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Enforced Expression of Gata3 in T Cells and Group 2 In innate Lymphoid Cells Increases Susceptibility to Allergic Airway Inflammation in Mice

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Airway inflammation in allergic asthma reflects a threshold response of the innate immune system, including group 2 innate lymphoid cells (ILC2), followed by an adaptive Th2 cell–mediated response. Transcription factor Gata3 is essential for differentiation of both Th2 cells and ILC2. We investigated the effects of enforced Gata3 expression in T cells and ILC2 on the susceptibility of mice to allergic airway inflammation (AAI). We used CD2-Gata3 transgenic (Tg) mice with enforced Gata3 expression driven by the CD2 promoter, which is active both in T cells and during ILC2 development. CD2-Gata3 Tg mice and wild-type (WT) littermates were analyzed in mild models of AAI without adjuvants. Whereas OVA allergen exposure did not induce inflammation in WT controls, CD2-Gata3 Tg mice showed clear AAI and enhanced levels of IL-5 and IL-13 in bronchoalveolar lavage. Likewise, in house dust mite–driven asthma, CD2-Gata3 Tg mice were significantly more susceptible to AAI than WT littermates, whereby both ILC2 and Th2 cells were important cellular sources of IL-5 and IL-13 in bronchoalveolar lavage and lung tissue. Compared with WT littermates, CD2-Gata3 Tg mice contained increased numbers of ILC2, which expressed high levels of IL-33R and contributed significantly to early production of IL-4, IL-5, and IL-13. CD2-Gata3 Tg mice also had a unique population of IL-33–responsive non-B/non-T lymphoid cells expressing IFN-γ. Enforced Gata3 expression is therefore sufficient to enhance Th2 and ILC2 activity, and leads to increased susceptibility to AAI after mild exposure to inhaled harmless Ags that otherwise induce Ag tolerance. The Journal of Immunology, 2014, 192: 1385–1394.

Asthma is characterized by reversible airway obstruction, pulmonary inflammation, and bronchial hyperreactivity (1). Patients manifest symptoms of asthma in response to inhalation of airborne allergens, such as house dust mite (HDM), molds, or animal dander. Many cell types participate in allergic airway inflammation (AAI), including eosinophils, dendritic cells (DCs), B and T lymphocytes, mast cells, macrophages, epithelial cells, smooth muscle cells, and fibroblasts. Nevertheless, CD4+ Th2 cells, which produce a unique profile of IL-4, IL-5, IL-9, and IL-13 proinflammatory cytokines, are thought to be central in the orchestration and amplification of allergic inflammatory events. Th2 cytokines explain many hallmarks of allergic asthma: IgE synthesis (IL-4), airway eosinophilia (IL-5), mast cell accumulation (IL-9), goblet cell hyperplasia (IL-4, IL-13), and bronchial hyperreactivity acting on bronchial smooth muscle cells (IL-13) (1).

Although adaptive Th2 cells have been identified as important sources of IL-4, IL-5, and IL-13, many studies emphasize the importance of innate cells such as mast cells, eosinophils, and basophils, in cytokine production. Most importantly, a population of non-B/non-T innate lymphoid cells (ILCs) that have the capacity to produce large amounts of IL-5 and IL-13 upon stimulation by IL-33 or IL-25 was recently identified in the intestine and the lung (2, 3). Because of their Th2 cytokine profile, they have been named group 2 ILCs (ILC2) and can be identified as lineage-negative cells expressing receptors for IL-2, IL-7, and IL-33 (4). The involvement of ILC2 in asthma was recently demonstrated in various mouse models, including HDM-driven allergic AAI (5–8). ILC2 have also been identified in human lung and are associated with chronic rhinosinusitis (9–11).

