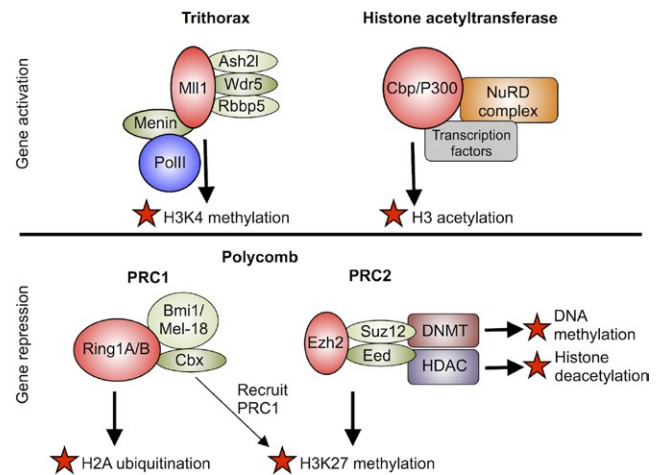


FIGURE 2 Complexes that control epigenetic regulation of Th cells. Epigenetic modification complexes such as polycomb and trithorax are responsible for methylating histones. Polycomb consists of two main complexes, polycomb repressive complex (PRC) 1 and PRC2. PRC2 is responsible for methylating H3K27. PRC1 can recognize this modification, resulting in recruitment of this complex. PRC1 can also ubiquitinate histone H2A. Trithorax is responsible for H3K4 methylation. In addition, PRC2 can interact with histone deacetylases (HDAC) and DNA methyltransferases (DNMT) that also promote genetic repression. Histone acetyltransferase complexes containing P300 and Cbp are recruited to genes by master transcription factors and facilitate transcription. Trithorax can also recruit the transcriptional machinery including RNA polymerase II (PolII) to promote transcription

complexes that both possess methyltransferase activity for histones, but function in a mutually antagonistic manner.^{3,92,93} Polycomb proteins are responsible for genetic repression, whereas Trithorax proteins mediate gene activation. Polycomb proteins form two major complexes (PRC1 and PRC2) that regulate genetic repression and are required for maintenance of cellular identity at several stages of development.^{92,94–96} The PRC2 component Enhancer of zeste (Ezh) proteins are methyltransferases and are enzymatically responsible for H3K27 methylation via their conserved SET domain.^{97–100} There are two Ezh proteins in mammals and Ezh2 is more highly expressed in T cells. PRC1 can recognize the H3K27me3 modification via chromobox (Cbx) proteins and is responsible for stable genetic repression.^{101,102} The PRC1 component RING finger protein 1 (Ring1) also has histone H2AK119 ubiquitin ligase activity and is thought to inhibit RNA polymerase II.¹⁰³ For a thorough review of polycomb function in gene regulation, see Aranda et al.¹⁰⁴ In contrast, the Trithorax protein Mixed lineage leukemia (Mll)1/2 methylates H3K4 and induces a chromatin structure permissive of transcription.⁹³

Both acquisition and maintenance of Th2 cell identity and Gata3 expression involves epigenetic changes at the *Gata3* gene locus.^{84,87} Occupancy of both polycomb and trithorax proteins is altered during Th2 cell differentiation at the *Gata3* gene locus, resulting in upregulation of Gata3. Transcription factors and epigenetic modifiers often work together to induce stable gene expression profiles in differentiated cells. For example, Gata3 can also be referred to as a pioneer

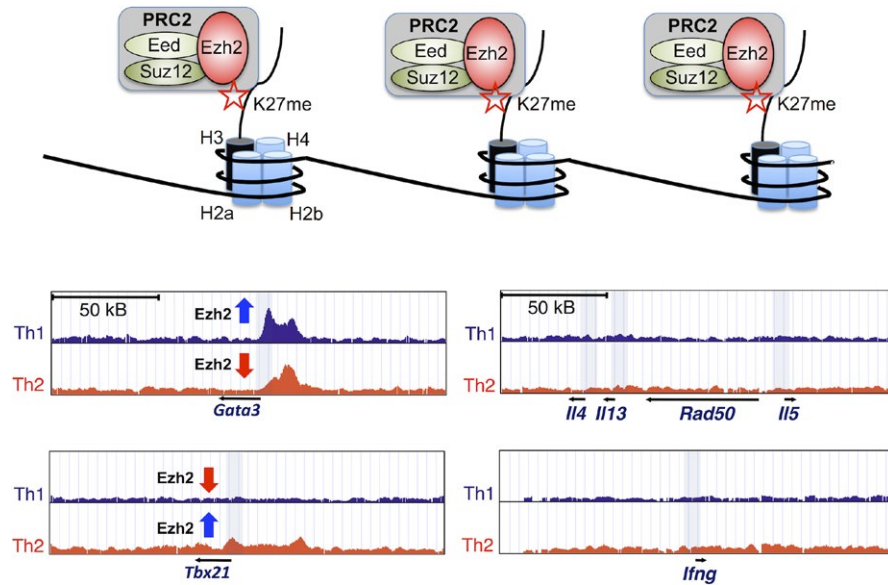


FIGURE 3 PRC2 in Th differentiation. DNA is packaged inside the nucleus wrapped around the core histones H2A, H2B, H4, and H4. Histone N-terminal tails are covalently modified by epigenetic modification complexes such as polycomb. PRC2 consists of Ezh2, EED, and Suz12. This complex is responsible for H3K27 methylation with Ezh2 possessing methyltransferase activity through its conserved SET domain. Ezh2, detected by chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq), localizes strongly to the genes *Tbx21* and *Gata3* that encode the master regulators of Th1 and Th2 differentiation, T-bet and Gata3, respectively; however, direct targeting of Ezh2 to *Ifng* and the Th2 cytokine loci is not obvious. Ezh2 is lost from the *Tbx21* upstream region in Th1 cells and the *Gata3* upstream region in Th2 cells. All ChIP-Seq figures represent 50 kilobases surrounding the genes of interest and the y-axes are all equivalent (Ezh2 ChIP-Seq data GEO accession number, GSE51079)

