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The Transcription Factor GATA3 Is Critical for the Development of All IL-7Rα-Expressing Innate Lymphoid Cells

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SUMMARY

Innate lymphoid cells (ILCs) are critical in innate immune responses to pathogens and lymphoid organ development. Similar to CD4$^+$ T helper (Th) cell subsets, ILC subsets positive for interleukin-7 receptor α (IL-7Rα) produce distinct sets of effector cytokines. However, the molecular control of IL-7Rα$^+$ ILC development and maintenance is unclear. Here, we report that GATA3 was indispensable for the development of all IL-7Rα$^+$ ILC subsets and T cells but was not required for the development of classical natural killer cells. Conditionally *Gata3*-deficient mice had no lymph nodes and were susceptible to *Citrobactor rodentium* infection. After the ILCs had fully developed, GATA3 remained important for the maintenance and functions of ILC2s. Genome-wide gene expression analyses indicated that GATA3 regulated a similar set of cytokines and receptors in Th2 cells and ILC2s, but not in ILC3s. Thus, GATA3 plays parallel roles in regulating the development and functions of CD4$^+$ T cells and IL-7Rα$^+$ ILCs.

INTRODUCTION

CD4$^+$ T helper (Th) cells are central in orchestrating adaptive immune responses; distinct Th cell subsets are involved in protective immune responses to a variety of pathogens (Kanno et al., 2012; Zhu et al., 2010). For example, type 1 T helper (Th1) cells are critical for eradicating intracellular bacteria and viruses, whereas type 2 Th (Th2) cells are indispensable for the expulsion of helminths. Interleukin-17 (IL-17)-producing Th cells (also known as Th17 cells) are critical for defending against extracellular bacterial and fungal infections.

It usually takes several (5–10) days for antigen-specific CD4$^+$ T cells to expand from rare precursors in the naive population and reach a meaningful number to execute host defense functions. Therefore, many innate effector cells, including natural killer (NK) cells, are responsible for early control of invading pathogens. Recently, a new class of innate effector cells whose development relies on signaling through the IL-2 receptor (IL-2R) common γ chain and IL-7Rα has drawn much attention. These cells, together with classical NK cells, are often referred to as innate lymphoid cells (ILCs) (Sonnenberg and Artis, 2012; Spits and Cupedo, 2012; Spits and Di Santo, 2011). Because distinct subsets of ILCs are capable of making the same characteristic effector cytokines as produced by different Th cell subsets, they are similarly classified into type 1 ILCs (ILC1s), including classical NK cells that produce interferon-γ (IFN-γ), type 2 ILCs (ILC2s), which produce IL-5 and IL-13, and type 3 ILCs (ILC3s), including lymphoid tissue inducer (LTi) cells, which produce IL-17 and IL-22 (Spits et al., 2013; Walker et al., 2013).

By producing Th2 cell effector cytokines such as IL-13, ILC2s play an important role during early immune responses to helminth infection (Fallon et al., 2006; Moro et al., 2010; Neill et al., 2010; Price et al., 2010; Saenz et al., 2010). Mice with dysfunctional ILC2s have a significant delay in worm expulsion in *Nippostrongylus brasiliensis* infection, whereas expanding the number of ILC2s by IL-25 injection can eliminate the need for Th2 cells in effective resistance to helminth infection. ILC2s are also important for allergen-induced airway inflammation and lung-tissue repair in animal models (Chang et al., 2011; Halim et al., 2012a; Monticelli et al., 2011), and human cells corresponding to the ILC2s found in mice have been identified (Mjösberg et al., 2011). The ILCs that produce IL-17 and IL-22 also participate in the early phase of responses to infections and in inflammatory disorders (Buonocore et al., 2010; Lee et al., 2012; Powell et al., 2012; Satoh-Takayama et al., 2008). Thus, understanding the molecular mechanisms controlling the development and functions of ILCs is essential to developing strategies to control responses to pathogens and autoimmunity.

GATA3 is the key transcription factor for Th2 cell differentiation (Yagi et al., 2011). GATA3 expression is indispensable for proper induction of Th2 cytokines, including IL-4, IL-5, and IL-13, both in vitro and in vivo (Zhu et al., 2004). Interestingly, GATA3 is critical not only for regulating Th2 cell differentiation but also for

CD4$^+$ T cell development in the thymus at multiple stages (Ho et al., 2009; Pai et al., 2003; Ting et al., 1996).

It has been reported that GATA3 is highly expressed by ILC2 cells (Moro et al., 2010; Price et al., 2010). Conditional inactivation of *Gata3* with a transgenic Cre whose expression is driven by the *Il13* locus completely eliminated IL-13-producing ILC2 cells (Liang et al., 2012). GATA3 has been shown to be critical for the maintenance of ILC2 number and IL-13 production by these cells both in mice and in humans (Furusawa et al., 2013; Hoyler et al., 2012; Klein Wolterink et al., 2013; Mjösberg et al., 2012; Yang et al., 2013). However, because GATA3 affects ILC2 number, IL-13 regulation by GATA3 in the previous studies requires more careful assessment, i.e., at a single-cell level with proper controls. Furthermore, other important target genes that are regulated by GATA3 in ILC2s are largely unknown. Finally, whether other ILC subsets require GATA3 to develop remains unclear.

Here, we report that GATA3 is not only critical for T cell development but also indispensable for the development of all the IL-7Rα$^+$ ILC lineages, including ILC2s, LTi cells, IL-7Rα-expressing NK cells, Nkp46$^+$RORγt$^+$ ILCs, and Nkp46$^+$RORγt$^-$ ILCs. Both ILC2 progenitors and LTi progenitors were diminished in the absence of GATA3. Genome-wide analysis of GATA3-regulated genes in ILC2s and ILC3s suggested that GATA3 function during ILC development is independent of many known key transcription factors, including Id2, RORα, RORγt, and Tcf7. GATA3 regulated many critical genes, including *Il5*, *Il13*, *Il1rl1*, *Il2ra*, *Il9r*, and *Ccr8*, but not *Klrg1*, in ILC2s. Comparing GATA3-regulated genes in Th2 cells, we found that although many genes, such as *Pth*, *Cysltr1*, *Htr1b*, and *Tph1*, were regulated by GATA3 in a cell-type-specific manner, most of the key type-2-immune-response-related genes were regulated by GATA3 in both ILC2s and Th2 cells. These results demonstrate that GATA3 plays parallel roles in regulating the development and functions of CD4$^+$ T cells and IL-7Rα$^+$ ILCs, i.e., the development of both CD4$^+$ T cells and all IL-7Rα$^+$ ILCs requires GATA3, and GATA3 is especially critical for the maintenance and function of ILC2s, similarly to in Th2 cells.

RESULTS

All ILCs Express GATA3 at Different Amounts

To determine the tissue distribution of ILCs (particularly ILC2s), we assessed lineage-negative (Lin$^-$) CD127$^+$ (IL-7Rα$^+$) cells by flow cytometry analysis of many lymphoid tissues and organs. Lin$^-$CD127$^+$ cells represented <1% of the total cells in the spleen, mesenteric lymph nodes, and the lung but were somewhat enriched in the small intestinal lamina propria (siLP, Figure S1A, available online). Some of these ILCs were ILC2s, identified by expression of T1/ST2 (IL-33R) (Moro et al., 2010; Neill et al., 2010). ILC2s (T1/ST2$^+$Lin$^-$CD127$^+$ cells) constituted the largest fraction of recovered ILCs in the lung-derived cells (>80% of total Lin$^-$CD127$^+$ cells, Figure S1B). In contrast, only ~16% of Lin$^-$CD127$^+$ cells in the spleen were T1/ST2$^+$. In the mesenteric lymph nodes, ~40% of the Lin$^-$CD127$^+$ cells were T1/ST2$^+$.