Differentiation of Th2 cells is induced by the key NF Gata3, which acts as a transcription regulator of the Th2 cytokines IL-4, IL-5, and IL-13, and concomitantly suppresses Th1 development (12–14). A suggested mechanism for this activity is that Gata3 causes chromatin remodeling in the Th2 cytokine locus, in conjunction with various other transcriptional regulators including c-maf, Irf4, SatB1, and Ctf (12, 15, 16). Apart from its function in Th cells, Gata3 is also essential for early stages of T cell development in the thymus (12, 17). Conditional deletion of the Gata3 gene in established Th2 cells demonstrated that Gata3 is critical for IL-5 and IL-13, but not IL-4 production (18). Deletion of the Gata3 gene in murine ILC2 also abolished their IL-13 production (19). Moreover, studies of Gata3-deficient hematopoietic chimeras and conditional targeting experiments showed that...
the Gata3 gene is essential for ILC2 development, as well as maintenance of mature ILC2 in mice (20, 21).

To date, >100 genes have been associated with asthma genome-wide, including the Th2 locus, thymic stromal lymphopoietin, IL-33, and IL-33R/T1ST2, but typically the impact of each of these genes is mild (22–24). Interestingly, haplotype analysis of the GATA3 locus showed associations with asthma and atopy-related phenotypes (25). Nakamura et al. (26) showed that GATA3 expression is increased in lung mucosa and bronchoalveolar lavage (BAL) cells of asthmatic patients, compared with healthy control subjects. Moreover, GATA3 expression was increased after local allergen provocation, in the absence of inflammatory cell recruitment (27).

Mouse studies indicated that blocking Gata3 expression by knockdown strategies can reduce allergic inflammation in mouse models (13), suggesting that Gata3 is an important target for therapeutic strategies (28). Conversely, we have previously shown that enforced expression of Gata3 in T cells under the control of the CD2 promoter in transgenic (Tg) mice resulted in enhanced CD2 expression by Gata3 (30). These CD2-Gata3 Tg mice lacked Th1-mediated hypersensitivity reactions and showed reduced differentiation or activity of the Th1 cell subset, and protection from autoimmune encephalomyelitis and rheumatoid arthritis (29, 31, 32). Overexpression of Gata3 in T cells has been associated with enhanced Th2 responses and eosinophilic airway inflammation in vivo (33–36). However, effects of Gata3 overexpression on the ILC2 population in AAI remain unknown.

We recently found that CD2-Gata3 Tg mice contained increased numbers of ILC2, which might be explained by increased Gata3 levels in ILC2 precursors (21). Importantly, we found increased Gata3 expression in CD2-Gata3 Tg common lymphoid progenitor (CLP) cells, which showed an increased capacity to develop into ILC2 in vitro (21). Together with the finding that in mice with one targeted Gata3 allele, the numbers of ILC2 were reduced, we concluded that Gata3 is essential for the development of ILC2 from CLP cells in a dose-dependent fashion (21). However, it remains unknown whether also the function of Gata3 in mature Th2 cells or ILC2 is dose dependent.

In this study, we investigated the effects of Gata3 overexpression on Th2 cytokine production by T cells and ILC2, and the effects on the susceptibility of mice to develop AAI. In particular, we found that enforced Gata3 expression enhanced eosinophilia in acute models of AAI, and increased both Th2 and ILC2 involvement.

**Materials and Methods**

**Mice and genotyping**

CD2-Gata3 Tg mice (29) were backcrossed on the C57BL/6 background for >10 generations and genotyped by PCR (5′-CAG CTC TGG ACT CTT CCC AC-3′ and 5′-GTT CAC ACA CTC CCT GGC TT-3′). Animals were kept under specific pathogen-free conditions, provided with water and food ad libitum, and were used at the age of 6–11 wk. All experiments were approved by the Ermoupolis MC Animal Ethics Committee.