transcription factor as it can mediate alterations in epigenetic modifications along with facilitating the recruitment of other transcriptional complexes. Gata3 binds in cis to regulatory elements at the Th2 cytokine gene loci, including *Il4*, *Il5*, and *Il13* promoters, and this is followed by chromatin remodeling and regulation of Th2 gene expression. PRC1 group proteins, including Mel-18, Bmi1 and Ring1B are important for Th cell differentiation and inhibit cell death pathways of T cells.^{105–108} Ezh2 occupancy at the Gata3 locus has been shown to progressively decrease during Th2 cell differentiation⁸⁷ and Ezh2 positioning spatially correlates with the presence of H3K27me3,⁸⁶ indicating that epigenetic repression via Ezh2 may be an important regulator of CD4⁺ T cell differentiation (Figure 3). We previously reported that Ezh2 is required to repress the spontaneous generation of cytokine-producing Th cells by facilitating the correct expression of lineage-specific transcription factor genes, including *Tbx21*, *Gata3*, and *Eomes*.¹⁰⁹ Ezh2 exhibits strong binding to these transcription factors (Figure 3) and loss of Ezh2 function resulted in large decreases in H3K27me3 at these loci. Interestingly, however, a decrease in the H3K27me3 levels was also observed at both Th1 and Th2 cytokine gene loci in T cells lacking Ezh2 function, indicating that Ezh2 may also regulate H3K27me3 at these loci. Regulation of H3K27me3 by Ezh2 at the *Il4* and *Il13* gene loci in Th1 cells has been shown to be important for transcriptional silencing of these cytokine genes.^{109–111} Ezh2 has also been shown to be important for the regulation of cellular proliferation and viability after activation of Th cells.^{110,111} It was also reported that TGF- β induces expression of IL-9 via Smad2 and Smad4-mediated displacement of Ezh2.¹¹² Interestingly, one group

reported that PcG binding to cytokine gene loci is associated with increased transcription,¹¹³ indicating that polycomb proteins may have functions other than just genetic repression in Th cells. Other histone methyltransferase family proteins have also been reported to control Th subset differentiation. G9a, a histone methyltransferase that methylates H3K9, also appears to be involved in both positive and negative regulation of Th effector cell differentiation and function.¹¹⁴ The functions of individual Trithorax proteins have also been investigated in models of Th cell differentiation. Mll1 is essential for maintenance of *Gata3* gene expression in memory Th2 cells, and Th2 recall cytokine responses are defective in cells lacking Mll1.¹¹⁵ In addition, Menin is important for the maintenance of *Rorc* expression in Th17 cells.¹¹⁶

Without normal function of the epigenetic machinery, the immune response shows alterations including an inability to control cytokine expression, and in some cases failure to effectively mount an immune response after antigen exposure. Epigenetic regulation of Th cell function is, therefore, a fundamental way that the immune response is controlled.

4 | EPIGENETIC REGULATION OF EFFECTOR AND MEMORY TH FUNCTION IN ASTHMA

Asthma prevalence appears to be influenced by a combination of genetic and environmental factors, with environmental factors likely susceptible to epigenetic regulation. Studies in human cohorts have

also demonstrated an association between epigenetic modifications and asthma. A genome-wide study of histone modifications identified disease-associated enhancers in CD4⁺ T cells from asthmatic patients.¹¹⁷ Epigenetics also intersects with genetic susceptibility to asthma. In asthmatic patients, enhancers that gain the H3K4me2 mark during Th2 differentiation are enriched for asthma-associated single-nucleotide polymorphisms (SNP).¹¹⁸ A SNP in the *IL-4R* has also been associated with asthma when assessed in the context of *IL-4R* DNA methylation,¹¹⁹ and asthmatic patients have hypomethylation at several genes including *IL13* in PBMCs.¹²⁰ In a mouse asthma model, CD4⁺ T cells also display increased DNA methylation of the *Ifng* promoter.¹²¹

The functions of several epigenetic modifiers in development of asthma have also been investigated in gene deletion studies in mice. Loss of Mel-18 results in reduced eosinophil accumulation in the lungs, likely due to a reduction in Gata3 protein expression in Th2 cells.¹⁰⁶ The PRC1 component Ring1B has been shown to inhibit Bim-dependent apoptosis of Th2 cells in the lung, and mice with a T-cell-specific loss of Ring1B have a reduction in airway inflammation.¹⁰⁷ In addition, the deletion of Bmi1 leads to impaired memory Th2 responses due to increased susceptibility to cell death.¹⁰⁸ Loss of PRC1 components Bmi1, Mel-18, or Ring1B, therefore, decreases allergic airway responses through a combination of increased cell death and decreased Th2 polarization in models of asthma. In contrast to PRC1, loss of PRC2 function results in increased Th2 cell formation and asthma-like pathology. Th2 cells lacking Ezh2 can cause increased pathology in an acute inflammation model, and T-cell-specific deletion of Ezh2 results in spontaneous development of memory phenotype Th2 cells accompanied by increased levels of circulating IgE.¹⁰⁹ Thus, Ezh2 may restrain the pathogenicity of CD4⁺ T cells in allergic airway inflammatory diseases such as asthma. Suv39h1, another genetic repressor that functions via methylation of H3K9, has also been shown to be important for development of asthma-like pathology. Suv39h1 deficiency results in reduced pathology in a mouse model of asthma.¹²²

As described above, HDACs negatively regulate permissive histone acetylation such as H3K9ac. Deletion of HDAC1 in T cells increases airway inflammation and Th2 cytokine production.⁸¹ In addition, inhibition of HDAC activity with trichostatin A induced an increase in the production of the Th2 cytokines IL-13 and IL-5 and a decrease in production of the Th1 cytokine IFN- γ from peripheral blood mononuclear cells isolated from asthmatics.¹²³