To assess GATA3 expression in the various subsets of ILCs, we performed ex vivo GATA3 intracellular staining of Lin$^-$CD127$^+$ cells harvested from different tissues. Most ILC2s expressed high levels of GATA3 whether they were from the spleen, mesenteric lymph nodes, lung, or siLP (Figure S1B). Interestingly, other ILCs (T1/ST2$^-$Lin$^-$CD127$^+$ cells) expressed intermediate amounts of GATA3. In the siLP, most of the Lin$^-$CD127$^+$GATA3^{int} cells were RORγt$^+$ ILCs, and all Lin$^-$CD127$^+$GATA3^{hi} cells expressed another ILC2 marker (KLRG1), but not RORγt (Figure S1C). Thus, all IL-7Rα ILCs expressed GATA3, albeit at different amounts.

GATA3 Is Indispensable for the Development of All IL-7Rα$^+$ ILCs

To study the role of GATA3 in ILC development in vivo, we generated *Gata3*$^{fl/fl}$-Vav-Cre mice in which *Gata3* exon 4 was deleted in hematopoietic stem cells. As expected, no T cells were detected in *Gata3*$^{fl/fl}$-Vav-Cre mice (Figure 1A), confirming a previous report on the critical role of GATA3 in T cell development as determined by germline *Gata3* deletion (Ting et al., 1996). In contrast, there was a modest but significant increase in B cells in *Gata3*$^{fl/fl}$-Vav-Cre mice (Figure 1A), arguing against a loss of stem cell function. In line with a previous report showing that GATA3 is critical for the development of IL-7Rα$^+$ NK cells (Vosshenrich et al., 2006), IL-7Rα$^+$NK1.1$^+$ cells, including CD3ε$^-$IL-7Rα$^+$NK1.1$^+$ cells, were absent in the spleen of *Gata3*$^{fl/fl}$-Vav-Cre mice, whereas classical IL-7Rα$^-$NK1.1$^+$ cells were not significantly affected (Figure 1A). Staining of cells from the lung of *Gata3*$^{fl/fl}$-Vav-Cre mice showed complete absence of ILC2s (Figure 1B). In the bone marrow of wild-type mice, T1/ST2$^+$ cells were detectable among the Lin$^-$CD127$^+$ population; these cells have been regarded as the progenitors of ILC2s and they were all GATA3$^+$ (Figure 1C). However, this population was absent in the bone marrow of *Gata3*$^{fl/fl}$-Vav-Cre mice (Figure 1C). Overall, our data indicate that GATA3 is indispensable for the development of ILC2s and T cells, but not B cells or IL-7Rα$^-$ NK cells.

We also noticed a defect in lymphoid organ development in the *Gata3*$^{fl/fl}$-Vav-Cre mice; similar to RORγt-deficient mice, these mice had no lymph nodes, including the brachial and axillary lymph nodes. This finding suggests that GATA3 regulates the development of LTi cells, which are Lin$^-$CD4$^+$RORγt$^+$ and critical for the development of lymph node structure (Eberl et al., 2004; Sun et al., 2000). Indeed, Lin$^-$CD4$^+$RORγt$^+$ LTi cells, which were found with a high frequency in the siLP of *Gata3*$^{fl/fl}$ mice, were markedly reduced in the *Gata3*$^{fl/fl}$-Vav-Cre mice (Figure 2A). Furthermore, total Lin$^-$RORγt$^+$ cells were reduced in the siLP of *Gata3*$^{fl/fl}$-Vav-Cre mice (Figure 2B). In fact, IL-7Rα$^+$ cells, whether they expressed RORγt or not, were abolished in *Gata3*$^{fl/fl}$-Vav-Cre mice, suggesting that GATA3 is critical for the development of all IL-7Rα-expressing ILCs. The loss of RORγt$^+$ ILCs was not due to a possible Cre-mediated genotoxicity given that all the *Gata3*$^{fl/+}$-Vav-Cre mice had normal lymph node structure and developed RORγt$^+$ ILCs normally (Figure S2).

LTi progenitors have been identified in the mouse fetal liver (Cherrier et al., 2012; Possot et al., 2011). To assess the function of GATA3 in the development of LTi progenitors, we stained fetal liver cells from *Gata3*$^{fl/fl}$-Vav-Cre embryos. Lin$^-$CD127$^+$ cells were present at a similar frequency and total cell number between the *Gata3*$^{fl/fl}$ and *Gata3*$^{fl/fl}$-Vav-Cre fetal livers (Figure 2C). However, Flt3$^-$α4β7$^+$CD127^{hi} LTi progenitors were greatly diminished in *Gata3*$^{fl/fl}$-Vav-Cre fetal livers, suggesting that

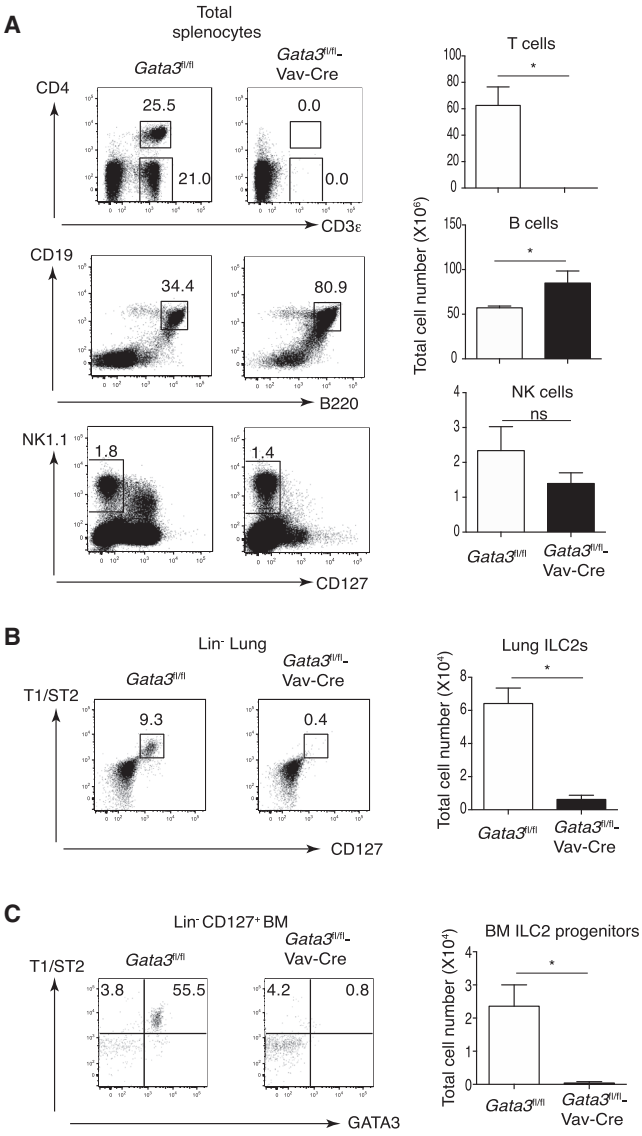

Figure 1. GATA3 Is Critical for the Development of T Cells and ILC2s, but Not B Cells and Classical NK Cells

(A) Total splenocytes from *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained for CD3ε, CD4, CD19, B220, NK1.1, and CD127. Dot plots (left panel) and total numbers of T, B, and NK cells (right panel) are shown.

(B) Cells prepared from the lungs of *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained with a cocktail of antibodies to CD127, T1/ST2, and various lineage markers. Dot plots gated on Lin$^-$ cells (left panel) and total numbers of lung ILC2s (right panel) are shown.

(C) Cells prepared from the bone marrow of *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained with a cocktail of antibodies to CD127, T1/ST2, GATA3, and various lineage markers. Dot plots gated on Lin$^-$ (left panel) and total numbers of ILC2 progenitors in the bone marrow (right panel) are shown.

