Immunity
Review

GATA-3 Function in Innate and Adaptive Immunity

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The zinc-finger transcription factor GATA-3 has received much attention as a master regulator of T helper 2 (Th2) cell differentiation, during which it controls interleukin-4 (IL-4), IL-5, and IL-13 expression. More recently, GATA-3 was shown to contribute to type 2 immunity through regulation of group 2 innate lymphoid cell (ILC2) development and function. Furthermore, during thymopoiesis, GATA-3 represses B cell potential in early T cell precursors, activates TCR signaling in pre-T cells, and promotes the $CD4^+$ T cell lineage after positive selection. GATA-3 also functions outside the thymus in hematopoietic stem cells, regulatory T cells, $CD8^+$ T cells, thymic natural killer cells, and ILC precursors. Here we discuss the varied functions of GATA-3 in innate and adaptive immune cells, with emphasis on its activity in T cells and ILCs, and examine the mechanistic basis for the dose-dependent, developmental-stage- and cell-lineage-specific activity of this transcription factor.

Introduction

Shortly after its identification in 1990, the zinc-finger transcription factor GATA-3 was found to be required both for early T cell development in the thymus and for differentiation of naive $CD4^+$ T cells into committed T helper type 2 (Th2) cells (Ting et al., 1996; Yamamoto et al., 1990; Zhang et al., 1997; Zheng and Flavell, 1997). The molecular function of GATA-3 has been most extensively studied in the context of transcriptional regulation of genes encoding the Th2 signature cytokines interleukin-4 (IL-4), IL-5, and IL-13, which are tightly clustered to form the Th2 cytokine locus. Within this locus, GATA-3 has a complex role: it binds not only to the *Il5* and *Il13* promoter regions, but also to Th2-cell-specific DNase I hypersensitive sites (DHSs) that engage chromatin remodeling machinery allowing GATA-3 to orchestrate a three-dimensional topography of type II cytokine transcription (Lee et al., 2006). GATA-3 can repress expression of other genes, for example *Ifng* encoding the Th1 signature cytokine interferon-γ (IFN-γ), although the mechanism for this repression remains less clearly understood.

The paradigm of GATA-3 as a central mediator of type II inflammation was recently extended by the finding that GATA-3 is also essential for group 2 innate lymphoid cell (ILC2) development and Th2 cell cytokine production (Furusawa et al., 2013; Hoyler et al., 2012; Klein Wolterink et al., 2013; Liang et al., 2012; Yagi et al., 2014). ILC2s are innate non-T and non-B lymphoid cells that produce large amounts of IL-5 and IL-13 upon activation by epithelial-derived proinflammatory cytokines (Moro et al., 2010; Neill et al., 2010; Saenz et al., 2010). However, it has become clear that GATA-3 function is not limited to innate and adaptive lymphocytes that mediate type II immunity. GATA-3 is also required in several mature T cell populations as well as in developmental cell fate decisions during lymphoid development. For example, in addition to controlling Th2 cell differentiation, GATA-3 controls survival and proliferation of $CD8^+$ T cells and is essential for regulatory T (Treg) cell function (Rudra et al., 2012; Wang et al., 2011, 2013). Beyond the T cell lineage, GATA-3 is also involved in hematopoietic stem cell (HSC) self-renewal and maintenance (Fitch et al., 2012; Frelin et al., 2013; Ku et al., 2012) and repression of B cell commitment in lymphoid precursors (Banerjee et al., 2013; García-Ojeda et al., 2013). GATA-3 is not needed for classical NK cell development, but is important for several specialized subsets of NK cells (Vosshenrich et al., 2006). In an analogous fashion to its critical role in early T lymphopoiesis, recent evidence shows that GATA-3 functions not only in mature ILC2s but also in ILC precursor cells (Constantinides et al., 2014; Klose et al., 2014; Serafini et al., 2014; Yagi et al., 2014) that give rise to various ILC populations (reviewed in Spits et al., 2013; Walker et al., 2013). Thus, GATA-3 is essential in the differentiation and function of multiple innate and adaptive lymphocytes.

Genome-wide analyses have identified a large number of GATA-3-binding sites in both active and silent genes in thymocytes and various mature T cell subsets (Jenner et al., 2009; Kanhere et al., 2012; Wei et al., 2011), suggesting that GATA-3 can both activate and repress gene expression. In contrast to the detailed knowledge of GATA-3 function in transcriptional regulation of Th2 cytokine genes, little is known about mechanisms of GATA-3-dependent gene regulation in developing T cells and ILCs. Because GATA-3 has crucial roles in a broad variety of cell types, it is logical to assume that GATA-3 function is context dependent. As such, the regulatory output of GATA-3 will be dictated by its distinct protein-protein interactions in a given cell type. In this review, we highlight recent reports describing functional roles for GATA-3 in several hematopoietic cell types and discuss how genome-wide identification of binding sites support a model in which GATA-3 is recruited to distinct subsets of its potential binding sites, in a dose-dependent, developmental-stage-specific, and cell-lineage-specific fashion.

GATA-3 and Its Family Members

In mammals the GATA family of transcription factors consists of six members, GATA-1 to GATA-6. GATA proteins contain two N-terminal transactivation domains and two characteristic Cys_4 DNA-binding zinc finger domains, each of which is followed

Table 1. Interacting Partners of GATA-3

Protein	Function	Cell Type	Reference
CHD4	Chromodomain-helicase-DNA-binding protein 4, ATP-dependent chromatin remodeler, subunit of the repressive NuRD complex	Th2	Hosokawa et al., 2013
CBP-p300	Histone acetyltransferease (HAT) complex; transcriptional activation	Th2	Hosokawa et al., 2013
NuRD	Nucleosome remodeling histone deacetylase repression complex; transcriptional repression	Th2	Hosokawa et al., 2013
Fog1	Friend of GATA-1; zinc-finger transcription factor that inhibits GATA-3 autoactivation and represses *Il5* gene transcription and Th2 cell differentiation	naive T cells	Zhou et al., 2001
Rog	Repressor of GATA (also known as Zbtb32); lymphoid-specific transcription factor that is rapidly induced in activated T cells and that represses GATA-3-induced transactivation	activated T cells	Miaw et al., 2000
T-bet	T-box protein; master regulator of Th1 cell differentiation, essential regulator of IFN-γ expression	Th1	Hwang et al., 2005
Eomes	T-box protein eomesodermin, highly homologous to T-bet and expressed in NK cells and inactivated $CD8^+$ T cells, Th1 cells, and Th2 cells	Th1	Endo et al., 2011
Runx3	Transcription factor that represses CD4 and activates CD8 expression and promotes Th1 cell differentiation in naive T cells and induces IFN-γ	Th1/Th2	Yagi et al., 2010
FoxP3	Master regulator of Treg cell differentiation and function	Treg	Wang et al., 2011
Smad3	Intracellular signal transducer of TGF-β	Th2	Blokzijl et al., 2002
YY1	Yin Yang 1, ubiquitously expressed zinc-finger transcription factor implicated in long-distance DNA interactions	Th2	Hwang et al., 2013
Fli1	Member of the ETS transcription factor family also known as ERGB	T cells	Wei et al., 2011

by a conserved basic region. The zinc finger closest to the C terminus mediates binding to the consensus DNA sequence (A/T)-GATA-(A/G), and the N-terminal zinc finger stabilizes this binding and physically interacts with the zinc finger coregulator protein friend of GATA (Fog) (Table 1 and discussed below; Fox et al., 1998).

GATA factors have pivotal roles during development, as shown by the fact that disruption of each of the GATA genes (except GATA-5) in mice results in embryonic lethality. Most GATA factors show a tissue- and cell-restricted pattern of expression. GATA-1 and GATA-2 are primarily expressed in the hematopoietic system, whereas GATA-4, GATA-5, and GATA-6 are mostly expressed in the cardiac, pulmonary, and digestive systems (Bresnick et al., 2012; Chlon and Crispino, 2012), although GATA-6 is also expressed in peritoneal macrophages in which it is required for proliferative renewal during homeostasis and in response to inflammation (Okabe and Medzhitov, 2014; Rosas et al., 2014) (Figure 1). The broad expression of GATA-3 in multiple cell types is an exception to the rule. There is a functional overlap among GATA family members: GATA-3 can partially restore erythroid development in GATA-1-deficient embryos (Tsai et al., 1998) and GATA-1, GATA-2, GATA-3, and GATA-4 all have the ability to enhance IL-4 and IL-5 and to inhibit IFN-γ production in differentiated T cells (Ranganath and Murphy, 2001).