**OVA and HDM AAI model**

To induce airway inflammation, we anesthetized mice with isofluorane and treated them intratracheally (i.t.) with 80 μl OVA (Worthington) solution (10 mg/ml in PBS; Life Technologies) or PBS as a control on day 0. Ten days later, all mice were exposed to OVA (Sigma OVA type III) aerosols 10 mg/ml for 30 min for 3 consecutive days. In some experiments, HDM (Greer Laboratories) extract was used as an allergen: on day 0, isofluorane-anesthetized mice were sensitized i.t. using 10 μg HDM extract in 80 μl PBS or using PBS only. Ten days later, anesthetized mice were challenged with 10 μg HDM in 50 μl PBS intranasally for 3 consecutive days. Twenty-four hours after the last challenge, mice were sacrificed and BAL was performed by flushing the lungs three times with 1 ml PBS containing EDTA (Sigma-Aldrich). Lungs were collected and digested using collagenase I or IV (Life Technologies) containing DNaseI (Life Technologies) for FACS analysis and partly snap-frozen or inflated with PBS/OCT (TissueTek; Sakura Finetek Europe, Zoeterwoude, the Netherlands), snap-frozen in liquid nitrogen, and stored at –80°C until further processing for histological analysis.

In some experiments, DCs were transferred i.t. to induce asthma. In brief, myeloid DCs were grown for 8 d from C57BL/6 bone marrow in the presence of rGM-CSF 200 ng/ml (kindly provided by Dr. K. Thielenmans, Belgium) and subsequently pulsed with OVA or PBS. A total of 106 DCs was injected i.t. and subsequently challenged three times using OVA aerosols as described earlier.

**Flow cytometric analysis**

BAL, lung, and mediastinal lymph node cells were collected for cellular differentiation by flow cytometry as previously described (37). In some experiments, a fraction of the isolated cells were stimulated with ionomycin (Sigma), PMA (Sigma), and GolgiPlug (BD Biosciences) at 37°C for 4 h. Next, cells were stained for CD3, CD4, and intracellularly for IL-4, IL-5, IL-10, IL-13, IL-17, and IFN-γ, after fixation with 2% PFA and permeabilization using a saponin-containing buffer (7, 21). Fixable Aqua Dead for 405 nm (Invitrogen, Molecular Probes) was used to distinguish live and dead cells.

For Gata3 protein detection in ILC2 and T cells, cells were stained for CD3, CD4, T1ST2, CD1, CD25, and lineage-positive cells (PE-conjugated Abs against CD8α, CD11b, CD11c, CD19, CD45R [B220] Ter-119, Gr-1, CD161 [NK1.1], and FcRlIe), fixed, and permeabilized by using the Fixp3 staining kit (eBioscience) according to the manufacturer’s protocol. Alexa Fluor 647–conjugated Abs against Gata3 and PECy7-conjugated Abs against T-Bet were purchased from BD Biosciences or eBioscience. Fixable Aqua Life Dead for 405 nm (Invitrogen, Molecular Probes) was used to distinguish live and dead cells. Cells were analyzed using a LSRII Flowcytometer (BD Biosciences) and with FlowJo software (Tree Star).

**Immunohistochemistry**

Immunohistochemical stainings were performed in a half-automatic stainer (Sequenza) as previously described (38). Acetone-fixed slides were washed with PBS, and incubated and blocked in diluted normal goat serum (CLB, Amsterdam, the Netherlands). Sections were stained with PE-conjugated anti–Siglec-F (E50-2440; BD Pharmingen). The primary Ab was detected using an appropriate alkaline phosphatase–conjugated secondary Ab. After rinsing, slides were incubated with New Fuchsin substrate. Finally, the sections were counterstained with Gill’s triple strength hematoxylin and mounted in VectaMount (Vector).

**Measurements of cytokine concentrations**

Cytokine levels in lung homogenates and BAL fluid were determined using commercial ELISA kit for IFN-γ (BD), IL-4 and IL-5 (both eBioscience), IL-13, IL-17, and IL-33 (all R&D) according to manufacturers’ protocol.

**Statistical analysis**

Reported values are shown as mean ± SEM. Statistical analyses were performed with SPSS (SSPS, Chicago, IL) using a Mann–Whitney U test. Resulting p values <0.05 (*), <0.01 (**), and <0.001 (****) are indicated and considered significant. Tests that did not reach significance (p > 0.05) are not indicated.