Numbers indicate the percentages in each box or quadrant. Error bars represent the mean ± SD. ns, not significant. *$p < 0.05$. Data are representative of three independent experiments. See also Figure S1.

GATA3 is critical for the development of such cells. Together with the finding that GATA3 is required for the generation of ILC2 progenitors in bone marrow (Figure 1C), our data suggest that GATA3 might act in a common innate lymphoid progenitor (CILP) that gives rise to all IL-7Rα-expressing ILCs.

IL-7Rα$^-$ ILCs found in the compartment of intraepithelial lymphocytes (IELs) have been reported, and these cells express both NK1.1 and Nkp46 (Fuchs et al., 2013). Interestingly, there were significantly more IEL ILCs in *Gata3*$^{fl/fl}$-Vav-Cre mice than in *Gata3*$^{fl/fl}$ mice (Figure 2D), indicating that GATA3 is critical for the development of IL-7Rα$^+$ ILCs, but not IL-7Rα$^-$ ILCs.

Gata3$^{fl/fl}$-Vav-Cre Mice Are Susceptible to *Citrobacter rodentium* Infection

IL-7Rα$^+$ ILCs also include IL-22-producing ILCs, some of which express Nkp46. These cells play a critical role in host defense against *Citrobacter rodentium* infection. Thus, we tested whether *Gata3*$^{fl/fl}$-Vav-Cre mice are susceptible to *Citrobacter rodentium* infection. On day 4 after infection, a substantial number of Lin$^-$IL-7Rα$^+$ cells, many of which expressed Nkp46, were detected in the large intestinal lamina propria (liLP) of littermate mice (Figures 3A and 3B); these cells were absent in the liLP of the *Gata3*$^{fl/fl}$-Vav-Cre mice. Accordingly, *Gata3*$^{fl/fl}$-Vav-Cre mice had much higher bacterial load than their littermates (Figure 3C). All of the *Gata3*$^{fl/fl}$-Vav-Cre mice died after *Citrobacter rodentium* infection, whereas all of the control mice survived (Figure 3D). These data further establish the role of GATA3 in the development of IL-22-producing ILCs.

The Effect of GATA3 on the Development of IL-7Rα$^+$ ILCs Is Cell Intrinsic

Vav-Cre deletes floxed genes specifically in hematopoietic stem cells; therefore, the failure of ILC development in *Gata3*$^{fl/fl}$-Vav-Cre mice is not due to a GATA3 function in nonhematopoietic cells. To further confirm this, we performed mixed bone marrow chimera experiments to directly examine whether the influence of GATA3 on ILC development is cell intrinsic. As expected, GATA3-sufficient and -deficient bone marrow cells efficiently repopulated CD45$^+$ cells in the spleen of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (Figure 4). However, GATA3-deficient bone marrow cells failed to give rise to IL-7Rα$^+$ ILCs in the mixed bone marrow chimeras. In contrast, B cells developed normally, whereas T cells failed to develop from GATA3-deficient bone marrow progenitors. These results indicate that the effect of GATA3 on the development of IL-7Rα$^+$ ILCs is cell intrinsic.

GATA3 Is Required for Maintaining ILC2 Number Both In Vivo and In Vitro

At first consideration, our results contradict an earlier report showing that GATA3 is required for the development of ILC2s, but not other ILCs (Hoyler et al., 2012). However, in that study, *Gata3* was deleted by an inducible Cre driven by the *Id2* locus after all the ILCs had fully developed. Therefore, it is likely that the survival of ILC2s is sensitive to *Gata3* deletion. To test this possibility, we generated *Gata3*$^{fl/fl}$-CreER^{T2} mice and treated them with tamoxifen. Three weeks after treatment, we observed that only KLRG1$^+$ ILC2s, but not RORγt$^+$ ILCs, in the siLP were diminished (Figure 5A), confirming that GATA3 is critical only for the maintenance of ILC2s in vivo.

Because GATA3 is required for the development and maintenance of ILC2s in vivo, it is difficult to assess the function of GATA3 in ILC2s without obtaining a pure ILC2 population.

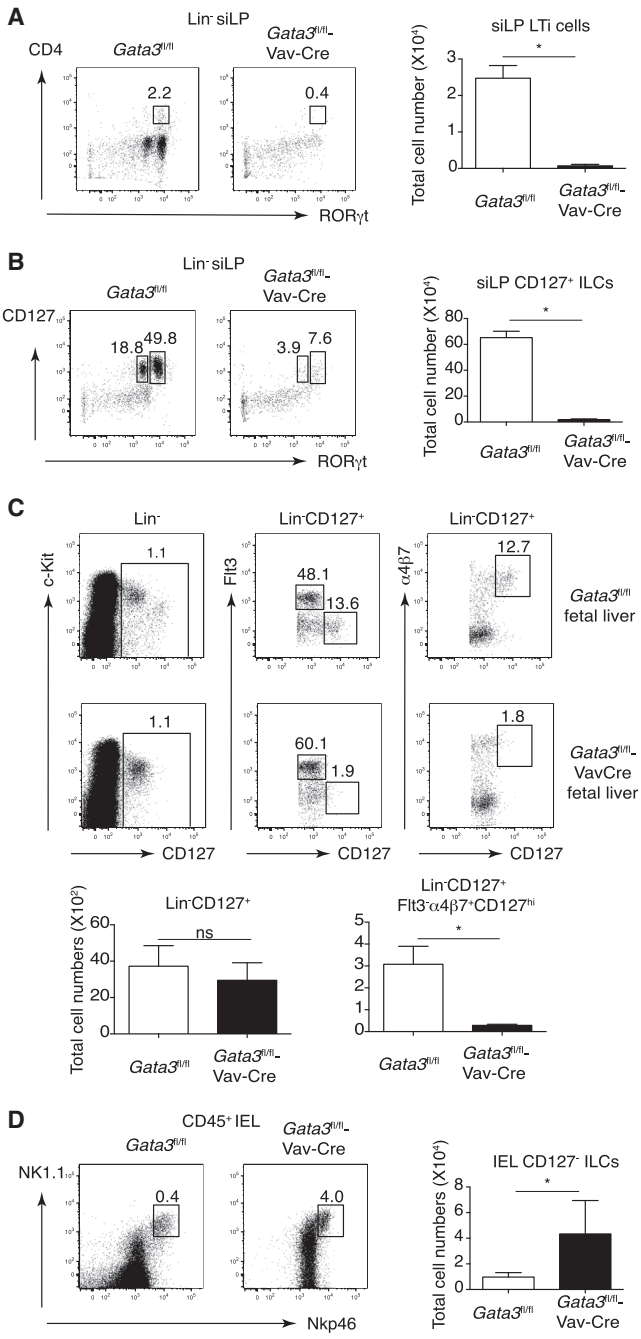

Figure 2. GATA3 Is Critical for the Development of All IL-7Rα⁺ ILCs, but Not IL-7Rα⁻ ILCs

(A) Cells prepared from the siLP of *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained with a cocktail of antibodies to CD4, RORγt, and various lineage markers. Plots gated on Lin$^-$ cells (left panel) and total numbers of LTi cells (right panel) are shown.

(B) Cells prepared from the siLP of *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained with a cocktail of antibodies to CD127, RORγt, and various lineage markers. Plots gated on Lin$^-$ cells (left panel) and total numbers of IL-7Rα$^+$ ILCs (right panel) are shown.