GATA-1 is critically involved in the development of erythrocytes, megakaryocytes, mast cells, dendritic cells (DCs), basophils, and eosinophils (Figure 1). GATA-2 is indispensible for efficient hematopoiesis, both for the production and expansion of HSCs in the embryonic aorta-gonad-mesonephros (AGM) region and for the proliferation of HSCs in adult bone marrow (Ling et al., 2004). During erythroid differentiation, GATA-1 and GATA-2 manifest dynamic reciprocal changes in their expression profiles (see for review Vicente et al., 2012). In addition, a key role for GATA-2 has been demonstrated in basophil development and mast cell generation (Figure 1; Fiedler and Brunner, 2012). Surprisingly, GATA-3 overexpression in early double-negative (DN1) and DN2 but not DN3 fetal thymocytes that were cultured in the absence of Notch ligands rapidly and efficiently induced mast cell specification (Taghon et al., 2007). Mast cell development usually occurs independently of GATA-3, but because GATA proteins can induce their own expression, it is likely that the ability of GATA-3 to upregulate *Gata2* gene expression accounts for the observed reprogramming of thymocytes into mast cells.

In the hematopoietic system, expression of GATA-3 is confined to specific lymphocyte populations (Figure 1), as will be discussed below. GATA-3 is also expressed in various nonhematopoietic tissues, including adrenal glands, kidneys, central nervous system, inner ear, hair follicles, skin, and mammary gland. GATA-3-deficient embryos die between embryonic days 11 and 12 and display internal bleeding, growth retardation, severe brain and spinal cord deformation, and aberrations in fetal liver hematopoiesis (Pandolfi et al., 1995). The embryonic lethality is secondary to noradrenalin deficiency of the sympathetic nervous system, and GATA-3 mutation-induced lethality is partially averted by feeding catechol intermediates to pregnant dams (Lim et al., 2000). Haploinsufficiency of *GATA3* in man results in an autosomal-dominant developmental disorder, referred to as hypoparathyroidism-deafness-renal (HDR) dysplasia, associated with various heterozygous germline GATA-3 abnormalities, including nonsense, frameshift, and missense mutations (Ali et al., 2007). Mutations in *GATA3* are commonly found in human breast cancers and low GATA-3 expression is associated with poor prognosis. GATA-3 is required for luminal epithelial cell differentiation and commitment in the mammary gland. Whereas GATA-3 expression suppresses lung metastasis, loss of GATA-3 triggers fibroblastic

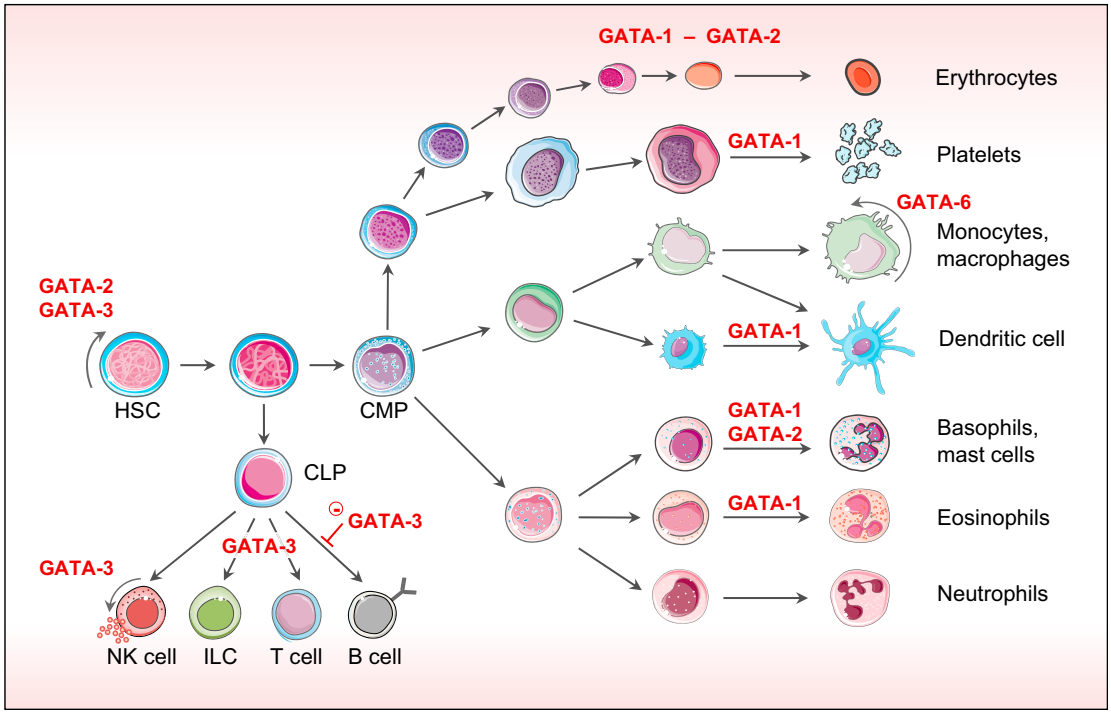

Figure 1. Function of GATA Factors in Hematopoietic Development

Overview of hematopoiesis, showing the main lineage commitment steps from HSC to fully maturated and functional blood cells. GATA transcription factors that are required for these processes are indicated in red: GATA-1 and GATA-2 are important for the development of the erythroid and myeloid cell lineages. In contrast, GATA-3 is involved only in the lymphoid cell lineage, wherein GATA-3 is crucial for NK cell maturation, for the development of ILCs and T cells, and for the repression of B cell potential. Abbreviations are as follows: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; ILC, innate lymphoid cell; NK, natural killer.

transformation and cell invasion (see for review Chou et al., 2010). Loss of Gata3 is also observed in high-grade invasive bladder cancer (Higgins et al., 2007).

Members of the GATA family have a highly conserved gene organization. Two distinct promoters drive lineage- and tissue-specific expression and alternative first exon usage generates a series of GATA mRNA isoforms. Regulation of *Gata3* expression is particularly complex and is dictated by individual tissue-specific enhancers. For example, a kidney enhancer element has been identified ~113 kb 5′ to the *Gata3* structural gene (Hasegawa et al., 2007), whereas a *cis*-acting element located ~280 kb 3′ to the *Gata3* structural gene regulates GATA-3 expression in the T and NK cell lineage in vivo (Hosoya-Ohmura et al., 2011).

The GATA-3 protein contains a classical nuclear localization signal motif and its localization between cytoplasm and nucleus is dependent on phosphorylation of critical serine residues by mitogen-activated protein kinase (MAPK) p38. MAPK is activated by T cell receptor (TCR) and IL-33R signaling in T cells and ILC2s, respectively, which facilitates binding to the nuclear transporter protein importin-α resulting in nuclear carriage (Frelin et al., 2013; Furusawa et al., 2013; Maneechotesuwan et al., 2007). Corticosteroids, which are highly effective in suppressing allergic airway inflammation, have the capacity to suppress GATA-3 nuclear import by competing for importin-α and by inducing MAPK phosphatase-1, an inhibitor of MAPK p38 (Maneechotesuwan et al., 2009). Finally, it is known that the acetylation status of GATA-3 affects its transactivation ability. The GATA-3 mutant KRR-GATA-3 is hypoacetylated and shows hypomorphic functions, resulting in reduced T cell survival and altered lymphocyte homing (Yamagata et al., 2000).

GATA-3 in Th2 Cell Differentiation

The role of GATA-3 in transcriptional regulation of the murine Th2 cytokine locus is well understood (Figure 2). This ~150 kb region contains *Il4*, *Il5*, and *Il13*, as well as a locus control region (LCR) that is crucial for appropriate Th2-specific cytokine expression and is located at the 5′ end of the interspersed *Rad50* gene, encoding a ubiquitously expressed DNA repair protein (Spilianakis and Flavell, 2004). GATA-3-mediated gene regulation and chromatin remodeling in the Th2 cytokine locus represents a model for understanding the molecular mechanisms of type II immune responses.

Initiation of Th2 Cell Differentiation and Inhibition of Th1 Cell Differentiation

Multiple distinct Th cell subsets (Th1, Th2, Th9, Th17, Th22, follicular T helper [Tfh], and Treg cells), characterized by unique cytokine production and transcription factor profiles, have been described (see for recent review Vahedi et al., 2013).