**Results**

**Enforced Gata3 expression does not enhance signs of AAI in a severe asthma model**

First, we compared asthma susceptibility of CD2-Gata3 Tg mice and wild-type (WT) littermates in an OVA-driven murine AAI model. Sensitization to inhaled OVA was induced by i.t. injection of bone marrow–derived DCs, using PBS-pulsed DCs as a control (39). Upon three OVA aerosol challenge days at days 10–12 after sensitization, mice were analyzed at day 13 (Supplemental Fig. 1A). Whereas only a few inflammatory cells were observed in the
BAL fluid of mice that received unpulsed PBS-DC, significant eosinophilic airway inflammation developed upon OVA aerosol challenge of mice that had received OVA-pulsed DCs (Supplemental Fig. 1B). However, we observed no significant differences between CD2-Gata3 Tg and WT mice in the numbers of the individual immune cell populations present in the BAL, determined by flow cytometry (Supplemental Fig. 1B). Likewise, using intracellular FACS staining, we found no differences in the numbers of Th1, Th2, or Th17 cells, typified by IFN-γ, IL-4, or IL-17 production, respectively (Supplemental Fig. 1C). Therefore, we conclude that enforced Gata3 expression in CD2-Gata3 Tg mice did not enhance AAI in a severe asthma model based on Th2 sensitization by OVA-pulsed DCs.

Enforced Gata3 expression induces susceptibility to AAI upon i.t. OVA sensitization

Next, we determined the susceptibility of CD2-Gata3 Tg mice to AAI in a very mild asthma model based on i.t. sensitization with OVA followed by OVA aerosol challenges (Fig. 1A). When we compared OVA-sensitized and PBS-sensitized WT mice 1 d after the last OVA aerosol challenge, we did not find evidence for eosinophilic inflammation by quantification of BAL cells by flow cytometry, or by immunohistochemical staining of the lungs for...
Enforced Gata3 expression increases susceptibility to AAI

Characterization of lung T cells and ILC2 in naive CD2-Gata3 Tg mice

Next, we investigated the effects of Gata3 overexpression in an HDM-driven allergic inflammation model, whereby TLR4 on epithelial cells helps drive the development of allergic reactions (41). As shown in Fig. 3A, we performed PBS or HDM sensitizations, followed by three HDM challenges at days 13–15. To focus on the early phase of inflammation, we analyzed CD2-Gata3 Tg mice and WT littermates 1 d after the last challenge. Histological analyses of lung tissue showed more peribronchial and perivascular eosinophilic infiltrates in CD2-Gata3 Tg HDM-sensitized and challenged (HDM/HDM) mice than in WT HDM/HDM or CD2-Gata3 Tg PBS/HDM mice. Quantification of BAL fluid cells showed that HDM sensitization and challenge did not induce any signs of eosinophilic AAI (Fig. 3B). In contrast, CD2-Gata3 Tg mice in the HDM/HDM group showed an ~5-fold increase in eosinophils in BAL fluid, when compared with WT HDM/HDM mice (Fig. 3B). In the HDM/HDM CD2-Gata3 Tg mice, this eosinophilia was accompanied by a more generalized inflammation of innate cells, evidenced by increased numbers of macrophages, neutrophils, and DCs (Fig. 3B). We observed a similar trend in the lungs: the highest numbers of eosinophils were present in the HDM/HDM CD2-Gata3 Tg mice (Fig. 3C).

Both in CD2-Gata3 Tg mice and WT littermates, we observed a significant increase of the total numbers of T lymphocytes in the HDM/HDM group compared with the PBS/HDM group (Fig. 3D). In BAL fluid of CD2-Gata3 Tg mice, we observed a substantial influx of IL-22 after HDM exposure, irrespective of PBS or HDM sensitization (Fig. 3D). In the lungs, the numbers of IL-22 (for gating strategy, see Supplemental Fig. 2B) upon HDM challenge was ~2-fold higher in CD2-Gata3 Tg mice than in WT mice (Fig. 3D). Collectively, these findings show that enforced CD2-driven Gata3 expression enhances HDM-driven AAL. In this model, the BAL fluid of CD2-Gata3 Tg mice contains increased numbers of leukocytes, in particular, eosinophils and ILC2.
Enforced Gata3 expression enhances cytokine production by Th2 cells and ILC2