(C) Fetal liver common lymphoid progenitors (CLPs, Lin$^-$CD127$^+$) from *Gata3*$^{fl/fl}$ (n = 6) or *Gata3*$^{fl/fl}$-Vav-Cre (n = 2) embryos at embryonic day 15.5 were fractionated by the expression of Flt3 and α4β7. Plots were gated on Lin$^-$ cells (upper left panels) or Lin$^-$CD127$^+$ cells (upper middle and right panels). Total numbers of CLPs (Lin$^-$CD127$^+$ cells) and LTi progenitors (Lin$^-$CD127$^+$ Flt3$^-$α4β7$^+$CD127^{hi} cells) were calculated (lower panels).

(D) Intraepithelial lymphocytes (IELs) prepared from *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three mice per group) were stained with a cocktail of antibodies to CD45, NK1.1, Nkp46, and various lineage markers. Plots gated on CD45$^+$ cells (left panel) and total numbers of IEL ILCs (right panel) are shown.

Numbers indicate the percentages in each box. Error bars represent the mean ± SD. ns, not significant. *p < 0.05. Data are representative of three (A and B) or two (C and D) independent experiments. See also Figure S2.

ILC2s, although present at a low frequency, expand during type 2 immune responses, presumably as a result of stimulation by IL-25 and/or IL-33, which are produced by epithelial cells. Indeed, injection of IL-25 induced substantial expansion of ILC2s (Neill et al., 2010; Saenz et al., 2013). We also observed that IL-25 injection induced the expansion of ILC2s. Culturing sorted Lin$^-$ cells harvested from mesenteric lymph nodes of IL-25-injected mice with IL-33, IL-25, and IL-7 in vitro further expanded these cells. After 5–7 days of culture of purified Lin$^-$ cells, virtually all cells were GATA3^{hi} (Figure S3A). These cells also expressed two cell-surface molecules, Sca-1 and KLRG1, which are highly expressed by ILC2s (Figures S3B and S3C). Therefore, we chose this approach in an effort to obtain large numbers of ILC2s at high purity; staining and/or sorting for T1/ST2- and IL-7Rα-expressing cells was not required until the end of the culture, alleviating the concern that antibody staining might affect IL-33 or IL-7 signaling.

To determine the function of GATA3 in ILC2s, we prepared ILC2s, as described in Figure S3, from *Gata3*$^{fl/fl}$-CreER^{T2} or *Gata3*$^{fl/fl}$ mice. After 5 days of culture, samples were split into two groups and treated either with 4-hydroxytamoxifen (4-OHT), the active metabolite of tamoxifen that binds to CreER^{T2} to induce Cre activity, or with a vehicle control, ethanol (EtOH). To test whether GATA3 affects ILC2 maintenance in culture in a cell-intrinsic manner, we mixed *Gata3*$^{fl/fl}$-CreER^{T2} or *Gata3*$^{fl/fl}$ ILC2s with ILC2s generated from CD45.1 congenic mice. After 4-OHT treatment, there was a progressive decrease in the percentage of *Gata3*-deficient ILC2s over a 6-day period (Figure 5B). To comprehensively profile the genes regulated by GATA3 in ILC2s at a genome-wide level, we sorted live ILC2s that had or had not undergone *Gata3* inactivation on day 2, a time point when no dramatic ILC2 loss was observed, and performed RNA sequencing (RNA-seq) analyses. Interestingly, we found that many TNF and TNFR superfamily genes, such as *Tnfrsf9* (encoding 4-1BB, which provides costimulation) and *Tnfsf21* (encoding DR6, which induces cell apoptosis), and NF$_K$b family members, including *Nfkb2* and *Relb*, showed altered expression patterns (Figure 5C). In addition, the cell-cycle inhibitor *Cdkn2b* was upregulated upon *Gata3* inactivation (Figure 5C). These changes are consistent with and might contribute to the loss of *Gata3*-deficient ILC2s.

GATA3 Regulates Many Critical Genes in ILC2s and Th2 Cells

RNA-seq results confirmed efficient *Gata3* inactivation by CreER^{T2} after 4-OHT treatment given that a nearly 100% reduction of the reads at *Gata3* exon 4 was observed (Figure 6A). Interestingly, reads at the other exons of *Gata3* were only modestly

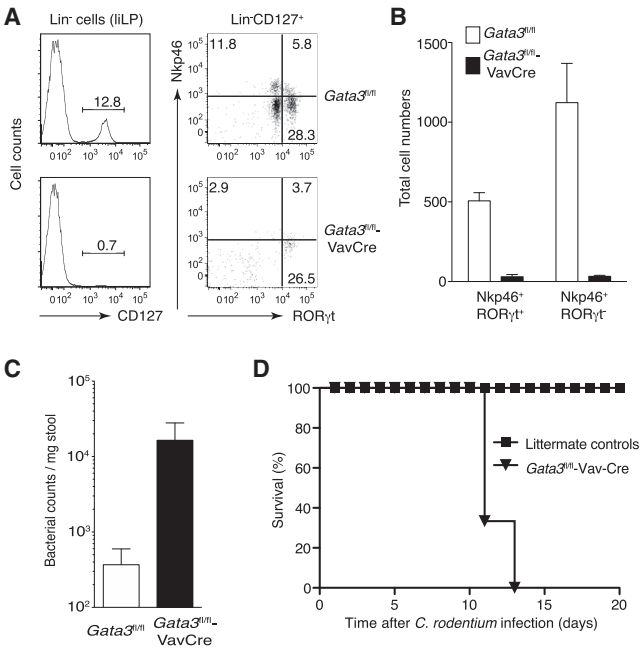

Figure 3. GATA3 Deficiency Results in Susceptibility to *Citrobacter rondentium* Infection

Gata3$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice (three to four mice per group) were orally infected with *Citrobacter rondentium*. Four days after infection (A–C), cells were harvested from the large intestinal lamina propria (liLP) and stained with a cocktail of antibodies to CD127, RORγt, Nkp46, and various lineage markers. Data in (A)–(C) represent one experiment, and data in (D) represent another experiment. Error bars represent the mean ± SD. Numbers indicate the percentages in each quadrant or gate.

(A) Plots were gated on Lin$^-$ cells (left panel) or Lin$^-$CD127$^+$ cells (right panel).

(B) The absolute number of Nkp46$^+$ populations from the liLP of each mouse was plotted.

(C) Feces were collected for assessing bacterial loads.

(D) Survival curves are shown for the *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice after infection.

affected, implying that GATA3 is not a major player in maintaining its own transcription in ILC2s (Figure 6A).

We then compared genes that are either positively or negatively regulated by GATA3 in ILC2s and Th2 cells (Wei et al., 2011). A total of 32 genes that are positively regulated by GATA3 in Th2 cells were also affected in ILC2s upon *Gata3* inactivation (Figures 6B and 6C and Table S1). *Il13* transcripts were dramatically reduced when GATA3 was absent in ILC2s (Figure 6C and Table S1). Other genes that are positively regulated by GATA3 in both ILC2s and Th2 cells include *Areg* (encoding Amphiregulin), *Il5*, *Ccr8*, and *Lif* (Figure 6C and Table S1). Interestingly, *Il1rl1* (encoding IL-33R) and *Il9r* were expressed at much higher levels in ILC2s than in Th2 cells; their expression was dramatically reduced when *Gata3* was disrupted in ILC2s (Figure 6C and Table S1).

We also identified 225 genes, including *Il4*, *Maf*, *Pth*, and *Ikzf3* (encoding Aiolos), that are positively regulated by GATA3 in Th2 cells but that are not affected by GATA3 in ILC2s (Figure 6B). On the other hand, 130 genes are positively regulated by GATA3 in ILC2s, but not in Th2 cells; they include *Icos*, *Il2ra*, *Kit*, *Il1r2*, *Cysltr1*, *Htr1b*, and *Tph1*, many of which are cell-surface markers of ILC2s (Figures 6B and 6D and Table S1). These data indicate that GATA3 has unique functions in ILC2s and Th2 cells in addition to its common regulation of many type 2 effector cytokines.