Th2 cells control helminth infections and allergic immune responses and are characterized by the expression of the proinflammatory cytokines IL-4, IL-5, and IL-13. The initiating signals that drive Th2 cell differentiation have been the subject of intense investigation and include IL-4, which induces phosphorylation and activation of STAT6, which in turn enhances GATA-3

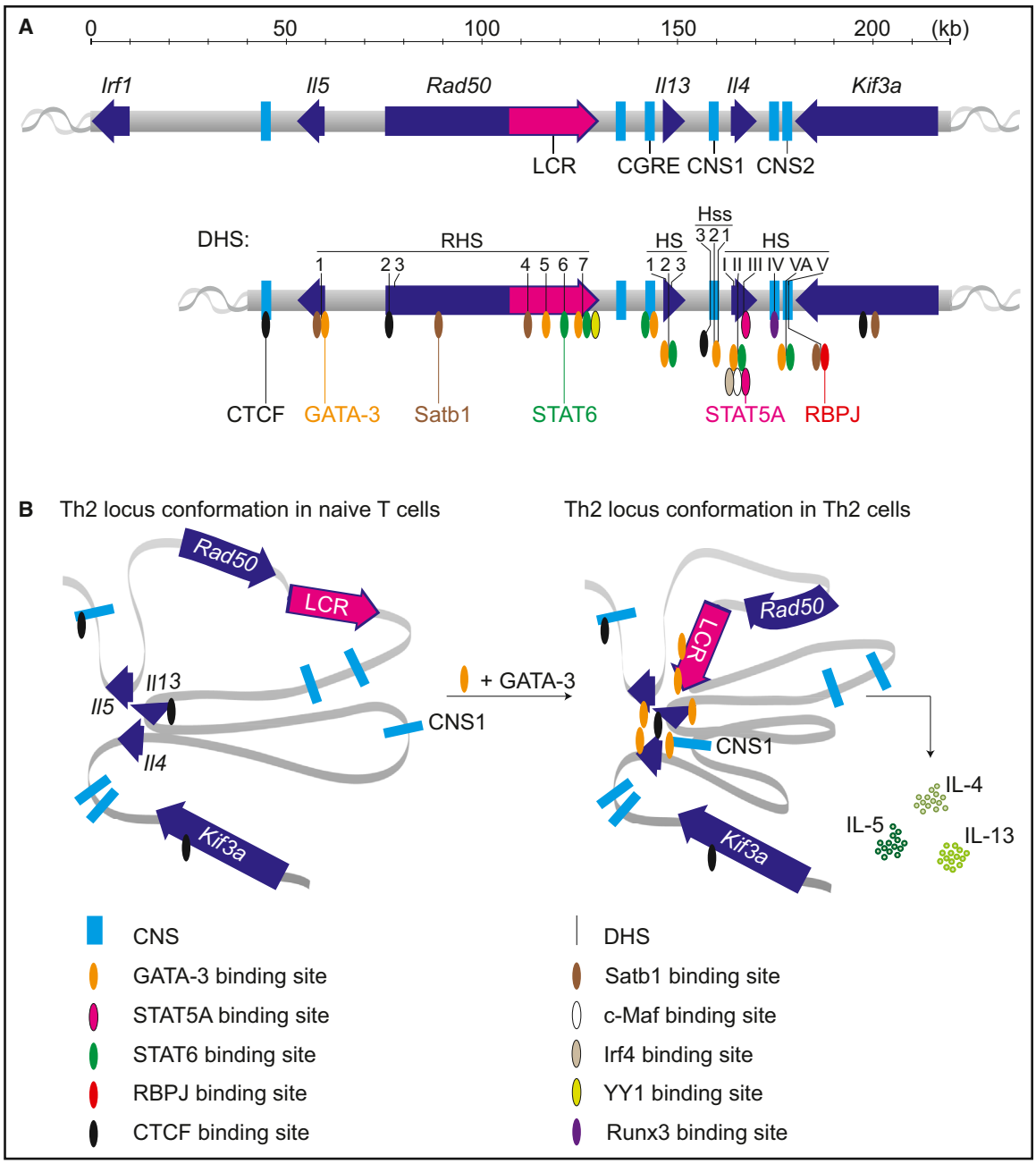

Figure 2. GATA-3-Mediated Regulation of the Th2 Cytokine Locus

(A) GATA-3 binds to the *Il5* and *Il13* promoter regions and to the *Il13* HS1-CGRE region (Kishikawa et al., 2001; Tanaka et al., 2011; Zhang et al., 1998). GATA-3 can also bind to a regulatory element in the first intron of the *Il4* gene (Tykocinski et al., 2005). GATA-3 helps establish long-range chromatin changes in the cytokine locus during Th2 cell differentiation, including the acquisition of four specific DHS sites: the HSII-intronic enhancer (IE), HSIII, HSVa, and HSV, whereby HSII-IE is crucial in GATA-3-mediated activation of *Il4* (Agarwal and Rao, 1998; Antebi et al., 2013; Lee et al., 2000; Yamashita et al., 2004). HSII, controlled by GATA-3, is strongest of the known *Il4* enhancers in Th2 cells (Kishikawa et al., 2001; Tanaka et al., 2011; Zhang et al., 1998). In the intergenic regulatory region CNS-1 located between the *Il4* and *Il13* genes, two additional Th2-cell-specific DHS sites HSS1 and HSS2 are occupied by GATA-3 in vitro (Takemoto et al., 2000). GATA-3 also binds to RHS5 and RHS7 in the LCR located within *Rad50* (Lee et al., 2003) in a STAT6-dependent manner (Lee and Rao, 2004). Additional nuclear factors that have been shown to bind in the Th2 locus are indicated (see text).

(B) Schematic representation of the Th2 locus in naive T cells (left) and in polarized Th2 cells (right). In naive T cells, the promoters for the Th2 cytokine genes are in close spatial proximity. During Th2 cell differentiation, activated STAT6 and Notch signaling induce GATA-3 upregulation, leading to the participation of the LCR and CNS1 elements in this "poised" chromatin core configuration, allowing for high transcription of IL-4, IL-5, and IL-13, which is dependent on the presence of CTCF (Ribeiro de Almeida et al., 2009).

Abbreviations are as follows: CGRE, conserved GATA-3 response element; CNS1, conserved noncoding region; CTCF, CCCTC-binding factor; DHS, DNaseI hypersensitive site; Il, interleukin; IRF4, interferon regulatory factor 4; LCR, locus control region; RBPjκ, recombination-signal-binding protein for immunoglobulin Jκ region; Runx3, Runt-related transcription factor 3; Satb1, special AT-rich sequence-binding protein-1; STAT, Signal Transducer and Activator of Transcription; YY1, Yin Yang 1.

expression via distal and proximal *Gata3* promoters and an upstream conserved regulatory region (Scheinman and Avni, 2009). GATA-3 is necessary and sufficient for Th2 cytokine gene expression in T helper cells (Zhang et al., 1997; Zheng and Flavell, 1997). Once induced, GATA-3 upregulates its own expression, either directly (Ouyang et al., 2000) or via the transcription factor Dec2 (Yang et al., 2009). GATA-3 is essential for the differentiation of naive T cells to Th2 cells, as well as for the activation of already established Th2 cells (Pai et al., 2004; Zhu et al., 2004). The induction of GATA-3 by the IL-4-STAT6 axis in differentiating Th2 cells, however, raises the paradox that IL-4 is essential for the generation of the cell type that is its major producer. The initial source of IL-4 that directs the Th2 cell response remains unclear. Although a range of cell types can produce IL-4, Th2 cell responses can be generated when IL-4 is exclusively produced by T cells or when mice lack functional IL-4R signaling, arguing against a requisite role for an external source of IL-4 (Jankovic et al., 2000; Schmitz et al., 1994).

Other pathways have been implicated in the initial production of IL-4. TCR triggering in naive T cells induces GATA-3 and IL-4 upregulation (Grogan et al., 2001; Yamane et al., 2005) and IL-2 secretion that in turn activates STAT5a in T cells (Lin and Leonard, 2000). STAT5a can bind to the DNase hypersensitive sites (DHSs) HSII and HSIII in the *Il4* locus (Figure 2), and high STAT5a activity can cooperate with GATA-3 to induce Th2 cell differentiation (Cote-Sierra et al., 2004). Several studies showed that GATA-3 and IL-4 expression can be directly regulated by Notch signaling in activated T cells (Amsen et al., 2007; Fang et al., 2007). The role of Notch signaling in the innate and adaptive immune system has recently been reviewed (Radtke et al., 2013). Differentially expressed Notch ligands on DCs are able to instruct differentiation of naive CD4 T helper cells: Delta-like (DLL) and Jagged ligands induce Th1 and Th2 cell differentiation, respectively (Amsen et al., 2004). Notch signaling induces Th2 cell differentiation (1) by directly activating the upstream *Gata3* gene promoter and (2) by regulating *Il4* gene transcription through activation of a 3′ enhancer. Both of these events are dependent on a nuclear complex that contains recombination-signal-binding protein for immunoglobulin Jκ region (RBPjκ). In the absence of GATA-3, Notch no longer induces Th2 cells but instead induces Th1 cell differentiation. Mice lacking RBPjκ or the Notch1 and Notch2 receptors fail to generate robust Th2 cell responses to parasite antigens (Amsen et al., 2007). Therefore, it can be concluded that the Notch signaling pathway is a STAT6-independent pathway that is crucial for Th2 cell induction via GATA-3. Although high amounts of exogenous IL-4 can induce normal Th2 cell differentiation in the absence of Notch signaling (Amsen et al., 2007; Ong et al., 2008; Tu et al., 2005), it is likely that under physiological conditions, Notch signaling and IL-4R signaling synergize to promote Th2 cell responses via the activation of GATA-3.