Next, we used intracellular FACS analyses to investigate cytokine profiles of Th2 and ILC2 in BAL fluid and lungs in HDM-driven AAI. Fig. 4A shows an example of the cytokine analysis of gated CD3+ T cells and ILC2 from the lungs WT and CD2-Gata3 Tg mice. When we quantified cytokine-expressing cells in BAL fluid, we observed that enforced expression of Gata3 increased the numbers of T cells expressing the Th2 cytokines IL-4, IL-5, and IL-13, whereby differences between CD2-Gata3 Tg and WT littermates reached significance in the HDM-sensitized group (Fig. 4B). In contrast, the presence of the CD2-Gata3 transgene did not affect the numbers of T cells positive for the key Th1 cytokine IFN-γ. Remarkably, enforced expression of Gata3 did not only increase the numbers of ILC2 expressing IL-5 and IL-13, both of which are typically produced by ILC2, but also resulted in the appearance of IL-4+ and IFN-γ+ cells within the gate of the IL-33R+T1ST2+ CD25+CD127+Sca-1+ population of ILC2 (Fig. 4B).

Whereas CD2-Gata3 Tg and WT littermates had similar numbers of IFN-γ T cells in the lung, CD2-Gata3 Tg mice showed an increase in the numbers of IL-4−, IL-5−, or IL-13−expressing T cells. Importantly, the average number of Th2-expressing T cells was not different between the groups of naive, PBS/HDM-treated and HDM/HDM-treated CD2-Gata3 Tg mice (Fig. 4C). Parallel to our findings in the BAL fluid, we observed significantly increased numbers of IL-4−, IL-5−, and IL-13−expressing ILC2 in HDM-sensitized/HDM-challenged CD2-Gata3 Tg mice, compared with WT littermates. We also detected the induction of IFN-γ+ ILC2-like cells in CD2-Gata3 Tg mice (Fig. 4B).

Next, we measured cytokine levels in BAL fluid by ELISA. We found a moderate increase for IFN-γ, IL-4, and IL-5 in CD2-Gata3 Tg mice compared with WT littermates, whereby observed differences were significant for IFN-γ and IL-5 (Fig. 4D). Finally, we
found significantly increased levels of serum IgE in HDM-treated CD2-Gata3 Tg mice, compared with HDM-treated WT control littermates (Fig. 4E). Taken together, these findings in our HDM-driven AAI show that the presence of the CD2-Gata3 transgene is associated with enhanced Th2 cytokine production by both T cells and ILC2 in BAL and lung. Enforced expression of Gata3 in vivo does not affect the numbers of IFN-γ-expressing T cells, but remarkably results in an accumulation of IFN-γ-producing ILC2-like cells in BAL and lung.

**Enforced Gata3 expression is associated with an IL-33–inducible population of T1ST2+ T-bet+Gata3+Lin2 cells**

Because ILC2 normally do not express IFN-γ, we further explored the phenomenon of IFN-γ production by CD2-Gata3 Tg ILC2-like cells. Because Gata3 can directly regulate the expression of IL-33R/T1ST2 in T cells and ILC2 (21, 29, 42), and because the CD2-Gata3 transgene is already expressed in CLP cells, it is conceivable that ectopic Gata3 expression in CLP-derived non-ILC2 cells may switch on IL-33R/T1ST2 expression. In particular, ILC1 cells that express the key transcription factor T-bet and the signature cytokine IFN-γ (4) may become surface IL-33R/T1ST2+ and therefore hard to distinguish from ILC2. Because HDM challenge induces IL-33 (1, 3, 43), such IL-33R/T1ST2+ ILC1 may become activated in our HDM airway inflammation model and respond to IL-33 by producing IFN-γ.