Gata3 inactivation in Th2 cells resulted in derepression of several Th1-cell-associated genes, such as *Fasl*, *Il12rb2*, and *Stat4* (Table S1). However, *Gata3* inactivation in ILC2s did not alter the expression of these genes; instead, it derepressed several other genes—such as *Cd244* (encoding 2B4), *Lta*, and *Tnf* (Figure 6E and Table S1)—that are characteristic of type I ILCs and/or NK cells.

Gata3 inactivation did not reduce *Klrg1* transcription (Figure 6F), suggesting that *Klrg1* is not a GATA3 target gene. Other cell-surface molecules expressed by ILC2s include CD127, Sca-1, and Thy1. The expression of *Il7r* (encoding CD127) and *Ly6a/e* (encoding Sca-1) was reduced less than 2-fold, whereas *Thy1* expression was increased upon *Gata3* inactivation (Table S1). The expression of *Rora* and *Id2*, encoding two transcription factors critical for the development of ILC2s, was not reduced upon *Gata3* inactivation (Figure 6F).

To determine whether GATA3 has a similar function in ILC2s at the steady state, we performed RNA-seq by using ex-vivo-purified KLRG1$^+$ ILC2s from tamoxifen-treated *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Cre-ER^{T2} mice. The results indicated that among the 162 genes positively regulated by GATA3 in activated ILC2s, 57 genes were also positively regulated by GATA3 in "naive" ILC2s (Figure 6G and Table S1). These included *Il5*, *Il13*, *Areg*, *Il1r1*, *Ccr8*, *Il2ra*, *Cysltr1*, *Tph1*, and *Htr1b*. Interestingly, *Il10* and *Il24* were regulated by GATA3 in activated ILC2s, but not in "naive" ILC2s (Figure 6G), suggesting that GATA3-mediated regulation of *Il10* and *Il24* expression requires cofactors that are only present in activated ILC2s. *Il4* expression was affected by *Gata3* deletion in "naive" ILC2s, but not in activated ILC2s (Figure 6G), consistent with our previous finding that GATA3 is required for the expression of IL-4 in developing Th2 cells but is not essential in fully differentiated Th2 cells.

To verify the RNA-seq data at the protein level, we performed flow cytometry analysis. Compared to control samples, 4-OHT-treated *Gata3*$^{fl/fl}$-CreER^{T2} ILC2 culture showed no evidence of GATA3 expression, as judged by intracellular staining (Figure S4A), implying that the deletion of *Gata3* was efficient and the pre-existing GATA3 was degraded in ILC2s over a 2-day period. By contrast, consistent with the RNA-seq data, two cell-surface molecules highly expressed by ILC2s (KLRG1 and Sca-1) were only modestly, if at all, affected by inactivation of *Gata3* (Figures S4B and S4C), demonstrating that ILC2s maintain some features of their phenotype in the absence of GATA3. Consistent with the role of GATA3 in Th2 cells, IL-13 production by ILC2s in response to IL-33 and IL-7 stimulation was dramatically reduced upon *Gata3* inactivation (Figure S4D). In agreement with RNA-seq data, T1/ST2 surface expression was also abolished when *Gata3* was inactivated in ILC2s (Figure S4E). This result raised the question of whether defective expression of IL-13 was due to a failure of the *Gata3*-deficient cells to respond to IL-33 or to a direct effect of GATA3 on *Il13*. Therefore, we stimulated these cells with PMA and ionomycin. Even with this potent stimulus, IL-13 production was dramatically decreased in *Gata3*-deficient cells (Figure S4F), suggesting that GATA3 directly regulates IL-13 production in ILC2s independently of its role in maintaining IL-33R expression. This is consistent

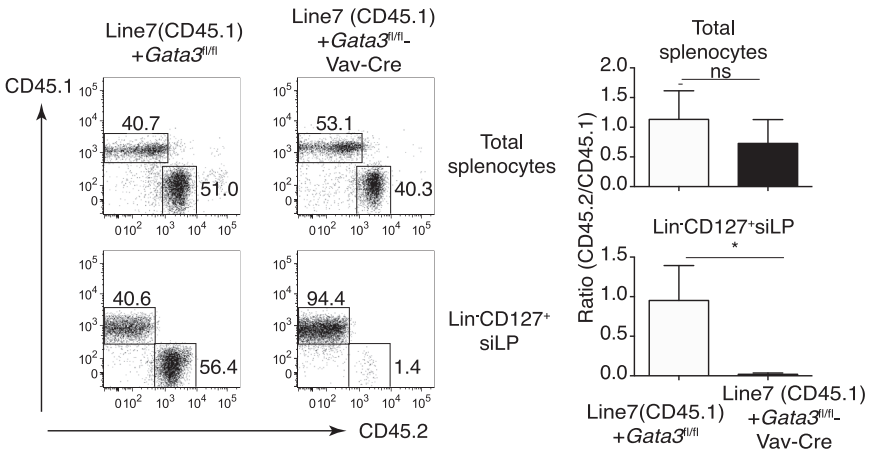

Figure 4. The Effect of GATA3 on the Development of IL-7Rα$^+$ ILCs Is Cell intrinsic

Bone marrow cells from *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice were mixed with bone marrow cells from CD45.1 congenic mice (line 7) and cotransferred into irradiated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. Eight weeks after mixed bone marrow transplant, cells were harvested from the spleen or siLP of chimeric mice (three mice per group) and stained with CD45.1 and CD45.2 together with a cocktail of antibodies to CD127 and various lineage markers. Plots were gated on total splenocytes (upper panel) or Lin$^-$CD127$^+$ cells from the siLP (lower panel). The ratio of CD45.2 to CD45.1 was calculated and is shown on the right. Numbers indicate the percentages in each box. Error bars represent the mean ± SD. ns, not significant. *$p < 0.05$.

with the established capacity of GATA3 to directly bind to the promoter of *Il13* in many T cell subsets (Wei et al., 2011).

GATA3 Regulates Common and Unique Sets of Genes in ILC2s and ILC3s

To determine the GATA3 target genes in ILC3s, we performed RNA-seq on ILC3s with or without *Gata3* deletion. Ninety-eight and 81 genes were positively and negatively regulated, respectively, by GATA3 in ILC3s. Among the 98 positively regulated genes, 29 (including *Atf3*, *Ccr9*, *Ets2*, and *Nr4a2*) were also positively regulated by GATA3 in ILC2s (Figure 7A and Table S1). Similarly, among the 81 negatively regulated genes, 17 were also negatively regulated by GATA3 in ILC2s. The functions of these 46 genes that were commonly regulated by GATA3 in ILC2s and ILC3s require further investigation. Many more genes are regulated by GATA3 in ILC2s than in ILC3s, possibly because GATA3 is expressed at much higher amounts in ILC2s than in ILC3s. Some cytokine-receptor-encoding genes regulated by GATA3 in ILC2s (such as *Il1rl1*, *Il2ra*, and *Il9r*) were not regulated by GATA3 in ILC3s. The expression of key transcription-factor-encoding genes reported to be critical for the development of ILC3s, including *Rorc*, *Runx1*, *Runx3*, *Ahr*, *Id2*, and *Tcf7*, was not affected by *Gata3* deletion in ILC3s (Figure 7B). Together with the finding that GATA3 does not affect the expression of *Id2* and *Rora* in ILC2s (Figure 6F), our data indicate that the mechanism of GATA3 in regulating the development of IL-7Rα-expressing ILCs is independent of, or in parallel with, all known key transcription factors.