GATA-3 and Notch signaling are also required for efficient Th9 cell development. Th9 cells produce IL-9 and can differentiate from naive T cells or Th2 cells under the influence of IL-4, IL-9, and TGF-β (Kaplan, 2013). It has been shown that IL-9R expression is regulated by GATA-3 (Wei et al., 2011). Conditional deletion of Notch1 and Notch2 led to decreased IL-9 production in Th9 cell cultures, and the Notch ligand Jagged2—but not Delta-like 1—induced IL-9 in cells cultured with TGF-β alone (Elyaman et al., 2012).

Whereas Th2 cells substantially upregulate GATA-3 levels during development, Th1 cells express very low amounts of GATA-3 (Ouyang et al., 1998; Usui et al., 2006; Zhang et al., 1997; Zheng and Flavell, 1997). During Th2 cell differentiation, GATA-3 inhibits T-bet function and IFN-γ expression. Moreover, GATA-3 suppresses Th1 cell development by downregulating STAT4 and IL-12Rβ2 chain expression (Ouyang et al., 1998; Usui et al., 2006). This was supported by genome-wide analyses demonstrating that GATA-3-deficient Th2 cells have increased expression of STAT4 and IL-12Rβ2 mRNA (Wei et al., 2011). Nevertheless, recent data show that Th1 and Th2 cell differentiation is not mutually exclusive (as previously thought); stable and functional GATA-3$^+$T-bet$^+$ T cells that produce both IL-4 and IFN-γ are generated in vitro and in vivo in parasite and lymphocytic choriomeningitis virus (LCMV) infection (Antebi et al., 2013; Fang et al., 2013; Hegazy et al., 2010; Peine et al., 2013). These GATA-3$^+$T-bet$^+$ T cells support both Th1- and Th2-cell-mediated immune responses but cause less immunopathology compared with committed T-bet$^+$ Th1 or GATA-3$^+$ Th2 cells, suggesting a regulatory role for these GATA-3$^+$T-bet$^+$ T cells. These studies are consistent with the notion of T helper cell flexibility and adaption depending on the characteristics of invading pathogens. Moreover, evidence is now emerging for frequent coexpression of the Th cell signature transcription factors GATA-3, T-bet, RORγt, Bcl6, or FoxP3 in specialized CD4$^+$ T cell subtypes, challenging the paradigm of stable T helper cell subsets defined by the expression of a single "master regulator" (Oestreich and Weinmann, 2012). As such, T helper cell differentiation appears quite diverse and perhaps less stable compared with developmental cell-fate decisions that accompany lineage commitment, e.g., to the B or T lymphocyte lineage.

GATA-3 and the Th2 Cytokine Locus

GATA-3 binds directly to the *Il5* and *Il13* promoters, as well as to a binding site in the first intron of the *Il4* gene (Figure 2). In addition, GATA-3 plays a crucial role in establishing long-range chromatin interactions, because it binds to almost all DHSs in the locus that Th2 cells acquire during their differentiation from naive T cells, including DHSs in the LCR, as well as four DHSs crucial for activation of *Il4* (Tanaka et al., 2011). GATA-3 can induce the latter DHSs in Th1 cells, which clearly demonstrates that GATA-3 is associated with chromatin remodeling activity (Lee et al., 2000). More recently, chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq) experiments showed that in Th2 cells, genomic regions surrounding the GATA-3 binding sites in *Il4* and *Il13* are associated with activating H3K4 methylation but lack extensive repressive H3K27 trimethylation. In contrast, GATA-3 binding sites in the *Tbx21* and *Ifng* loci are associated with H3K27 trimethylation in Th2 cells (Chang and Aune, 2007; Wei et al., 2011). The finding that deletion of *Gata3* resulted in decreased H3K4 dimethylation at specific sites in the Th2 cytokine locus and decreased H3K27 trimethylation around its binding sites in the *Tbx21* and *Ifng* loci showed that GATA-3 mediates gene activation and repression by chromatin remodeling (Wei et al., 2011). Although T-bet is not expressed by Th2 cells, T-bet and GATA-3 are coexpressed in polarized human and mouse Th1 cells. Interestingly, ChIP-seq experiments showed that many of the T-bet and GATA-3 binding

sites in the *Ifng* locus in Th1 cells were coincident (Jenner et al., 2009; Kanhere et al., 2012), as discussed below.

Chromosome conformation capture (3C) studies show that the promoters for the Th2 cytokine genes are in close spatial proximity in various cell types, and in $CD4^+$ T cells specifically the LCR participates in this ''poised'' chromatin core configuration (Spilianakis and Flavell, 2004). GATA-3 and STAT6 have the capacity to directly remodel the LCR (Lee and Rao, 2004) and are essential for the establishment or maintenance of these long-range interactions, but additional nuclear factors have been implicated in the 3D organization of the Th2 cell locus. These include Th2-cell-specific transcription factors involved in *Il4* gene regulation, such as interferon regulatory factor 4 (IRF4), nuclear factor of activated T cells NFATc2, and c-Maf, but also general chromatin organizers, such as special AT-rich binding protein 1 (SATB1), Yin Yang1 (YY1), CCCTC-binding factor (CTCF), and cohesin (Figure 2; Barski et al., 2007; Cai et al., 2006; Hwang et al., 2013; Parelho et al., 2008). Further studies are required to elucidate the exact mechanisms by which GATA-3 contributes to the formation of chromatin loops in the Th2 cytokine locus.

In addition to Th2 cells, diverse myeloid cells, including mast cells, basophils, and eosinophils, are potent producers of IL-4, IL-5, and IL-13 in vivo. The finding that GATA-1 and GATA-2 bind HSII in the *Il4* locus in mast cells (Kwan et al., 2005) indicates that in type II immunity, GATA-3 function in lymphoid cells is, at least partly, substituted by GATA-1 and/or GATA-2 in myeloid cells.

GATA-3 in T Cell Development

Because GATA-3 plays critical roles in several tissues and deletion of *Gata3* results in embryonic lethality in mice (Pandolfi et al., 1995), analysis of GATA-3 function in immune development has been challenging. However, the use of diverse technical approaches (including blastocyst complementation, fetal liver hematopoietic stem cell reconstitution, and conditional gene targeting) has provided important clues as to how GATA-3 functions during the various stages of T cell development.

One dramatic result of GATA-3 deletion is the complete absence of T cell development (Hendriks et al., 1999; Hosoya et al., 2009; Ting et al., 1996). In the thymus, the T cell program is initiated by differentiation of early thymic progenitors (ETPs) derived from multipotent hematopoietic precursors in the bone marrow (Rothenberg and Taghon, 2005). Thymopoiesis requires several regulatory pathways for early thymocyte differentiation, including activation of the Notch1 receptor (Pui et al., 1999; Radtke et al., 1999) by its ligand delta-like 4 expressed on thymic epithelial cells (Hozumi et al., 2008; Koch et al., 2008). Notch1 triggering initiates and sustains the T cell program via activation of its transcription factor targets Tcf1 and Bcl11b (Chi et al., 2009). GATA-3 is also upregulated at this stage, although it is not clear whether this event is Notch dependent. As such, the interrelationship between Notch1 and GATA-3 pathways remains unclear. Without Notch1 signals, ETPs do not develop and thymic progenitors can be diverted into the B cell pathway (Feyerabend et al., 2009; Sambandam et al., 2005; Wilson et al., 2001). This Notch1-mediated repression of B cell development also involves GATA-3, as shown by the fact that GATA-3-deficient pro-T cells retain a latent B cell potential despite active Notch1 triggering (Figure 3; García-Ojeda et al., 2013). This is not the case with pro-T cells deficient for the Notch1 targets Tcf1 or Bcl11b (Li et al., 2010; Weber et al., 2011), and thus GATA-3 appears unique in its ability to ''seal'' Notch1-induced T cell commitment (Rothenberg, 2013). The mechanism by which GATA-3 represses the B cell program is unknown, but it is striking that repression of GATA-3 by the transcription factor early B cell factor-1 (Ebf1) is a critical component of normal B cell development (Banerjee et al., 2013). In the absence of Ebf1, lymphoid progenitors exhibit increased T cell lineage potential associated with increased *Gata3* gene transcription (Banerjee et al., 2013). Ebf1 ablation can divert lymphoid precursors into the ILC pathway as well (Nechanitzky et al., 2013). As such, the relative balance between GATA-3 and Ebf1 pathways critically determines the B versus T cell or ILC fate decision.