Increased cytokine recall is one of the hallmark characteristics of memory T cells. Memory Th2 cells produce markedly increased amounts of Th2 cytokines compared with naive CD4⁺ T cells.¹²⁴ This increase in cytokine production corresponds to increased levels of H3K9ac and H3K4me3 at Th2 cytokine loci. Trithorax proteins, therefore, function to support cytokine memory in Th cells via increased H3K4me3. Mll1 and Menin are members of the trithorax complex and loss of these proteins results in defective maintenance of Th2 memory responses while initial Th2 differentiation is unaffected.^{87,115} Cells lacking one copy of the gene that encodes Mll1 (*Kmt2a*) also failed to induce lung inflammation in a mouse model of asthma.¹¹⁵ We have

also recently found shown that Menin appears to be involved in the development of neutrophilic and dexamethasone resistant asthma; neutrophil accumulation in the lung is attenuated in mice transferred with Th17 cells lacking Menin.¹¹⁶

Epigenetic regulation is, therefore, important in the control of Th function and cytokine memory in development of asthma. Alterations in epigenetic modifications such as DNA and histone methylation have been detected in Th cells from asthmatics. In addition, numerous studies using gene deletion in T cells in mice have described essential functions for epigenetic regulators, such as members of the polycomb and trithorax complexes, in the development of asthma.

5 | ALTERING TH FUNCTION BY ENVIRONMENTAL AND PHARMACOLOGICAL REGULATION OF EPIGENETICS

As outlined above, epigenetic regulation of gene expression is a central mechanism that regulates differentiation of T cells and normal immune function. In addition, epigenetics could provide a direct link between environmental influences and gene expression. Some environmental influences have been suggested to influence epigenetic modifications and alter T-cell function. One study reported that secondhand smoke in combination with air pollution is associated with increased DNA methylation and decreased expression of *IFNG* in T effector cells and *FOXP3* in Treg cells in children.¹²⁵ Another study found that the protection from asthma, conferred by prenatal exposure to the bacterium *Acinetobacter lwoffii* F78, is associated with increased H4 acetylation at the *Ifng* locus in mice, and that protection can be abolished by treatment with a histone acetyltransferase inhibitor.¹²⁶ Notably, the short-chain fatty acids butyrate and propionate that are produced by commensal bacteria have intrinsic HDAC inhibitory potential and can promote iTreg differentiation.¹²⁷ Global levels of epigenetic regulation may also alter T-cell function. The HDAC to HAT ratio is lower in lung biopsies from asthmatics, and this has been found to return to normal levels with control of asthma symptoms.^{128,129} There have been some studies investigating the ability of drugs targeting epigenetic regulators to modulate asthma-like pathology in mice. Chemical inhibition of Suv39h1 can reduce pathology in a mouse model of asthma.¹²² In addition, administration of a DNA methyltransferase inhibitor can reduce airway inflammation in mice.¹²¹ Altering epigenetic modifiers is a promising avenue for the control of T-cell function and potentially control of asthma symptoms; however, methyltransferases and acetylases function at numerous different genes and also within the cytosol.¹³⁰ It is, therefore, likely that drugs that can alter epigenetic modifications at specific genetic loci will need to be developed in future.

6 | PLASTICITY OF EFFECTOR AND MEMORY TH CELLS

It is becoming apparent that Th cells can also display appreciable amounts of plasticity when exposed to strong re-polarizing signals

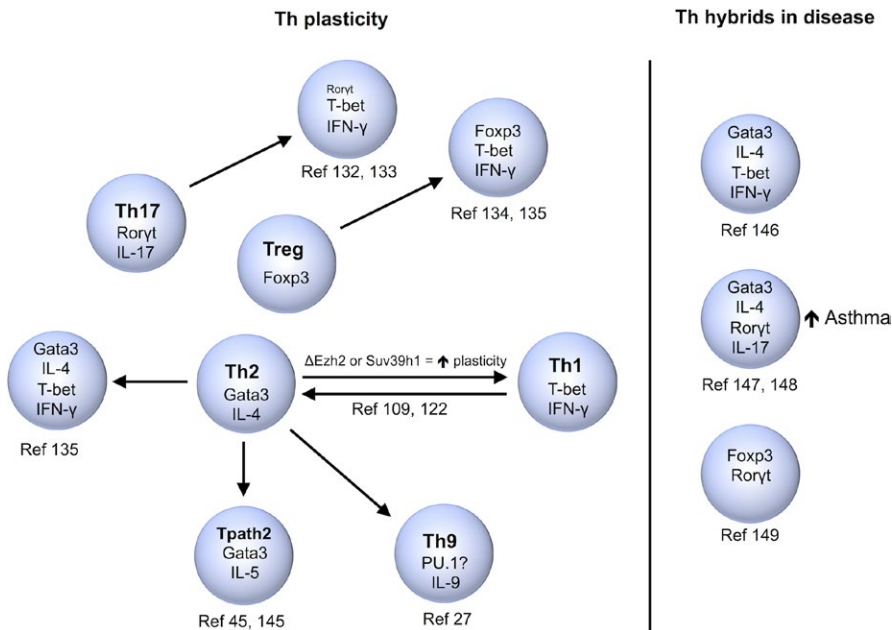


FIGURE 4 Th hybrids and plasticity. It is now appreciated that some Th cells, particularly Th17 and pTreg cells, have the capacity for substantial plasticity. This usually manifests in the generation of hybrid Th subsets that co-express subset-specific Th cell signature cytokines and transcription factors. Inhibition of the epigenetic machinery, either by genetic deletion of chromatin modifiers or chemical inhibition of their function, also appears to render Th cells hyper-susceptible to re-polarization. In addition, discreet populations of Th hybrid populations have recently been identified *in vivo* (right side of figure), and in some cases, these hybrids have been shown to differentiate directly from naive CD4⁺ T cells