To address this hypothesis experimentally, we administered IL-33 intranasally to CD2-Gata3 Tg mice and WT littermates, resulting in eosinophilia in the BAL fluid of both mouse groups (Fig. 5A). Analysis of BAL fluid revealed that the influx of ILC2 and T cells was significantly higher in IL-33–treated CD2-Gata3 Tg mice than in WT littermates (Fig. 5A). Flow cytometric analyses of the lung did not reveal differences in total ILC2 numbers, but demonstrated the presence of a clear and distinct population of IFN-γ Il33low ILC2-like cells exclusively in CD2-Gata3 Tg mice (Fig. 5B, Supplemental Fig. 2C for gating strategy). When we gated for total Sca1+Lin2 cells in the lung, we identified significant populations of IFN-γ+ cells only in CD2-Gata3 Tg mice, but not in WT littermate controls; these IFN-γ+ Lin2 Sca1+ cells were either T1ST2− or T1ST2+ and were CD25low (Fig. 5C). Finally, gating for T1ST2+Lin2 Gata3+ cells showed that these ILC2-like cells contained a unique population of T-bet+ cells that were only observed in the lungs of IL-33–treated CD2-Gata3 Tg mice and not in WT mice (Fig. 5D, Supplemental Fig. 2D for gating strategy). Thus, the presence of CD2-Gata3 gene is associated with an IL-33–inducible population of T1ST2+T-bet+Gata3+ Sca1+Lin2 cells, consistent with ILC1 cells that have gained IL-33R/T1ST2+ expression.

**Discussion**

The transcription factor Gata3 is highly expressed in Th2 cells and ILC2, is required for the differentiation of these cells, and acts as a key regulator of Th2 cytokine expression. In this study, we found that increased expression of Gata3 in the T cell and ILC2 lineage in mice enhanced their susceptibility to AAI.
In the presence of the CD2-Gata3 transgene eosinophilic airway inflammation was observed in mild models (involving i.t. sensitizations with OVA or HDM both without adjuvant), which fail to induce AAI in WT mice. Remarkably, the presence of the CD2-Gata3 Tg transgene resulted in a significant increase of the expression of IL-4, IL-5, and IL-13 (but not INF-γ) in pulmonary T cells already in naive mice (Fig. 2E). Furthermore, the increased numbers of Th2 cytokine-expressing T cells did not change upon HDM challenge in PBS-sensitized or HDM-sensitized mice (Fig. 4C). Together with the findings that the T cell population in CD2-Gata3 Tg mice contains increased proportions of T cells with a memory phenotype and increased expression of IL-33R/T1ST2 (29), our results suggest that Gata3 supports the formation of a Th2-committed IL-33R/T1ST2+ memory T cell compartment in vivo in the lung. ILC2 in the lungs of naive CD2-Gata3 Tg mice manifested slightly increased cytokine expression, compared with WT mice (Fig. 2E), and upon HDM challenge their cytokine expression is induced to levels that are significantly higher than those of WT ILC2 (Fig. 4C). Consistent with the innate characteristics of ILC2, that is, the absence of an Ag receptor, their

**FIGURE 4.** Enforced Gata3 expression enhances cytokine production by airway Th2 cells and ILC2. (A) Cytokine expression profiles of gated lung T cells (left) and ILC2 (right) of naive WT or CD2-Gata3 Tg mice upon 4 h of PMA/ionomycin stimulation. Data are shown as contour plots; proportions of cells in the indicated quadrants are given (in %). (B) Quantification of flow cytometric analysis of T cells (left) and ILC2 (right) positive for the indicated cytokines upon 4 h of PMA/ionomycin stimulation from BAL WT (white bars) and CD2-Gata3 Tg mice (gray bars). Results are expressed as means ± SEM. (C) Quantification of flow cytometric analysis of T cells (left) and ILC2 (right) positive for the indicated cytokines upon 4 h of PMA/ionomycin stimulation from collagenase-treated lung WT (white bars) and CD2-Gata3 Tg mice (gray bars). Results are expressed as means ± SEM. (D and E) Quantification of levels of INF-γ, IL-4, and IL-5 in BAL fluid (D) and IgE in serum (E) from WT (white bars) and CD2-Gata3 Tg mice (gray bars) as measured by ELISA. Data are shown as average values ± SEM. Results represent one of two independent experiments with three to six animals per group. *p < 0.05, **p < 0.01.
do not detect differences in IL-33 expression between CD2-Gata3 Tg and WT littermates in lung homogenates and observed a similar increase upon HDM challenge in both mouse groups (data not shown). An important role of Gata3 in enhancing IL-33R–mediated ILC2 activation would be consistent with the finding that in our asthma model based on i.t. instillation of OVA-primed DCs (thus bypassing the role of IL-33–expressing epithelial cells or activated macrophages), we did not detect differences between CD2-Gata3 Tg and WT littermates (Supplemental Fig. 1). The differences between the DC installation and OVA/HDM sensitization models would point to an important role of IL-33 as a trigger in AAI in susceptible individuals. In this context, it was shown that IL-33 is a steroid-resistant cytokine, and because levels are increased in steroid-resistant asthma patients (44), the IL-33–IL-33R/T1ST2 axis could be a potential therapeutic target to inhibit Th2 cytokine induction in susceptible individuals (44, 45). Finally, we found that already in the absence of sensitization, HDM-challenged CD2-Gata3 Tg mice manifested significant recruitment of DCs, next to some eosinophilic inflammation (Fig. 3B). It is therefore conceivable that ILC2 may act in parallel with epithelial cells that are induced by HDM to produce CCL20 and GM-CSF to support recruitment and activation of DCs (46). Further experiments should identify chemokines and cytokines that are produced by activated ILC2. Just like IL-5 and IL-13, such factors may be regulated by Gata3.