Using Lck-Cre transgenic *Gata3*-floxed mice to ablate GATA-3 expression in early DN cells, it was established that GATA-3 is critical for β-selection (Figure 3) and thus for the generation of T cell receptor (TCR-β)-expressing DN4 thymocytes (Pai et al., 2003). This is consistent with findings in GATA-3 reporter mice that demonstrate upregulation of GATA-3 during pre-TCR-mediated β-selection (Hendriks et al., 1999). Continued Notch1 triggering is also required for this transition (Bender et al., 2004; Wolfer et al., 2002), which again suggests a close relationship between Notch1 and GATA-3 pathways at this juncture.

Using ChIP-seq technology and expression profiling in developing thymocytes, GATA-3 binding was detected at 1,500 loci with marked differences in GATA-3 occupancy between early and later stages of T cell development (Wei et al., 2011; Zhang et al., 2012). In fact, GATA-3 binding sites did not show significant similarities between DN1 and DP cells, but rather binding was particularly enriched at ''stemness'' genes and ''T cell identity'' genes, in these respective stages (Zhang et al., 2012). These analyses suggest that GATA-3 is involved in the control of the expression of many genes that play a crucial role in T cell development, including key transcription factors, Notch1 and Notch2, TCR components, and the RAG enhancer. Because in DN thymocytes GATA-3 occupancy of regulatory elements in *Cd3d*, *Rag1*, *Rag2*, and *Zbtb7b* loci preceded their expression, GATA-3-mediated chromatin remodeling may function to prepare loci for interactions with nuclear factors at later stages of T cell development.

Conditional ablation of GATA-3 at later stages of T cell development (at the DP stage, using CD4-Cre transgenic *Gata3*-floxed mice) demonstrates the necessity of GATA-3 for promoting $CD4^+$ lineage choice (Pai et al., 2003). In the absence of GATA-3, differentiation of the $CD4^+$ lineage is blocked before CD4/CD8 commitment, because MHC class II-restricted GATA-3-deficient thymocytes are redirected to the $CD8^+$ T cell lineage, albeit inefficiently (Wang et al., 2008). In this context, GATA-3 is required for the expression of the transcription factor ThPOK, which promotes $CD4^+$ lineage differentiation (He et al., 2005; Sun et al., 2005). The finding that enforced ThPOK expression does not rescue $CD4^+$ lineage differentiation of GATA-3-deficient thymocytes indicates that GATA-3 also acts as a key $CD4^+$ lineage differentiation factor, independently of its capacity to induce ThPOK (Wang et al., 2008). Expression of GATA-3 is induced by TCR signaling during positive selection at the DP stage, and because GATA-3 enhances TCR upregulation, it is

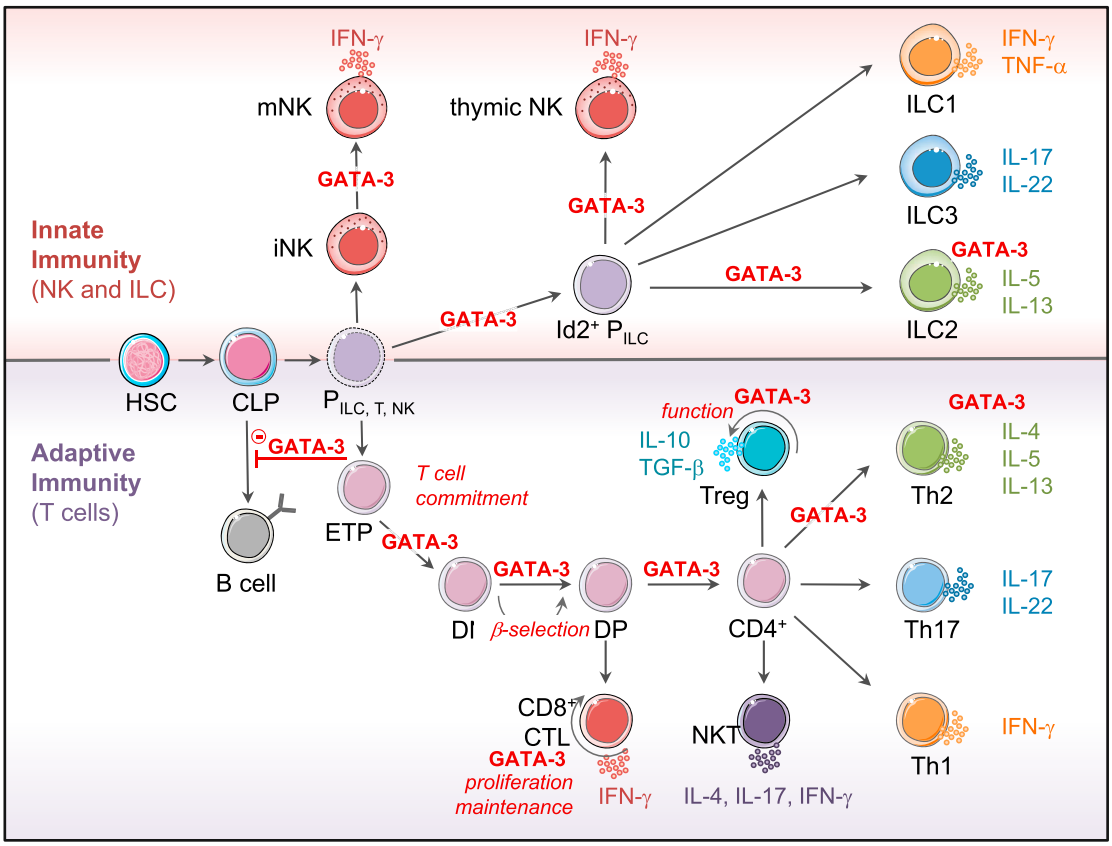

Figure 3. GATA-3 Has Multiple Roles in ILC and T Cell Development and Function

HSC-derived CLPs give rise to both adaptive and innate lymphoid cells. ILC development (top) mirrors T cell development (bottom). Developmental steps or cellular functions that absolutely require GATA-3 are indicated. These include the generation of a common ILC precursor and the differentiation into thymic NK cells and ILC2s, as well as NK cell maturation. GATA-3 represses B cell potential and is crucial in various stages of T cell development. In addition, GATA-3 is important for the function of $CD8^+$ T cells, Treg cells, and Th2 cells, as indicated. T effector cells and ILC subsets are grouped according to their ability to produce different cytokines. Abbreviations are as follows: CLP, common lymphoid progenitor; CTL, cytotoxic T lymphocyte; HSC, hematopoietic stem cell; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; iNK, immature NK cell; mNK, mature NK cell; NK, natural killer cell; NKT, natural killer T cell; P, progenitor; TGF, transforming growth factor; Th, T helper cell; Treg, regulatory T cell.

likely that GATA-3 establishes a positive-feedback loop that increases TCR surface expression in developing CD4-lineage cells (Hernández-Hoyos et al., 2003; Ling et al., 2007). Accordingly, GATA-3 binds strongly to the *Tcra*, *Tcrb*, *Cd3d*, and *Cd3g* loci and expression of CD3δ and CD3ε mRNA is decreased in GATA-3-deleted $CD69^+$ DP cells. Although it has been suggested that Notch activity directly influences CD4/CD8 lineage commitment, it now seems that DP cells have very low levels of Notch signaling and that Notch does not play a direct role in lineage commitment (Laky and Fowlkes, 2008; Radtke et al., 2013).