¹³¹ (Figure 4). For example, Th17 cells^{132,133} and induced Treg (iTreg) cells^{134,135} can acquire the ability to produce IFN-γ *in vitro* and in models of autoimmunity and microbial infection, and recent evidence indicates that Th2 cells may also display some degree of functional plasticity during viral infection *in vivo*.¹³⁶ Allergen-specific Th2 cells can be driven toward an IFN-γ-producing phenotype when exposed to strong signaling via Toll-like receptor ligands *in vitro*.^{137,138} In addition, interferon signaling has been proposed as a driver of development of Th1/Th2 hybrid cells from Th2 cells.¹³⁶ Th1 and Th17 cells can also be driven to produce IL-4 during a mouse helminth infection.¹³⁹ It has also been suggested that Th9 cells develop from Th2 cells in response to TGF-β.²⁷ The stability of allergen-specific iTreg cells will also be an important area of future research, as these cells are able to recognize allergens and under normal conditions ensure immune tolerance.

Some genes simultaneously exhibit both activating (H3K4me3) and repressive histone (H3K27me3) modifications and are termed bivalent. These transcription factors are often central to cellular differentiation in processes such as the initial differentiation of immune cell subsets from hematopoietic stem cells. Interestingly, both *Tbx21* and *Gata3* are bivalent in Treg cells, indicating that this may be one-way Treg cells maintain plasticity.⁸⁹ *Gata3* also exhibits co-occupancy of the trithorax protein Menin (required for H3K4me3) and the polycomb protein Ezh2 (required for H3K27me3) in differentiated CD4⁺ T cells, and evidence suggests that the precise position of these proteins with respect to the transcriptional start site of genes may alter transcriptional activity and the ability of both of these complexes to control gene expression.¹⁴⁰ Th cells display higher levels of plasticity when the activities of repressive epigenetic regulators are inhibited, indicating that epigenetic mechanisms play a prominent role in the regulation of cellular plasticity. We found enhanced plasticity of both Th1 and Th2 cells if Ezh2 function is lost, indicating that this PRC2 protein is important for mediating subset stability. In Th1 cells lacking Ezh2, H3K27me3 is lost at several key genes including *Gata3*, and *Gata3*

is particularly sensitive to IL-4-induced upregulation in the absence of Ezh2, even in differentiated Th1 cells.¹⁰⁹ It was also shown that Suv39h1-dependent H3K9me3 maintains the stability of Th2 cells.¹²² Compared with wild-type cells, Suv39h1-deficient Th2 cells more readily expressed Th1 genes when exposed to Th1 polarizing stimuli. Ezh2 has also been found to influence iTreg stability.^{110,111,141,142} Loss of Ezh2 function in Foxp3-expressing cells results in multi-organ autoimmunity and production of proinflammatory cytokines.^{141,142}

Epigenetic control of Th plasticity could have important consequences for the regulation of immune responses to allergens and other disease antigens. In addition, *in vivo* and *in vitro* re-polarization of antigen-specific Th populations by drugs targeting epigenetic modifiers could be a novel way to control Th function in inflammatory disease.

7 | PATHOGENIC MEMORY TH CELLS; BEYOND THE CLASSICAL TH SUBSET MODEL

Despite the recent findings that Th cells can alter their phenotype under some circumstances, it is clear that memory Th cells have cytokine memory, with a single clone of antigen-specific T cells generally maintaining the cytokine production profile that was acquired during its initial differentiation.³ This system of cytokine memory has the advantage that the appropriate cytokine is produced quickly during the recall phase. This can also have adverse consequences during the recall response to some environmental antigens such as pollen or foods. Given the increased appreciation that Th cells, and particularly memory Th cells, can produce cytokines in a fashion that cannot be reconciled within the Th1/Th2/Th9/Th17/Th22/iTreg subset model, we recently proposed a 'pathogenic T-helper population disease-induction model'^{143,144} that recognizes the existence of memory Th cell populations with defined pathogenic activities and antigenic specificities. The model proposes that pathogenic subpopulations of

antigen-specific memory Th cells are primarily responsible for driving allergic pathology, regardless of the overall Th cytokine bias within a given tissue. Consistent with this model, several distinct subpopulations of T-helper cells have been identified in mice and humans that are capable of driving pathogenesis of inflammatory diseases, including asthma. In particular, pathogenic Th2 cells or Tpath2 cells appear important for development of allergic lung inflammation.^{143,144} A subset of IL-5 producing Tpath2 cells was originally identified within the CD62L and CXCR3 non-expressing portion of memory Th2 cells.¹⁴⁵ This CD62L and CXCR3 non-expressing subpopulation has high levels of H3K4me3 at the *Il5* promoter, indicating that these cells are epigenetically primed for IL-5 production.¹⁴⁵ It was subsequently shown that exposure of memory Th2 cells to IL-33 can induce chromatin remodeling of the *Il5* gene, independent of antigenic stimulation, resulting in enhanced Tpath2 cell function and eosinophilic inflammation.⁴⁵

Th populations that appear to stably possess characteristics of more than one of the canonical Th subsets have also been defined in both mice and humans. Th1/Th2 hybrids were found to differentiate directly from naive precursors and arise during parasite infection in mice.¹⁴⁶ These Th1/Th2 hybrids could induce inflammation in an allergic asthma model, albeit less compared with defined Th2 cells. GATA-3⁺ RORγt⁺ co-expressing, and IL-4 and IL-17 double-producing T cells have also been detected in BAL samples from asthmatics, and the presence of these cells was correlated with more severe forms of asthma. Interestingly, in vitro dexamethasone treatment suppressed Th2 cell function, but did not alter the function of the IL-4/IL-17 double-producing T cells.¹⁴⁷ Moreover, the IL-4 and IL-17 double producers can cause enhanced levels of eosinophilic and neutrophilic inflammation in a mouse model of asthma.¹⁴⁸ The intestinal microbiota has also been shown to induce iTreg cells that express RORγt and are capable of reducing Th2 inflammation.¹⁴⁹

It will be important to determine which of these populations are stable Th subsets and which are cells with a changeable phenotype. In addition, it is unknown to what extent cytokine committed cells can become plastic, and if there are differences between cytokine committed Th cells and Th cells that are capable of altering their phenotype. Addressing these questions will allow better understanding of the mechanisms that drive chronic Th-cell-mediated inflammatory diseases.