DISCUSSION

Research on the ILCs has exploded since the description and characterization of the ILC2s in 2010 by four different groups (Moro et al., 2010; Neill et al., 2010; Price et al., 2010; Saenz et al., 2010), and it is now well accepted that ILCs play important roles in innate immunity by producing effector cytokines (Spits et al., 2013). Because these effector cytokines are virtually identical to those produced by Th cells, ILCs can be divided into ILC1s, ILC2s, and ILC3s, representing innate versions of Th1-, Th2-, and Th17-like cells, respectively (Spits et al., 2013). There are also similarities in the development of ILCs and Th cells because the key transcription factor that determines a particular Th cell fate is also critically involved in the development of its ILC counterpart (Bernink et al., 2013; Eberl et al., 2004; Hoyler et al., 2012; Klose et al., 2013; Mjösberg et al., 2012; Sciumé et al., 2012; Spits et al., 2013; Vonarbourg et al., 2010). However, much less is known about the early development of ILCs. Our observation that the development of all IL-7Rα$^+$ ILCs, but not classical NK cells, requires GATA3 suggests that all IL-7Rα$^+$ ILC subsets might have a CILP. Furthermore, by comparing gene expression in mature ILC2s with or without GATA3 expression at a genome-wide level, we have provided important data that are crucial for further understanding the development and functions of ILC2s during type 2 immune responses.

Previous reports have suggested that ILC2s can be identified by a combination of several cell-surface markers, including T1/ST2 (IL-33R), IL-25R, ICOS, Sca-1, and KLRG1, in addition to IL-2Rα and IL-7Rα (Halim et al., 2012b; Hoyler et al., 2012; Moro et al., 2010; Neill et al., 2010; Price et al., 2010; Saenz et al., 2010; Wong et al., 2012). We have shown that GATA3 regulates most of these molecules. However, the expression of IL-7Rα and Sca-1 is only modestly reduced, and KLRG1 is not affected by *Gata3* inactivation. We also identified other cell-surface molecules, including IL-9R, CCR8, cysteinyl leukotriene receptor 1 (Cysltr1), 5-hydroxytryptamine (serotonin) receptor 1B (Htr1b), and TNFR superfamily members 4-1BB and RANK, whose expression in ILC2s is GATA3 dependent, in addition to known ILC2 cell-surface markers. Cysltr1 is expressed by ILC2s, and leukotriene D_4 is able to induce the expression of IL-5, IL-13, and IL-4 (Doherty et al., 2013). Therefore, GATA3 might indirectly affect type 2 cytokine production in vivo by regulating two stimulating receptors, T1/ST2 and Cysltr1. Overall, our data indicate that although GATA3 regulates many of the distinguishing cell-surface markers on ILC2s, other transcription factors are also involved. Identification of other factors that regulate KLRG1 expression might add important insights into the transcription factor networks controlling the development and maturation of ILC2s.

GATA3 plays critical roles in the differentiation and maintenance of both Th2 cells and ILC2s and thus is important for mediating type 2 immune responses at different stages. Through genome-wide profiling of the genes regulated by GATA3 in

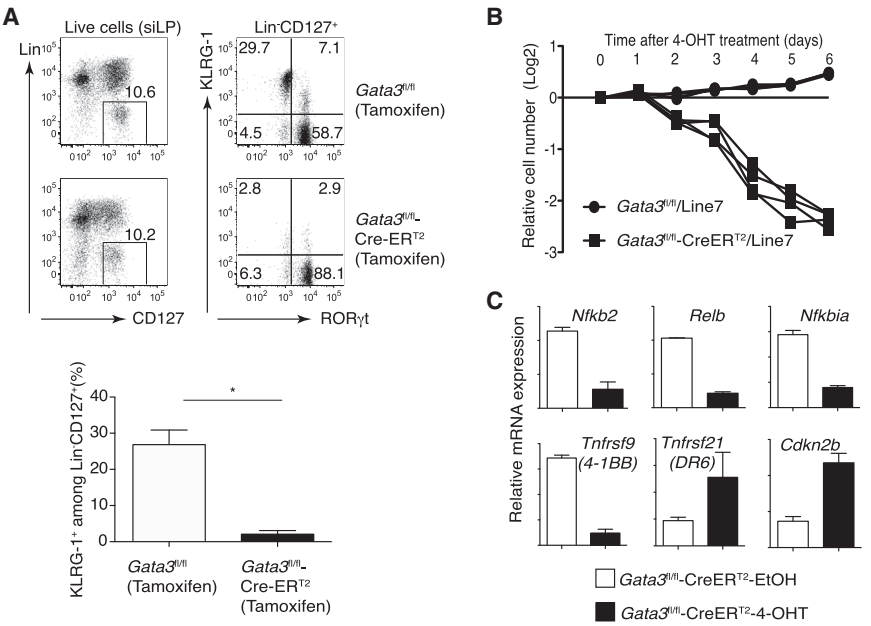

Figure 5. GATA3 Is Critical for the Maintenance of ILC2 Numbers Both In Vivo and In Vitro

(A) *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-CreER^{T2} mice (two to three mice per group) were intraperitoneally injected five times with tamoxifen every other day. Three weeks after the first injection, siLP cells were prepared and stained with a cocktail of antibodies to CD127 (IL-7Rα), KLRG1, RORγt, and various lineage markers. Plots were gated on live cells (left panel) or Lin$^-$CD127$^+$ cells (right panel). Numbers indicate the percentages in each quadrant.

(B) Lin$^-$ cells were purified from the mesenteric lymph nodes of IL-25-treated *Gata3*$^{fl/fl}$-CreER^{T2}, *Gata3*$^{fl/fl}$, and CD45.1 congenic (line 7) mice (two to three mice per group) by cell sorting. After being cultured with IL-7, IL-25, and IL-33 for 5 days, they were mixed as indicated at a 1:1 ratio and then treated with 4-hydroxytamoxifen (4-OHT). The relative cell numbers were calculated on the basis of the change in ratio over a period of 6 days. Four independently mixed cultures were tested in both groups. Data are representative of two independent experiments.

(C) Lin$^-$ cells were purified from the mesenteric lymph nodes of IL-25-treated *Gata3*$^{fl/fl}$-CreER^{T2} mice (two to three mice per group in duplicates) by cell sorting. After being cultured with IL-7, IL-25, and IL-33 for 5 days, they were then treated with either 4-OHT or ethanol (EtOH) for 2 days. RNA-seq analysis was carried out. Relative mRNA expression of several genes was calculated on the basis of the RNA-seq results with duplicates.

Error bars represent the mean ± SD. See also Figure S3.

ILC2s, we found a substantial overlap of the genes regulated by GATA3 in Th2 cells and in ILC2s. Most strikingly, these commonly regulated genes included many key effector cytokines and receptors, such as *Il13*, *Il5*, *Areg*, *Il10*, *Il24*, *Lif*, *Il1rl1*, *Il9r*, and *Ccr8*. Except for *Il10* and *Il24*, which were expressed only in activated ILC2s, all other key cytokines and receptors were also regulated by GATA3 in "naive" ILC2s at the steady state. IL-4 was regulated by GATA3 in "naive" ILC2s, but not cultured ILC2s, consistent with our previous findings in T cells that GATA3 is important for the induction of IL-4 during Th2 cell differentiation but plays a minor role in IL-4 production in already developed Th2 cells. Thus, our data indicate that ILC2s resemble the innate counterpart of Th2 cells at a genomic scale and that GATA3 regulates the key functions shared by ILC2s and Th2 cells, possibly through a similar mechanism.