Development of $CD8^+$ SP thymocytes is not affected by lack of GATA-3. However, peripheral mature naive $CD8^+$ T cells constitutively express GATA-3, albeit to lower levels than found in $CD4^+$ T cells, and expression is upregulated by TCR activation and cytokine signaling. GATA-3 is important in $CD8^+$ T cells because it controls proliferation by regulating c-Myc, but it is dispensable for IFN-γ production (Figure 3; Wang et al., 2013). GATA-3-deficient $CD8^+$ T cells manifest defective long-term maintenance, which is attributed to lower IL-7R expression. By contrast, GATA-3 expression does not appear to be critical for the response to IL-7 in thymocytes. This is inferred from the finding that in GATA-3-deficient mice, $CD8^+$ SP thymocytes develop normally, although IL-7R signaling is essential for positively selected thymocytes to express the transcription factor Runx3 to specify CD8-lineage choice and promote $CD8^+$ SP differentiation (Park et al., 2010).

Although GATA-3 is required for multiple stages of T cell lineage development (Figure 3), an additional role for GATA-3 in prethymic lympho-hematopoietic progenitor cells is as yet unclear. HSCs express GATA-3 (Ku et al., 2012), although early reports found that absence of GATA-3 did not affect the generation, maintenance, or self-renewal of HSCs in fetal and adult mice (Buza-Vidas et al., 2011; Hosoya et al., 2009; Kanhere et al., 2012). In contrast, it was recently shown that *Gata3* deletion enhances expansion of long-term multipotent HSCs, consistent with a role for GATA-3 as an autonomous regulator of the balance between HSC self-renewal and differentiation (Frelin et al., 2013). Although in the absence of *Gata3* the production of functional definitive HSCs in the embryonic AGM region is impaired, this is largely independent of a cell-intrinsic role of GATA-3 and secondary to abnormalities

in the developing sympathetic nervous system (Fitch et al., 2012).

GATA-3 in ILC Development and Function

Recent work has demonstrated that GATA-3 serves as a central regulator of ILC development and function (Figure 3). ILCs contribute to the first-line immune defense against invading pathogens and have the capacity to promptly produce large amounts of proinflammatory cytokines, prior to the generation of adaptive immunity. ILCs have been categorized into three major groups, based on transcription factor dependency and cytokine production profiles, which strikingly mirror the various T helper cell subsets (Spits et al., 2013). Group 1 ILCs (or ILC1s) consist of NK cells and other IFN-γ-producing innate lymphocytes that express T-bet. ILC1s have been shown to play a major role in the defense against viruses, intracellular bacteria, and protozoa (Klose et al., 2014; Kupz et al., 2013; Orr and Lanier, 2010). ILC2s secrete IL-5 and IL-13 in response to stimulation with the cytokines IL-25, IL-33, or thymic stromal lymphopoietin (TSLP). ILC2s are important in the immune response against helminths but are also associated with allergic airway inflammation and hyperreactivity and maintenance of epithelial barrier integrity during influenza infection (see for review Li and Hendriks, 2013). Group 3 ILCs (ILC3s) include several phenotypically distinct cells that express and require the transcription factor RORγt for their development and for production of IL-17A and IL-22. ILC3s are enriched at mucosal sites and appear to regulate barrier function and epithelial cell homeostasis (Walker et al., 2013).

ILC1s

Several transcription factors drive bone-marrow- and tissue-resident NK cell development from lymphoid precursors, including the T-box factors T-bet (encoded by *Tbx21*) and eomesodermin (Eomes), nuclear-factor interleukin-3 related (Nfil3), and GATA-3 (reviewed in Vosshenrich and Di Santo, 2013). *Gata3* ablation affects the development of mature $CD11b^+$ splenic NK cells and reduces their capacity to produce IFN-γ (Samson et al., 2003). In the thymus, GATA-3 deletion ablates the generation of IL-$7R\alpha^+$ NK cells (Vosshenrich et al., 2006) and more recent results show that GATA-3 is also critical for the development of a particular subset of $CD49a^+Eomes^-$ $NKp46^+NK1.1^+$ ILC1s in the gut (Klose et al., 2014) but not in the liver (Sojka et al., 2014). This differential impact of GATA-3 deletion on diverse NK cell subsets suggests distinct developmental pathways for conventional and tissue-resident NK cells (Figure 3; Daussy et al., 2014; Di Santo and Vosshenrich, 2006; Klose et al., 2014). Consistent with this hypothesis, a bone marrow $PLFZ^+GATA\text{-}3^+$ ILC precursor was described that can develop into $CD49a^+$ hepatic but not conventional NK cells (Constantinides et al., 2014). The signals that generate these apparently distinct ILC precursors from CLP and their capacity to promote NK cell development in the bone-marrow- versus tissue-specific sites remains an area of active research.

Recent work has demonstrated that other IFN-γ-producing $NK1.1^+$ cells are present in mucosal sites in humans (Bernink et al., 2013) and in mice (Fuchs et al., 2013; Klose et al., 2014). These ILC1 subsets require the transcription factors T-bet, Nfil3, and GATA-3 for their generation and are phenotypically distinct from conventional NK cells that express NKp46, CD49a, IL-7Rα, and CD27, but not CD11b. These ILC1 subsets seem to be an important source of IFN-γ and TNF-α in both the intestinal epithelial layer and the lamina propria under steady-state conditions and during intestinal inflammation (Fuchs et al., 2013; Klose et al., 2014). The molecular mechanism through which GATA-3 contributes to ILC1 development or whether its maintained expression is needed for functional attributes is unclear.

ILC2s

GATA-3 plays an essential role in ILC2 development in mice (Furusawa et al., 2013; Hoyler et al., 2012; Klein Wolterink et al., 2013; Yagi et al., 2014) and man (Mjösberg et al., 2012) (Figure 3). ILC2s are generated from CLPs in vivo (Wong et al., 2012; Yang et al., 2011) and alterations in *Gata3* gene dosage positively correlates with ILC2 development from CLPs in vitro (Klein Wolterink et al., 2013), suggesting that GATA-3 transcriptional activity is a major determinant of ILC2 cell fate in uncommitted lymphoid precursors. Accordingly, bone marrow and lung ILC2 homeostasis in naive mice correlates in vivo with *Gata3* gene copy number (Klein Wolterink et al., 2013). The transition from CLP to ILC2 is associated with upregulation of the transcription factors inhibitor of DNA-binding 2 (Id2) and RORα, both of which are essential for ILC2 differentiation (Halim et al., 2012; Wong et al., 2012). Although the gene encoding Id2, which is involved in the development of all known ILC lineages (Moro et al., 2010; Yokota et al., 1999), is occupied by GATA-3 in early thymocyte precursors (Zhang et al., 2012), inactivation of GATA-3 in mature ILC2s did not affect the expression of Id2 or RORα (Yagi et al., 2014). In vitro generation of ILC2s from CLPs is dependent on Notch signaling (Wong et al., 2012; Yang et al., 2011), although it remains to be demonstrated whether ILC2 development in vivo requires canonical Notch signaling. Nevertheless, certain parallels between ILC2 development and early T cells are striking: Notch1, GATA-3, and Tcf1 are critical for ILC2 and T cell development (Yang et al., 2013), with Tcf1 being a Notch1-induced target for T cell specification (Weber et al., 2011) that probably serves a similar role in ILC2 generation.

In mature ILC2s, GATA-3 controls cell activation and function, including IL-5 and IL-13 cytokine secretion, cytokine responsiveness (IL-25 and IL-33 receptors), and production of amphiregulin, an epidermal growth factor family protein essential for airway epithelium integrity (Hoyler et al., 2012; Klein Wolterink et al., 2013; Liang et al., 2012; Monticelli et al., 2011; Yagi et al., 2014). The finding that ILC2 effector function directly correlates with *Gata3* gene expression suggests that GATA-3 modulation impacts pathological conditions that involve dysregulation of type II immunity. This notion gains support from genome-wide association studies showing a significant association of the *Il33* and *Il1Rl1* (encoding the IL-33R subunit T1ST2) loci with asthma in human and the increased susceptibility to allergic airway inflammation observed in mice with enforced expression of GATA-3 in T cells and ILC2s (KleinJan et al., 2014). In mouse models of allergic lung inflammation, ILC2s along with classical Th2 cells are major producers of IL-5 and IL-13 that orchestrate and amplify allergic inflammatory events in asthma (Klein Wolterink et al., 2012). Moreover, ILC2-derived IL-13 can promote migration of activated lung DCs that drive differentiation of naive T cells into Th2 cells (Halim et al., 2014).

Thus, GATA-3 plays a crucial role in the induction of IL-5 and IL-13 cytokine production both in ILC2s and in Th2 cells, which synergize in type II immunity and are activated through different mechanisms and with different kinetics.