8 | CONCLUSIONS

Over the past 25 years, many of the cytokines and Th subsets that drive development of allergic asthma have been defined. In this review, we have outlined the Th subsets that participate in asthmatic inflammation and described some of the main cytokines and intracellular signaling molecules that regulate their development. It is becoming clear that transcriptional control of Th differentiation by epigenetic regulation is an important way that differentiation and cytokine memory of Th subtypes is controlled. New Th subsets including Th hybrids and discreet populations of pathogenic Th cells (e.g. Tpath2 cells) that are likely to be involved in allergic conditions such as asthma continue to be defined. How epigenetic regulation controls these subsets and

the extent to which these subsets overlap and can be altered by epigenetic mechanisms will be an important area of future research.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

- Shahid SK, Kharitonov SA, Wilson NM, Bush A, Barnes PJ. Increased interleukin-4 and decreased interferon-gamma in exhaled breath condensate of children with asthma. *Am J Respir Crit Care Med*. 2002;165:1290-1293.
- Kim CK, Kim SW, Park CS, Kim BI, Kang H, Koh YY. Bronchoalveolar lavage cytokine profiles in acute asthma and acute bronchiolitis. *J Allergy Clin Immunol*. 2003;112:64-71.
- Nakayama T, Yamashita M. Initiation and maintenance of Th2 cell identity. *Curr Opin Immunol*. 2008;20:265-271.
- Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol*. 2010;184:1663-1674.
- Wenzel S, Ford L, Pearlman D, et al. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med*. 2013;368:2455-2466.
- Tumes DJ, Wong AC, Sewell WA, McColl SR, Connolly A, Dent LA. Differential rates of apoptosis and recruitment limit eosinophil accumulation in the lungs of asthma-resistant CBA/Ca mice. *Mol Immunol*. 2008;45:3609-3617.
- Tumes DJ, Cormie J, Calvert MG, et al. Strain-dependent resistance to allergen-induced lung pathophysiology in mice correlates with rate of apoptosis of lung-derived eosinophils. *J Leukoc Biol*. 2007;81:1362-1373.
- Tumes DJ, Connolly A, Dent LA. Expression of surviving in lung eosinophils is associated with pathology in a mouse model of allergic asthma. *Int Immunol*. 2009;21:633-644.
- Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med*. 1996;183:195-201.
- Rosenberg HF, Dyer KD, Foster PS. Eosinophils: Changing perspectives in health and disease. *Nat Rev Immunol*. 2013;13:9-22.
- Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med*. 2014;371:1198-1207.
- Doherty T, Broide D. Cytokines and growth factors in airway remodeling in asthma. *Curr Opin Immunol*. 2007;19:676-680.
- Trevor JL, Deshane JS. Refractory asthma: Mechanisms, targets, and therapy. *Allergy*. 2014;69:817-827.
- Molet S, Hamid Q, Davoine F, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol*. 2001;108:430-438.

15. Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK. Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. *Am J Respir Crit Care Med*. 2005;171:12-18.
16. Liang SC, Tan XY, Luxenberg DP, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med*. 2006;203:2271-2279.
17. Duhon T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol*. 2009;10:857-863.
18. Eyerich S, Eyerich K, Pennino D, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest*. 2009;119:3573-3585.
19. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol*. 2009;10:864-871.
20. Sonnenberg GF, Fouser LA, Artis D. Border patrol: Regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol*. 2011;12:383-390.
21. Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *J Exp Med*. 2010;207:1293-1305.
22. Pennino D, Bhavsar PK, Effner R, et al. IL-22 suppresses IFN-gamma-mediated lung inflammation in asthmatic patients. *J Allergy Clin Immunol*. 2013;131:562-570.
23. Takahashi K, Hirose K, Kawashima S, et al. IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation. *J Allergy Clin Immunol*. 2011;128:1067-1076 e1061-1066.
24. Konradsen JR, Nordlund B, Levanen B, Hedlin G, Linden A. The cytokine interleukin-26 as a biomarker in pediatric asthma. *Respir Res*. 2016;17:32.
25. Zhang J, Roberts AI, Liu C, et al. A novel subset of helper T cells promotes immune responses by secreting GM-CSF. *Cell Death Differ*. 2013;20:1731-1741.
26. Llop-Guevara A, Chu DK, Walker TD, et al. A GM-CSF/IL-33 pathway facilitates allergic airway responses to sub-threshold house dust mite exposure. *PLoS ONE*. 2014;9:000 e88714.
27. Veldhoen M, Uytendove C, van Snick J, et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat Immunol*. 2008;9:1341-1346.
28. Jones CP, Gregory LG, Causton B, Campbell GA, Lloyd CM. Activin A and TGF-beta promote T(H)9 cell-mediated pulmonary allergic pathology. *J Allergy Clin Immunol*. 2012;129:1000-1010 e1003.
29. 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.
30. Temann UA, Ray P, Flavell RA. Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. *J Clin Invest*. 2002;109:29-39.
31. Louahed J, Zhou Y, Maloy WL, et al. Interleukin 9 promotes influx and local maturation of eosinophils. *Blood*. 2001;97:1035-1042.
32. Yao W, Zhang Y, Jabeen R, et al. Interleukin-9 is required for allergic airway inflammation mediated by the cytokine TSLP. *Immunity*. 2013;38:360-372.
33. Nakajima H, Iwamoto I, Yoshida S. Aerosolized recombinant interferon-gamma prevents antigen-induced eosinophil recruitment in mouse trachea. *Am Rev Respir Dis*. 1993;148:1102-1104.
34. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3⁺ regulatory T cells in the human immune system. *Nat Rev Immunol*. 2010;10:490-500.
35. Tulic MK, Andrews D, Crook ML, et al. Changes in thymic regulatory T-cell maturation from birth to puberty: Differences in atopic children. *J Allergy Clin Immunol*. 2012;129:199-206 e191-194.
36. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol*. 2011;32:402-411.
37. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: Phenotype shaped by innate and adaptive immunity. *Nat Immunol*. 2010;11:577-584.
38. Gelfand EW, Kraft M. The importance and features of the distal airways in children and adults. *J Allergy Clin Immunol*. 2009;124:S84-S87.
39. Korematsu S, Tanaka Y, Hosoi S, et al. C8/119S mutation of major mite allergen Derf-2 leads to degenerate secondary structure and molecular polymerization and induces potent and exclusive Th1 cell differentiation. *J Immunol*. 2000;165:2895-2902.
40. Traidl-Hoffmann C, Jakob T, Behrendt H. Determinants of allergenicity. *J Allergy Clin Immunol*. 2009;123:558-566.
41. Chambers L, Brown A, Pritchard DI, Sreedharan S, Brocklehurst K, Kalsheker NA. Enzymatically active papain preferentially induces an allergic response in mice. *Biochem Biophys Res Commun*. 1998;253:837-840.
42. Halim TY, Steer CA, Matha L, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*. 2014;40:425-435.
43. Halim TY, Hwang YY, Scanlon ST, et al. Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. *Nat Immunol*. 2016;17:57-64.
44. Hoshino K, Kashiwamura S, Kuribayashi K, et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J Exp Med*. 1999;190:1541-1548.
45. Endo Y, Hirahara K, Iinuma T, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity*. 2015;42:294-308.
46. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol*. 2002;3:673-680.
47. Ito T, Wang YH, Duramad O, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med*. 2005;202:1213-1223.
48. 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.
49. 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.
50. 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.
51. Zhu J, Min B, Hu-Li J, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat Immunol*. 2004;5:1157-1165.
52. Yamashita M, Ukai-Tadenuma M, Miyamoto T, et al. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. *J Biol Chem*. 2004;279:26983-26990.
53. 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.
54. Ouyang W, Lohning M, Gao Z, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity*. 2000;12:27-37.
55. Zhang DH, Yang L, Cohn L, 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.

56. Krug N, Hohlfeld JM, Kirsten AM, et al. Allergen-induced asthmatic responses modified by a GATA3-specific DNase. *N Engl J Med*. 2015;372:1987-1995.
57. Akimoto T, Numata F, Tamura M, et al. Abrogation of bronchial eosinophilic inflammation and airway hyperreactivity in signal transducers and activators of transcription (STAT)6-deficient mice. *J Exp Med*. 1998;187:1537-1542.
58. 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.
59. Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN- γ production in CD4 and CD8 T cells. *Science*. 2002;295:338-342.
60. Afkarian M, Sedy JR, Yang J, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4⁺ T cells. *Nat Immunol*. 2002;3:549-557.
61. Lighvani AA, Frucht DM, Jankovic D, et al. T-bet is rapidly induced by interferon- γ in lymphoid and myeloid cells. *Proc Natl Acad Sci USA*. 2001;98:15137-15142.
62. 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.
63. Ivanov I, McKenzie BS, Zhou L, et al. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell*. 2006;126:1121-1133.
64. Ghoreschi K, Laurence A, Yang XP, et al. Generation of pathogenic T(H)17 cells in the absence of TGF- β signalling. *Nature*. 2010;467:967-971.
65. Korn T, Bettelli E, Gao W, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*. 2007;448:484-487.
66. Nurieva R, Yang XO, Martinez G, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*. 2007;448:480-483.
67. Tanaka J, Watanabe N, Kido M, et al. Human TSLP and TLR3 ligands promote differentiation of Th17 cells with a central memory phenotype under Th2-polarizing conditions. *Clin Exp Allergy*. 2009;39:89-100.
68. Kuwahara M, Yamashita M, Shinoda K, et al. The transcription factor Sox4 is a downstream target of signaling by the cytokine TGF- β and suppresses T(H)2 differentiation. *Nat Immunol*. 2012;13:778-786.
69. Kaplan MH. The transcription factor network in Th9 cells. *Semin Immunopathol*. 2017;39:11-20.
70. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science*. 2003;299:1057-1061.
71. Yao Z, Kanno Y, Kerenyi M, et al. Nonredundant roles for Stat5a/b in directly regulating Foxp3. *Blood*. 2007;109:4368-4375.
72. Chen W, Jin W, Hardegen N, et al. Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J Exp Med*. 2003;198:1875-1886.
73. Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity*. 2002;16:649-660.
74. Floess S, Freyer J, Siewert C, et al. Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol*. 2007;5:e38.
75. Yu Q, Zhou B, Zhang Y, et al. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc Natl Acad Sci USA*. 2012;109:541-546.
76. 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.
77. Wei L, Laurence A, Elias KM, O'Shea JJ. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *J Biol Chem*. 2007;282:34605-34610.
78. Yamashita M, Ukai-Tadenuma M, Kimura 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.
79. Hosokawa H, Tanaka T, Suzuki Y, et al. Functionally distinct Gata3/Chd4 complexes coordinately establish T helper 2 (Th2) cell identity. *Proc Natl Acad Sci USA*. 2013;110:4691-4696.
80. Hammitzsch A, Tallant C, Fedorov O, et al. CBP30, a selective CBP/p300 bromodomain inhibitor, suppresses human Th17 responses. *Proc Natl Acad Sci USA*. 2015;112:10768-10773.
81. Grausenburger R, Bilic I, Boucheron N, et al. Conditional deletion of histone deacetylase 1 in T cells leads to enhanced airway inflammation and increased Th2 cytokine production. *J Immunol*. 2010;185:3489-3497.
82. Beier UH, Wang L, Han R, Akimova T, Liu Y, Hancock WW. Histone deacetylases 6 and 9 and sirtuin-1 control Foxp3⁺ regulatory T cell function through shared and isoform-specific mechanisms. *Science Signaling*. 2012;5:ra45.
83. Kanno Y, Vahedi G, Hirahara K, Singleton K, O'Shea JJ. Transcriptional and epigenetic control of T helper cell specification: Molecular mechanisms underlying commitment and plasticity. *Annu Rev Immunol*. 2012;30:707-731.
84. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol*. 2006;24:607-656.
85. Lohning M, Richter A, Radbruch A. Cytokine memory of T helper lymphocytes. *Adv Immunol*. 2002;80:115-181.
86. 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.
87. Onodera A, Yamashita M, Endo Y, et al. STAT6-mediated displacement of polycomb by trithorax complex establishes long-term maintenance of GATA3 expression in T helper type 2 cells. *J Exp Med*. 2010;207:2493-2506.
88. Schoenborn JR, Dorschner MO, Sekimata M, et al. Comprehensive epigenetic profiling identifies multiple distal regulatory elements directing transcription of the gene encoding interferon- γ . *Nat Immunol*. 2007;8:732-742.
89. Wei G, Wei L, Zhu J, 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.
90. Heintzman ND, Hon GC, Hawkins RD, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*. 2009;459:108-112.
91. Zhang JA, Mortazavi A, Williams BA, Wold BJ, Rothenberg EV. Dynamic transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity. *Cell*. 2012;149:467-482.
92. Nakayama T, Yamashita M. Critical role of the Polycomb and Trithorax complexes in the maintenance of CD4 T cell memory. *Semin Immunol*. 2009;21:78-83.
93. Onodera A, Nakayama T. Epigenetics of T cells regulated by Polycomb/Trithorax molecules. *Trends Mol Med*. 2015;21:330-340.
94. Boyer LA, Plath K, Zeitlinger J, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature*. 2006;441:349-353.
95. Ezhkova E, Pasolli HA, Parker JS, et al. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell*. 2009;136:1122-1135.
96. Hirabayashi Y, Suzuki N, Tsuboi M, et al. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron*. 2009;63:600-613.
97. Cao R, Wang L, Wang H, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*. 2002;298:1039-1043.

98. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell*. 2002;111:185-196.
99. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev*. 2002;16:2893-2905.
100. Muller J, Hart CM, Francis NJ, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell*. 2002;111:197-208.
101. Eskeland R, Leeb M, Grimes GR, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol Cell*. 2010;38:452-464.
102. Shao Z, Raible F, Mollaaghababa R, et al. Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell*. 1999;98:37-46.
103. Zhou W, Zhu P, Wang J, et al. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol Cell*. 2008;29:69-80.
104. Aranda S, Mas G, Di Croce L. Regulation of gene transcription by Polycomb proteins. *Science Advances*. 2015;1:e1500737.
105. Hosokawa H, Kimura MY, Shinnakasu R, et al. Regulation of Th2 cell development by Polycomb group gene bmi-1 through the stabilization of GATA3. *J Immunol*. 2006;177:7656-7664.
106. Kimura M, Koseki Y, Yamashita M, et al. Regulation of Th2 cell differentiation by mel-18, a mammalian polycomb group gene. *Immunity*. 2001;15:275-287.
107. Suzuki A, Iwamura C, Shinoda K, et al. Polycomb group gene product Ring1B regulates Th2-driven airway inflammation through the inhibition of Bim-mediated apoptosis of effector Th2 cells in the lung. *J Immunol*. 2010;184:4510-4520.
108. Yamashita M, Kuwahara M, Suzuki A, et al. Bmi1 regulates memory CD4 T cell survival via repression of the Noxa gene. *J Exp Med*. 2008;205:1109-1120.
109. Tumes DJ, Onodera A, Suzuki A, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. *Immunity*. 2013;39:819-832.
110. Yang XP, Jiang K, Hirahara K, et al. EZH2 is crucial for both differentiation of regulatory T cells and T effector cell expansion. *Sci Rep*. 2015;5:10643.
111. Zhang Y, Kinkel S, Maksimovic J, et al. The polycomb repressive complex 2 governs life and death of peripheral T cells. *Blood*. 2014;124:737-749.
112. Wang A, Pan D, Lee YH, Martinez GJ, Feng XH, Dong C. Cutting edge: Smad2 and Smad4 regulate TGF-beta-mediated IL9 gene expression via EZH2 displacement. *J Immunol*. 2013;191:4908-4912.
113. Jacob E, Hod-Dvora R, Schif-Zuck S, Avni O. Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. *J Biol Chem*. 2008;283:13471-13481.
114. Lehnertz B, Northrop JP, Antignano F, et al. Activating and inhibitory functions for the histone lysine methyltransferase G9a in T helper cell differentiation and function. *J Exp Med*. 2010;207:915-922.
115. Yamashita M, Hirahara K, Shinnakasu R, et al. Crucial role of MLL for the maintenance of memory T helper type 2 cell responses. *Immunity*. 2006;24:611-622.
116. Watanabe Y, Onodera A, Kanai U, et al. Trithorax complex component Menin controls differentiation and maintenance of T helper 17 cells. *Proc Natl Acad Sci USA*. 2014;111:12829-12834.
117. Gerasimova A, Chavez L, Li B, et al. Predicting cell types and genetic variations contributing to disease by combining GWAS and epigenetic data. *PLoS ONE*. 2013;8:e54359.
118. Seumois G, Chavez L, Gerasimova A, et al. Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nat Immunol*. 2014;15:777-788.
119. Soto-Ramirez N, Arshad SH, Holloway JW, et al. The interaction of genetic variants and DNA methylation of the interleukin-4 receptor gene increase the risk of asthma at age 18 years. *Clin Epigenetics*. 2013;5:1.
120. Yang IV, Pedersen BS, Liu A, et al. DNA methylation and childhood asthma in the inner city. *J Allergy Clin Immunol*. 2015;136:69-80.
121. Brand S, Kesper DA, Teich R, et al. DNA methylation of TH1/TH2 cytokine genes affects sensitization and progress of experimental asthma. *J Allergy Clin Immunol*. 2012;129:1602-1610 e1606.
122. Allan RS, Zueva E, Cammas F, et al. An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature*. 2012;487:249-253.
123. Su RC, Becker AB, Kozyrskyj AL, Hayglass KT. Epigenetic regulation of established human type 1 versus type 2 cytokine responses. *J Allergy Clin Immunol*. 2008;121:57-63 e53.
124. Yamashita M, Shinnakasu R, Nigo Y, et al. Interleukin (IL)-4-independent maintenance of histone modification of the IL-4 gene loci in memory Th2 cells. *J Biol Chem*. 2004;279:39454-39464.
125. Kohli A, Garcia MA, Miller RL, et al. Secondhand smoke in combination with ambient air pollution exposure is associated with increased CpG methylation and decreased expression of IFN-gamma in T effector cells and Foxp3 in T regulatory cells in children. *Clinical Epigenetics*. 2012;4:17.
126. Brand S, Teich R, Dicke T, et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *J Allergy Clin Immunol*. 2011;128:618-625 e611-617.
127. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504:451-455.
128. Ito K, Caramori G, Lim S, et al. Expression and activity of histone deacetylases in human asthmatic airways. *Am J Respir Crit Care Med*. 2002;166:392-396.
129. Su RC, Becker AB, Kozyrskyj AL, Hayglass KT. Altered epigenetic regulation and increasing severity of bronchial hyperresponsiveness in atopic asthmatic children. *J Allergy Clin Immunol*. 2009;124:1116-1118.
130. Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. *Gene*. 2005;363:15-23.
131. Krawczyk CM, Shen H, Pearce EJ. Functional plasticity in memory T helper cell responses. *J Immunol*. 2007;178:4080-4088.
132. Hirota K, Duarte JH, Veldhoen M, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol*. 2011;12:255-263.
133. Lee YK, Turner H, Maynard CL, et al. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009;30:92-107.
134. Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3⁺ regulatory T cells in human autoimmune disease. *Nat Med*. 2011;17:673-675.
135. Oldenhove G, Bouladoux N, Wohlfert EA, et al. Decrease of Foxp3⁺ Treg cell number and acquisition of effector cell phenotype during lethal infection. *Immunity*. 2009;31:772-786.
136. Hegazy AN, Peine M, Helmstetter C, et al. Interferons direct Th2 cell reprogramming to generate a stable GATA-3(+)T-bet(+) cell subset with combined Th2 and Th1 cell functions. *Immunity*. 2010;32:116-128.
137. Brugnolo F, Sampognaro S, Liotta F, et al. The novel synthetic immune response modifier R-848 (Resiquimod) shifts human allergen-specific CD4⁺ TH2 lymphocytes into IFN-gamma-producing cells. *J Allergy Clin Immunol*. 2003;111:380-388.
138. Fili L, Ferri S, Guarna F, et al. Redirection of allergen-specific TH2 responses by a modified adenine through Toll-like receptor 7 interaction and IL-12/IFN release. *J Allergy Clin Immunol*. 2006;118:511-517.