Several other groups have generated Tg mice with overexpression of Gata3 and have consistently reported enhanced eosinophilic airway inflammation (33–36). In chronic models that involve repeated allergen exposure, enforced Gata3 expression was associated with increased subepithelial fibrosis and airway smooth muscle hyperplasia (35). Although in most of these published models Gata3 expression was also driven by the human CD2 promoter, the effects of Gata3 overexpression on cytokine profiles appeared variable: limited effects on IL-4 but increased IL-5/IL-13 expression (33), increased IL-4 but similar IL-5 levels (35), and increased production of IL-4, IL-5, and IL-13 (36) have all been described. Because we observed the largest effects of Gata3 in naive or nonsensitized mice, it is conceivable that acute and more chronic models for allergic inflammation may show diverging effects of Gata3. Furthermore, Gata3 overexpression may differentially affect Th2 cells and ILC2. In this context, a paradoxical finding was that enforced Gata3 expression, which does not affect IFN-γ production by circulating (29, 31, 32) or pulmonary T cells (Fig. 4A, 4C), resulted in increased expression of IFN-γ by cells that had characteristics of pulmonary ILC2 (Fig. 4A, 4C). Our finding of a population of IL-33–inducible T1ST2+ T-bet+Gata3+ Lin− cells exclusively in CD2-Gata3 Tg mice would support our hypothesis that ectopic expression of Gata3 in T-bet+ ILC1 may induce T1ST2+ expression in these cells. Stimulation of these cells by IL-33, induced by HDM challenge (1, 3, 43), would then signal for production of IFN-γ in these Gata33 T-bet+ ILC1. Our findings also imply that ectopic expression of Gata3 is not sufficient to downregulate T-bet and IFN-γ expression in ILC1. Nevertheless, we cannot formally exclude the alternative explanation for the finding of IFN-γ expression in cells with ILC2 characteristics, that is, ILC2 plasticity. It remains possible that ILC2 show plasticity in cytokine expression, because this has also been demonstrated for ILC3 (47–51). This plasticity is also reflected by the apparently variable capacity of ILC2 to produce IL-4 (3, 9, 43) (Fig. 4B, 4C).

In summary, we have shown that enforced expression of Gata3 is sufficient to enhance Th2 and ILC2 activity. As a result, because of joint efforts between Th2 cells and ILC2, production of the proinflammatory cytokines IL-4, IL-5, and IL-13 is enhanced and mice become remarkably susceptible to eosinophilic inflammation,
even after mild exposure to inhaled harmless Ags that usually leads to inhalational tolerance. Interestingly, because IL-33/ST2 expression is directly controlled by Gata3 (42), our findings suggest that the observed association in human GWAS studies of asthma with the IL-33/ST2 gene might be based on effects in the T cell, as well as the ILC2 lineage.

Disclosures
The authors have no financial conflicts of interest.

References