GATA3 is thought to be critical for controlling the cell fate of ILC2s, but not other ILCs (Hoyler et al., 2012). The discrepancy between this report and our study can be explained by the timing of *Gata3* inactivation. In that study, Id2-driven Cre-ER^{T2} was used for conditional deletion of *Gata3* by tamoxifen in otherwise normal mice. Because all the ILCs had already developed in these mice before *Gata3* inactivation and the half-life of ILCs might be longer than 3 weeks, the specific effect on ILC2s 3 weeks after GATA3 removal only confirms that GATA3 is critical for the maintenance of ILC2s, but not other ILCs. Indeed, we were also able to show that tamoxifen-induced *Gata3* deletion in *Gata3*$^{fl/fl}$-CreER^{T2} mice abolished ILC2s but had no effect on RORγt$^+$ ILCs once they had developed. Therefore, acute *Gata3* ablation after ILCs have developed obscures the role we have revealed here for GATA3 during development of ILCs. Vav-Cre-mediated gene inactivation disrupted *Gata3* before ILCs could develop, allowing us to conclude that GATA3 is critical for the development of all IL-7Rα$^+$ ILCs, including ILC2s.

All known subsets of ILCs and NK cells depend on Id2 expression for their development (Moro et al., 2010; Yokota et al., 1999). Although E4BP4 (also known as NF-IL3) is critical for the development of NK cells (Gascoyne et al., 2009; Kamizono et al., 2009), no severe ILC developmental defect has been reported in E4BP4-deficient mice. For the development of IL-7Rα$^+$ ILC subsets, RORα is a transcription factor specifically required for inducing ILC2s (Halim et al., 2012b; Wong et al., 2012), but not RORγt-expressing ILCs (Halim et al., 2012b). On the other hand, RORγt is only needed for the development of RORγt-expressing ILCs (Eberl et al., 2004; Halim et al., 2012b), but not ILC2s (Halim et al., 2012b; Moro et al., 2010). Up until now, the transcription factor critical for the development of all IL-7Rα$^+$ ILC lineages, but not NK cells, has not been identified. Here, we report that GATA3 is such a transcription factor. GATA3 does not seem to regulate Id2, RORα, or RORγt, suggesting that GATA3 might collaborate with Id2 for ILC development in general, with RORα for ILC2 development, and with RORγt for ILC3 development. Our data indicate that different IL-7Rα$^+$ ILC subsets might develop through a GATA3-dependent mechanism from a common progenitor. Thus, a hematopoietic branch point, with E4BP4 committing progenitors to the NK cell lineage and GATA3 leading to the development of IL-7Rα$^+$ ILCs, might exist. Therefore, it is reasonable to consider that IL-7Rα$^+$ ILC subsets resemble innate version of CD4$^+$ Th cell subsets, whereas conventional NK cells, which have higher cytotoxic activity than other IFNγ-expressing ILCs, represent innate version of CD8$^+$ T cells.

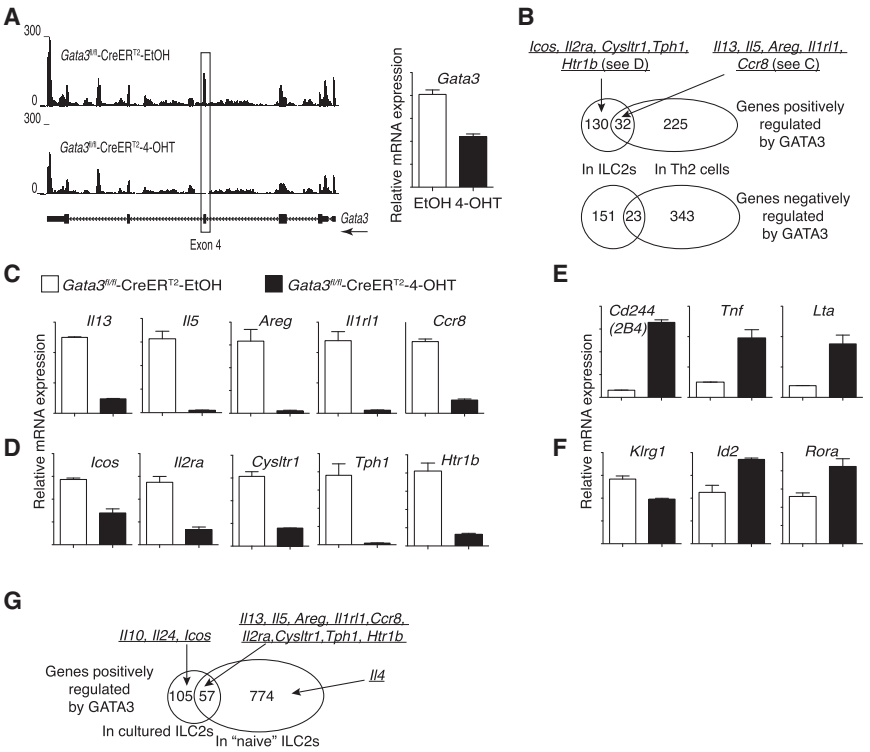

Figure 6. GATA3 Positively and Negatively Regulates the Expression of Many Critical Genes in ILC2s

(A–F) Lin$^-$ cells were purified from the mesenteric lymph nodes of IL-25-treated *Gata3*$^{fl/fl}$-CreER^{T2} mice (two to three mice per group in duplicates) by cell sorting. After being cultured with IL-7, IL-25, and IL-33 for 5 days, they were then treated with either 4-OHT or EtOH for 2 days. (A) UCSC Genome Browser view of the RNA-seq data at the *Gata3* locus (left panel). The boxed area indicates the deletion of exon 4 by Cre-ER^{T2}. Relative *Gata3* mRNA expression was calculated on the basis of the RPKM (reads per kilobase of exon per million reads) values obtained from RNA-seq results with duplicates (right panel). (B) Venn diagrams show the overlap of total genes positively (upper) or negatively (lower) regulated by GATA3 in ILC2s and Th2 cells. (C–F) Representative genes regulated by GATA3 in different categories are shown. Relative mRNA expression was calculated on the basis of the RPKM values obtained from RNA-seq results with duplicates. (C) Examples represent 32 genes positively regulated by GATA3 in both ILC2s and Th2 cells. (D) Examples represent 130 genes positively regulated by GATA3 in ILC2s, but not in Th2 cells. (E) Examples represent 151 genes negatively regulated by GATA3 in ILC2s, but not in Th2 cells. (F) Expression of *Klrg1*, *Id2*, and *Rora* was not affected by *Gata3* inactivation in ILC2s. (G) *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-CreER^{T2} mice (four mice per group) were treated with tamoxifen in vivo for 2–3 days, and then ILC2s were sorted on the basis of KLRG1 expression. RNA-seq analyses were performed with *Gata3*-sufficent and *Gata3*-deficient cells. The Venn diagram shows the overlap of total genes positively regulated by GATA3 in cultured ILC2s and ILC2s at the steady state.

Error bars represent the mean ± SD. See also Figure S4 and Table S1.

Our study shows that there is an excellent symmetry in ILC and Th cell development: although GATA3 is regarded as the master regulator for Th2 cells, it is also critical for the maintenance and functions of ILC2s, and although GATA3 is critical for the development of all CD4$^+$ T cells (Ho et al., 2009), it is also indispensable for the development of all IL-7Rα$^+$ ILC subsets. GATA3, therefore, plays parallel roles in establishing and regulating both adaptive and innate lymphocyte populations.

EXPERIMENTAL PROCEDURES

Mice

Gata3$^{fl/fl}$ mice on a C57BL/6 background were previously described (Yagi et al., 2010). These mice were bred with either CreER^{T2} (Taconic line 10471) or Vav-Cre (The Jackson Laboratory line 8610) transgenic mice on a C57BL/6 background for the generation of *Gata3*$^{fl/fl}$-CreER^{T2} or *Gata3*$^{fl/fl}$-Vav-Cre lines. C57BL/6 mice were ordered from Taconic. CD45.1 congenic mice (line 7) and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (line 4111) were from a contract between Taconic and the National Institute of Allergy and Infectious Diseases (NIAID). All mice were bred and/or maintained in the NIAID-specific pathogen-free animal facility, and the experiments were done when mice were 8 to 16 weeks of age under protocols approved by the NIAID Animal Care and Use Committee.