ILC3s

ILC3s are a heterogeneous population and include CCR6$^+$ lymphoid tissue inducer (LTi) cells that are needed for lymphoid tissue organogenesis in lymph nodes and Peyer's patches during fetal life and for postnatal formation of intestinal lymphoid clusters (van de Pavert et al., 2014). Moreover, adult CD4$^+$ ILC3s that can be found in secondary lymphoid tissues and in mucosal sites produce IL-17A and IL-22 that can contribute to immune defense (Takatori et al., 2009). Another subset of ILC3s that express the NK cell receptor NKp46 is noncytotoxic and produces IL-22 but not IFN-γ (Cella et al., 2009; Sanos et al., 2009; Satoh-Takayama et al., 2008). NKp46$^+$ ILC3s are CCR6$^-$, are found primarily in the intestinal lamina propria, and cross-talk with epithelial cells to stimulate cell proliferation and production of antimicrobial peptides that regulate communities of commensal bacteria (Satoh-Takayama et al., 2008; Sonnenberg et al., 2012). Heterogeneous CD4$^-$NKp46$^-$ "double-negative" ILC3s have also been described and produce IL-17A, IL-22, IFN-γ, and TNF-α and are involved in intestinal inflammation (Buonocore et al., 2010; Klose et al., 2013; Sawa et al., 2010). Both NKp46$^+$ and a subset of double-negative ILC3s express the transcription factor T-bet, which is critical for their development (Klose et al., 2013; Rankin et al., 2013; Sciumé et al., 2012).

More recently, GATA-3 was shown to be crucial for development of both LTi cells and T-bet$^+$ ILC3s (Serafini et al., 2014; Yagi et al., 2014). ILC3s express abundant GATA-3 protein, albeit in lower amounts than observed in mature ILC2s. Hematopoietic chimeric mice derived from GATA-3-deficient fetal liver cells failed to generate intestinal ILC3 subsets and showed defective IL-22 production and maintenance of mucosal barrier homeostasis upon infection (Serafini et al., 2014). Conditional *Gata3* ablation using Vav1-Cre generates a similar defect in ILC3 development (Yagi et al., 2014). Moreover, in the fetus GATA-3 is critical for differentiation of CD135$^+$α4β7$^+$ CLP-like cells and cell-intrinsic GATA-3 expression is essential to generate fetal liver RORγt^{hi}IL-7Rα^{hi} precursor cells (Serafini et al., 2014). Although the GATA-3-dependent transcriptional pathways that drive ILC3 development remain unclear, RNA-seq analyses revealed that GATA-3 ablation does not modify *Rorc*, *Runx1*, *Runx3*, *AhR*, *Id2*, or *Tcf7* expression in mature ILC3s (Yagi et al., 2014). This result probably reflects the context-dependent role for GATA-3 in early ILC development that is not recapitulated in mature ILC3s, because previous reports demonstrate that GATA-3 expression in mature Id2$^+$ ILC3 is not essential for their homeostasis (Hoyler et al., 2012).

GATA-3 as a Central Regulator of ILC Development

The observation that GATA-3 ablation severely affects development of several distinct ILC subsets (ILC2s, ILC3s, intestinal CD49a$^+$NK1.1$^+$ ILC1s, thymic NK cells) suggests that GATA-3 could operate at an early stage of ILC differentiation and perhaps via the generation of common ILC precursors (Figure 3). In the fetus, a subset of α4β7$^+$ fetal liver precursor (Lin$^-$IL-7Rα$^+$Sca-1^{int}c-Kit^{lo}) cells have been described that fail to give rise to B and T cells but retain NK cell and ILC3 potential (Sawa et al., 2010; Yoshida et al., 2001). A similar α4β7$^+$Lin$^-$IL-7Rα$^+$ bone marrow subset exists, although it includes more mature ILC2s (Hoyler et al., 2012; Klein Wolterink et al., 2013). Recently, two reports identified that this α4β7$^+$ subset contains committed ILC progenitors (Constantinides et al., 2014; Klose et al., 2014). Both groups used GFP reporter mice (in either the *Zbtb16* [PLZF] or *Id2* loci) to show that putative fetal and adult BM ILC precursors could give rise to ILC1–ILC3 subsets in vivo and in vitro at the single-cell level. These ILC precursors gave rise to ILC2s, ILC3s (especially NKp46$^+$ ILC3s), and the peculiar CD49a$^+$NKp46$^+$ ILC1 subset that has been identified in the liver and gut. Interestingly, these ILC precursors had reduced capacity to generate conventional NK cells. As such, these ILC precursors had the developmental potential for the same ILC subsets that require GATA-3 for their development (Constantinides et al., 2014; Klose et al., 2013; Rankin et al., 2013; Samson et al., 2003; Serafini et al., 2014). Accordingly, one group found that PLZF$^+$α4β7$^+$ cells coexpressed GATA-3 protein, suggesting a link between GATA-3 expression in these ILC precursors and their cell fate potential (Constantinides et al., 2014).

Because CLPs are GATA-3$^-$Id2$^-$ and PLZF$^-$ (Constantinides et al., 2014; Klein Wolterink et al., 2013), identifying the signals that allow for the emergence of Id2$^+$PLZF$^+$GATA-3$^+$ ILC precursors from CLPs will be of considerable interest. Soluble factors (such as IL-7) and cell-intrinsic transcription factors (including Notch1, Tox, and Runx1) are important for the normal development of distinct ILC subsets (Aliahmad et al., 2010; Cherrier et al., 2012; Eberl et al., 2004; Lee et al., 2012; Rankin et al., 2013; Tachibana et al., 2011). One possibility is that GATA-3 upregulation is an early event in CLPs that effectively restricts B lineage fate and thereby generates T cell and ILC precursors (Figure 3). Such "bipotent" precursors would then further differentiate to more restricted T cell lineage precursors (ETP) or ILC precursors; upregulation of Id2 would be a dominant factor in promoting the development of the latter. This model is consistent with the existing data and would clearly distinguish the developmental pathway of conventional NK cells (GATA-3 independent) from other ILC subsets (GATA-3 dependent).

Although GATA-3 function in ILC precursors and ETP may be partly overlapping, e.g., to repress B cell potential, it is conceivable that collaboration of GATA-3 with other transcription factors, such as RBPjκ, Id2, Tcf1, Nfil3, T-bet, or RORγ, may enforce differentiation into the distinct ILC subsets or the T cell lineage.

GATA-3 Cofactors and Target Genes

GATA-3 and Its Interacting Partners

Because GATA factors are expressed in a variety of cell types and can act as transcriptional activators or repressors, it was expected that their functional outcome would depend on their interactions with other transcriptional regulators. Indeed, using a biotinylation tagging/proteomics approach in erythroid cells, the association of specific interacting partners were linked to activating versus repressive functions of GATA-1 (Rodriguez et al., 2005). Likewise, it was recently shown that GATA-3 and chromodomain helicase DNA binding protein 4 (Chd4) complex form functionally distinct activating and repressive assemblies with histone acetyltransferase (HAT) and histone deacetylase

(HDAC) activity, respectively (Hosokawa et al., 2013). Many other proteins are known to interact with GATA-3 (Table 1). Genome-wide identification of GATA-3 binding sites in thymocytes and various effector T cell populations using ChIP-seq technology showed that in addition to the primary 5′-(A/T)GATA(A/G)-3′motif, they contained several secondary motifs, including binding sites for the Ets, Runx, AP1, and TCF11 transcription factors, or even contained only secondary motifs (Wei et al., 2011). Therefore, GATA-3 can be recruited through physical interactions with another transcription factor or protein interactions may stabilize binding of GATA-3 to noncanonical target sequences.

T-bet represses Th2 cell lineage commitment through a physical interaction with GATA-3 that is enhanced by T-bet tyrosine phosphorylation and interferes with GATA-3 binding to its target DNA (Hwang et al., 2005). However, recent genome-wide comparison of T-bet and GATA-3 binding sites revealed that many of the Th1-cell-specific GATA-3 binding sites overlapped with T-bet binding motifs (Jenner et al., 2009; Kanhere et al., 2012). As in Th1 cells, GATA-3 binds to T-box motifs and not to cognate GATA-3 sites, and GATA-3 occupancy is mediated through association with T-bet and T-box motifs (Kanhere et al., 2012). On the other hand, the T-box factor Eomes interacts with GATA-3 and suppresses the transcriptional activity of GATA-3 (and IL-5 expression) in memory T cells (Endo et al., 2011). Interaction of GATA-3 and the transcription factor Runx3 actively represses production of IFN-γ in Th1 cells. These examples demonstrate how GATA-3 targets repression and can be the target of repression.