139. Panzer M, Sitte S, Wirth S, Drexler I, Sparwasser T, Voehringer D. Rapid in vivo conversion of effector T cells into Th2 cells during helminth infection. *J Immunol.* 2012;188:615-623.
140. Onodera A, Tumes DJ, Watanabe Y, et al. Spatial interplay between polycomb and trithorax complexes controls transcriptional activity in T lymphocytes. *Mol Cell Biol.* 2015;35:3841-3853.
141. DuPage M, Chopra G, Quiros J, et al. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. *Immunity.* 2015;42:227-238.
142. Sarmento OF, Svingen PA, Xiong Y, et al. The role of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) in the pathobiological mechanisms underlying inflammatory bowel disease (IBD). *J Biol Chem.* 2017;292:706-722.
143. Nakayama T, Hirahara K, Onodera A, et al. Th2 cells in health and disease. *Annu Rev Immunol.* 2017;35:53-84.
144. Endo Y, Hirahara K, Yagi R, Tumes DJ, Nakayama T. Pathogenic memory type Th2 cells in allergic inflammation. *Trends Immunol.* 2014;35:69-78.
145. Endo Y, Iwamura C, Kuwahara M, et al. Eomesodermin controls interleukin-5 production in memory T helper 2 cells through inhibition of activity of the transcription factor GATA3. *Immunity.* 2011;35:733-745.
146. Peine M, Rausch S, Helmstetter C, et al. Stable T-bet(+)GATA-3(+) Th1/Th2 hybrid cells arise in vivo, can develop directly from naive precursors, and limit immunopathologic inflammation. *PLoS Biol.* 2013;11:e1001633.
147. Irvin C, Zafar I, Good J, et al. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. *J Allergy Clin Immunol.* 2014;134:1175-1186 e1177.
148. Wang YH, Voo KS, Liu B, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med.* 2010;207:2479-2491.
149. Ohnmacht C, Park JH, Cording S, et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T cells. *Science.* 2015;349:989-993.

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