Citrobacter rodentium Infections

Citrobacter rodentium (formerly *Citrobacter freundii*, biotype 4280) strain DBS100 was prepared by selection of a single colony and culture in Luria-Bertani broth for 8 hr. Mice were inoculated with approximately 1 × 10^{10} colony-forming units (cfu) in 200 μl of PBS via oral gavage. Cells from the liLP were prepared as previously described (Sun et al., 2007). cfu were determined via serial dilutions on MacConkey's agar from overnight cultures of homogenized fecal pellets.

Cell Preparation

Single-cell suspensions were prepared directly from mouse fetal livers or different lymphoid organs, including lymph nodes, spleen, and bone marrow. Cells from the siLP and IELs were prepared as previously described (Sun et al., 2007). For preparing cells from tissues such as lung, mice were perfused with PBS before organs were harvested. The lung was cut into small pieces and digested with DNase I (Roche) and Liberase (Roche) for 30 min at 37°C. Single-cell suspension from digested lung was subjected to Percoll density gradient centrifugation. In some experiments, recombinant murine IL-25 (from either eBioscience or PeproTech, 0.4 μg per mouse) in PBS was injected intraperitoneally into mice for 3 consecutive days before mesenteric lymph nodes were harvested. In other experiments, tamoxifen (Sigma) was intraperitoneally injected five times into mice every other day (5 mg tamoxifen in 150 μl corn oil per mouse per injection). In mixed bone marrow chimera experiments, bone marrow cells from *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-Vav-Cre mice were mixed with bone marrow cells from CD45.1 congenic mice (line 7) at a 1:1 ratio and injected (10 million cells per mouse) into sublethally irradiated (450 Rad) *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (line 4111). Cells were stained with a cocktail of antibodies to various lineage markers, including antibodies to CD3, CD5, CD45R, CD11b, CD11c, NK1.1, Gr-1, TER119, FcεRI, and TCRγ/δ for identifying Lin$^-$ cells. Lin$^-$ cells were sorted with a FACS Aria and then cultured with IL-7 (10 ng/ml), IL-33 (10 ng/ml), and IL-25 (10 ng/ml) for ~1 week. In some experiments, cells were treated with 100 nM 4-OHT after 5 days of culture for the deletion of *Gata3* from *Gata3*$^{fl/fl}$-CreER^{T2} cells.

Flow Cytometry Analysis

Cell-surface molecule staining and cytokine intracellular staining were performed as previously described (Zhu et al., 2004). Staining for transcription

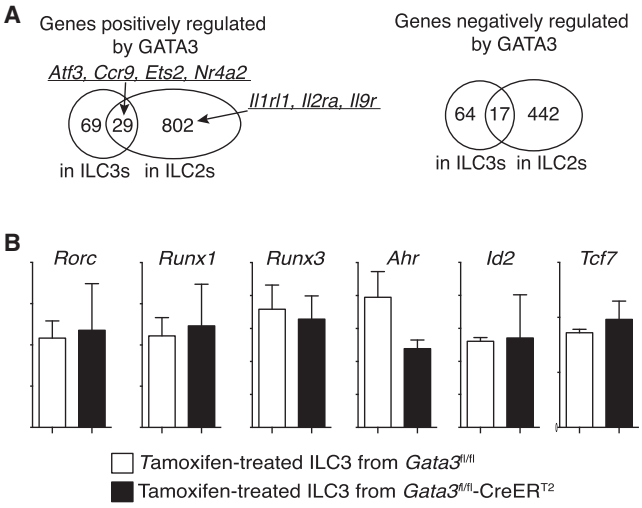

Figure 7. GATA3 Does Not Regulate Known Key Transcription Factors in ILC3s

(A) *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-CreER^{T2} mice (four mice per group) were treated with tamoxifen in vivo for 2–3 days, and then KLRG1$^-$ ILCs (mostly ILC3s) were sorted. RNA-seq analyses were performed with *Gata3*-sufficent and *Gata3*-deficient ILC3s. RNA-seq analyses were also performed with *Gata3*-sufficent and *Gata3*-deficient ILC2s, as described in Figure 6G. The Venn diagram shows the overlap of total genes positively or negatively regulated by GATA3 in ILC2s and ILC3s.

(B) Expression of *Rorc*, *Runx1*, *Runx3*, *Id2, Ahr*, and *Tcf7* was not affected by *Gata3* inactivation in ILC3s. Error bars represent the mean ± SD.

factors was carried out with the Foxp3 Staining Buffer Set (eBioscience) according to the manufacturer's instructions. Flow cytometry data were collected with the LSR II (BD Biosciences), and the results were analyzed with FlowJo software (Tree Star). Antibodies specific to mouse CD5 (53-7.3), CD45R (RA3-6B2), Gr-1 (RB6-8C5), NK1.1 (PK136), and TCRγ/δ (UC7-13D5) and a mouse erythroid cell marker (TER119) were purchased from BioLegend; antibodies specific to mouse CD3 (2C11), CD4 (RM4-5), CD11b (M1/70), CD11c (N418), CD127 (A7R34), CD45.1 (A20), CD45.2 (104), FcεRI (MAR-1), IL-13 (eBio13A), KLRG1 (2F1), NKp46 (29A1.4), RORγt (AFKJS-9), Sca-1 (D7), Flt3 (A2F10), α4β7 (DATK32), and c-Kit (2B8) were purchased from eBiosciences; antibodies specific to Thy1.2 (53-2.1) and GATA3 (L50-823) were purchased from BD Biosciences; and antibodies specific to IL-33R (DJ8) were purchased from MD Bioproducts.

RNA-Seq and Data Analysis

Lin$^-$ cells were sorted from *Gata3*$^{fl/fl}$-CreER^{T2} mice that had been injected with IL-25 for 3 days. After 5-day culture in IL-7, IL-25, and IL-33, cells were treated with either 4-OHT or EtOH as a control for 2 days. *Gata3*-sufficient ILC2s were sorted from EtOH-treated samples. *Gata3*-deficient cells were sorted from 4-OHT-treated samples, excluding residual T1/ST2-expressing cells. In other experiments, *Gata3*$^{fl/fl}$ or *Gata3*$^{fl/fl}$-CreER^{T2} mice were injected with tamoxifen (5 mg/mouse). After 2–3 days, Lin$^-$Thy1$^+$CD127$^+$KLRG1$^+$ cells (mostly ILC2s) and Lin$^-$Thy1$^+$CD127$^+$KLRG1$^-$ cells (mostly ILC3s) were sorted from the siLP. RNA-seq experiments were performed. In brief, 100 ng of total RNA was amplified with the Ovation RNA-Seq System V2 (NuGEN). The resulting double-stranded DNA was sonicated to 200–400 bp. Two hundred fifty nanograms of sonicated DNA was used for preparing sequencing libraries for multiplex sequencing (Illumina), and 50 bp reads were generated by the NHLBI DNA Sequencing and Computational Biology Core. Sequencing reads were mapped to the mm9 genome (UCSC Genome Browser). Gene expression levels were measured by RPKM (reads per kilobase of exon per million reads) in the library (Mortazavi et al., 2008). Differentially expressed genes were identified by edgeR (Robinson et al., 2010) with the following criteria: false discovery rate < 0.001, fold change > 2, and RPKM > 3 in either *Gata3*-sufficient or -deficient samples.

ACCESSION NUMBERS

RNA-seq data are available in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/gds) under the accession number GSE47851.

SUPPLEMENTAL INFORMATION

Supplemental Information include four figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2014.01.012.

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