FoxP3 forms a complex with GATA-3 specifically in activated Treg cells. Under inflammatory settings, GATA-3 limits RORγt expression and thus restrains excessive polarization and inflammatory cytokine production by Treg cells (Rudra et al., 2012; Wohlfert et al., 2011). GATA-3 is induced upon TCR and IL-2 stimulation and is required for the accumulation of Treg cells at inflamed sites. In Treg cells, GATA-3 specifically binds to regulatory elements of the *Foxp3* locus and thereby directly controls FoxP3 expression (Wang et al., 2011). Conversely, FoxP3 binds to the promoter and intronic regions of the *Gata3* locus, and thus FoxP3 and GATA-3 proteins not only physically interact, but also reciprocally control each other's expression. Because FoxP3-GATA-3 complex formation is dependent on TCR stimulation, GATA-3$^+$ Treg cells are subject to immune control in response to environmental changes.

YY1 occupies regulatory elements in the Th2 locus and is required for subsequent GATA-3 binding (Hwang et al., 2013). Thus, cooperation of YY1 with GATA-3 is essential for regulation of the Th2 cytokine locus and Th2 cell differentiation (Table 1; Figure 2). The Ets family member Fli1 binds to ~70% of all GATA-3-bound sites in Th2 cells (Wei et al., 2011). Upon *Gata3* deletion, Fli1 occupancy at the majority of shared GATA-3 and Fli1 binding sites is lost (including binding to the *IL13* and *Rad50* loci), indicating mutualistic binding of these two factors.

The differential complex formation provides a mechanism by which GATA-3 can be directed to a distinct subset of its potential binding sites in a cell-type-specific fashion and can help explain GATA-3 function as a transcriptional activator or repressor. Moreover, context-dependent cooperative binding of GATA-3 with different transcription factors dramatically increases the regulatory complexity. Indeed, even closely related T cell subsets (e.g., DN2 and DP thymocytes or Th1 and Th17 effector cells) exhibited different GATA-3 binding patterns, despite nearly identical amounts of total GATA-3 protein present (Wei et al., 2011; Zhang et al., 2012).

GATA-3 Target Genes

To identify GATA-3 target genes genome-wide, GATA binding sites have been identified by ChIP-seq and changes in gene expression upon *Gata3* deletion were evaluated in various thymocyte and mature T cell subsets (Kanhere et al., 2012; Wei et al., 2011; Zhang et al., 2012). In these studies, >7,000 and >14,000 GATA-3 binding sites were identified genome-wide in murine and human Th2 cells, respectively. Although GATA-3 binding was enriched in gene regions just upstream of transcription start sites and 5′ UTR, a majority of binding sites was >2 kb distal to known gene promoters. These distal sites were frequently at conserved sequences, coinciding with DHS, and enriched for both activating H3K4 methylation marks, indicative for *cis*-regulatory elements (Kanhere et al., 2012). Gene ontology analysis revealed that genes harboring distal GATA-3 binding sites—but not genes bound by GATA-3 only at their promoter region—were enriched for immune response functions. Upon deletion of *Gata3*, only a minority of GATA-3 bound genes (<10%) showed a significant increase or decrease in expression, but ~46% of its bound genes manifested significant changes in H3K4 or H3K27 histone methylation marks. Therefore, the observed epigenetic modifications are most likely regulated by GATA-3 activity and are not a consequence of transcriptional activation or silencing.

In addition to GATA-3 targets in the Th2 locus, these genome-wide studies identified several other complex loci controlled by GATA-3 in Th2 cells. These include the ~145 kb cytokine cluster containing the *Il10* gene and the homologs *Il19*, *Il20*, and *Il24* (Jones and Flavell, 2005). Consequently, *Gata3* deletion resulted in significantly decreased expression of IL-10 and IL-24. Furthermore, the large genomic regions encompassing the chemokine receptor genes (*CCR9*, *CXCR6*, *XCR1*, *CCR1*, *CCR3*, *CCR2*, *CCR5*, *CCRL2*) or the *Il1r1*, *Il1rl2*, *Il1l1* (encoding IL-33R or T1ST2), *Il18r1*, and *Il18rap* genes contains many GATA-binding sites, whereby GATA-3 binds to sites in the *Il18r1* gene in both Th1, Th2, Th17, and iTreg cells (Kanhere et al., 2012; Wei et al., 2011). As described above, expression of IL-33R is crucial for ILC2 activation and enforced expression of GATA-3 in transgenic mice induced the formation of Th2 memory cells expressing high amounts of IL-33R (Nawijn et al., 2001a).

To date, the identification of GATA-3 targets in the various mature ILC subsets and in developing ILCs is hampered not only by the low cell numbers of the individual ILC subsets and their precursors, but also by the fact that ILC precursors are still poorly defined. Nevertheless, comparison of genes expressed in Th2 cells and mature ILC2s showed that only 55 genes were positively or negatively regulated by GATA-3 both in Th2 cells and ILC2s, including *Il5*, *Il13*, *Areg* (encoding amphiregulin), and *Il1rl1* (Yagi et al., 2014). In contrast, 281 unique targets were regulated by GATA-3 in ILC2s (e.g., *Icos*, *Il2ra*, and *Kit*), and 568 targets were regulated in Th2 cells (e.g., *Il4* and *Maf*). Although these may represent both direct and indirect targets of GATA-3, these findings indicate that GATA-3 has mostly unique functions in these two functionally related cell types. In

a similar fashion, less than 4% of genes regulated by GATA-3 in ILC2s were also regulated by GATA-3 in ILC3s.

Concluding Remarks

More than two decades of research on GATA-3 biology has demonstrated that within the hematopoietic system, GATA-3 has multiple and diverse roles that are mediated in a complex, dose-dependent, developmental-stage-specific, and cell-lineage-specific fashion. Context-dependent activating or repressive functions of GATA-3 are provided by differential cooperative binding of GATA-3 with several different transcription factors, whereby related T cell or ILC subsets exhibit very different genome-wide GATA-3 occupancy.

Highly sensitive approaches to examine genome-wide GATA-3 binding sites in small populations of ILC subsets and their precursors may help to elucidate the critical GATA-3-dependent developmental pathways from CLPs to individual lymphocyte precursors. Moreover, such analyses should identify crucial GATA-3 targets as well as the complex relationships between GATA-3, Notch signaling, and key transcription factors such as Id2, RORα, RORγ, Tcf1, and Nfil3 in lymphocyte cell-fate decisions.

Parallels exist between ILC in the bone marrow and T cell development in the thymus, as they both require—in addition to GATA-3—similar transcription factors (e.g., Tcf-1) and Notch signaling. Obviously, instructive signals from the microenvironment such as cytokines, Notch ligands, or Wnt signaling are different between bone marrow and thymus, but also ETP and ILC precursors will have different intrinsic developmental capacities. Therefore, future experiments should show common and unique GATA-3 targets and their epigenetic configurations in ILC precursors and ETP.

In mice, GATA-3 functions in a dose-dependent fashion in both ILC2s and T cells (Klein Wolterink et al., 2013; KleinJan et al., 2014; Nawijn et al., 2001b). Human GATA-3 haploinsufficiency affects T cell function, but its effects on ILC subsets are not known. Enforced expression of GATA-3 during T cell development induces DP T cell lymphoma, whereby malignant transformation involves cooperation with c-Myc and the induction of activating Notch1 mutations (van Hamburg et al., 2008). Likewise, recent genome-wide germline SNP analysis identified *GATA3* gene variants that influence susceptibility to Philadelphia chromosome-positive acute lymphoblastic leukemia and risk of relapse (Perez-Andreu et al., 2013). It will be important to understand how dysregulated GATA-3 influences neoplastic transformation in hematopoietic cells and nonhematopoietic lineages (Chou et al., 2010).

Although to date the role of GATA-3 in the regulation of Th2 cytokine expression is known in molecular detail, one of the unresolved questions that remains is how signals from the microenvironment cooperate to induce GATA-3 expression in activated T cells. Next to TCR, IL-4R, and Notch signaling, very recent studies indicate that also nucleic acids (NA) released from dead cells and complexes with antimicrobial peptides or histones can upregulate GATA-3 expression, independently of known NA sensors (Imanishi et al., 2014). GATA-3 expression is sufficient and required for development and function of Th2 cells and ILC2s that play a central role in allergic inflammation. Therefore, inhibiting GATA-3 function, e.g., by inhibition of its translocation to the nucleus (Maneechotesuwan et al., 2009), could be an excellent starting point for drug discovery strategies.

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