Essential and Instructive Roles of GATA Factors in Eosinophil Development

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Abstract

GATA transcription factors are major regulators of hematopoietic and immune system. Among GATA factors, GATA-1, GATA-2, and GATA-3 play crucial roles in the development of erythroid cells, hematopoietic stem, and progenitor cells, and T helper type 2 (Th2) cells, respectively. A high level of GATA-1 and GATA-2 expression has been observed in eosinophils, but their roles in eosinophil development remain uncertain both in vitro and in vivo. Here we show that enforced expression of *GATA-1* in human primary myeloid progenitor cells completely switches myeloid cell fate into eosinophils. Expression of *GATA-1* exclusively promotes development and terminal maturation of eosinophils. Functional domain analyses revealed that the COOH-terminal finger is essential for this capacity while the other domains are dispensable. Importantly, GATA-1–deficient mice failed to develop eosinophil progenitors in the fetal liver. On the other hand, GATA-2 also showed instructive capacity comparable to GATA-1 in vitro and efficiently compensated for GATA-1 deficiency in terms of eosinophil development in vivo, indicating that proper accumulation of GATA factors is critical for eosinophil development. Taken together, our findings establish essential and instructive roles of GATA factors in eosinophil development. GATA-1 and GATA-2 could be novel molecular targets for therapeutic approaches to allergic inflammation.

Key words: GATA-1 • GATA-2 • eosinophil • myeloid cell • allergy

Introduction

Eosinophils play important roles in host immune defense against helminthic parasites and in the pathophysiology of allergic diseases. Eosinophils develop from progenitor cells in the bone marrow under the control of cytokines including IL-3, GM-CSF, and IL-5 (1). In allergic diseases, eosinophils are specifically recruited to the site of allergic inflammation through specific upregulation of adhesion and chemoattractive molecules as well as increased production of eosinophils in the bone marrow (2).

Transcriptional regulation is a key step in the commitment and differentiation of hematopoietic cells (3, 4). Through detailed analyses of eosinophil-specific promoters, GATA-1, CCAAT/enhancer binding protein (C/EBP)* factors, and RFX have been implicated in the eosinophil-specific gene expression (5–7). Among them, C/EBP and GATA-1 have been characterized in the chicken transformed hematopoietic system, in which C/EBP factors drive eosinophil differentiation of the chicken transformed

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**Abbreviations used in this paper:* C/EBP, CCAAT/enhancer binding protein; EGFP, enhanced GFP; EPO, eosinophil peroxidase; FOG-1, friend of GATA-1; GFP, green fluorescent protein; MBP, major basic protein; RT, reverse transcription.

hematopoietic progenitor cells (8) and an intermediate level of GATA-1 reprograms chicken myelomonocytic cell lines into eosinophils (9). On the other hand, friend of GATA-1 (FOG-1), a cofactor for GATA-1, inhibits eosinophil differentiation (10). These findings indicate that GATA-1, C/EBP, and FOG-1 act within a regulatory loop during the chicken eosinophil development. Consistent with these findings, C/EBPα-deficient mice shows a selective block in the development of eosinophils as well as neutrophils (11). Furthermore, we have recently shown that enforced expression of C/EBPα in human $CD34^+$ myeloid progenitor cells facilitates both eosinophil and neutrophil development (12). In contrast, the role of GATA-1 in eosinophil development has not yet been characterized in primary hematopoietic cells or in genetically altered mice.

GATA factors comprise a family of transcription factors that have highly conserved zinc finger DNA binding domains. Among six members, GATA-1, GATA-2, and GATA-3 play major roles in hematopoietic and immune system, especially in the development of erythroid cells, hematopoietic stem and progenitor cells, and Th2 cells, respectively (13–17). In addition, expression of GATA-1 and GATA-2 are specifically observed in eosinophils and mast cells during myeloid differentiation (18). We have previously demonstrated that GATA-1 and GATA-2 are differentially expressed during mast cell development and play important roles in establishing diverse mast cell population (19). In this study, we analyzed the roles of GATA-1 and GATA-2 in eosinophil development both in vitro and in vivo and disclosed their novel functions in organizing immune system.

Materials and Methods

Mice. Generation of mice with an erythroid promoter-specific "knock-down" allele of the *GATA-1* gene (*GATA-1.05* mice) has been described previously (14). *IE3.9int* is the mouse GATA-1 gene regulatory region that contains 3.9 kb of 5′ sequence to the IE exon, the IE exon itself, the first intron, and a part of the second exon of the *GATA-1* gene. *IE3.9int* is capable of conferring the complete *GATA-1* expression profile to a reporter transgene in hematopoietic cells (20). Generation of *IE3.9int*-directed green fluorescent protein (GFP) transgenic mice and rescue experiments of *GATA-1.05* mice by *IE3.9int*-directed GATA factor transgenes have been described (20, 21).

Production of Retrovirus. A series of mouse GATA-1 mutant cDNAs were described previously (21). Dominant negative-type GATA genes were generated by replacing NH_2-terminal portion of GATA-1 (amino acids 1–167) and GATA-2 (amino acids 1–259) with the engrailed repression domain (amino acids 2–298; reference 22). All cDNAs were FLAG-tagged at the NH_2 terminus, then subcloned into the retrovirus vector GCsam, which has an LTR derived from MSCV and intact splice donor and splice acceptor sequences for generation of subgenomic mRNA (23). To produce the recombinant retrovirus, the retrovirus vector was transfected into 293gp cells (293 cells containing the *gag* and *pol* genes but lacking an envelope gene) along with 10A1 *env* expression plasmid (pCL-10A1) by $CaPO_4$ coprecipitation and the supernatant from the transfected cells was collected to infect cells.

Preparation of Human Primary and Cultured Cells. Human umbilical cord blood samples were obtained, with informed consent, from placentas of full-term normal newborn infants. After isolation of mononuclear cells by density gradient centrifugation, $CD34^+$ hematopoietic progenitors were obtained using magnetic bead separation (Miltenyi Biotech). In all experiments, ~95% of purified cells were positive for CD34 as judged by specific antibody (23). For reverse transcription (RT)-PCR analysis, cord blood $CD34^+$ cells and glycophorin A^+ erythroblasts were purified by cell sorting on a FACS Vantage™ (Becton Dickinson). To obtain eosinophils, cord blood $CD34^+$ cells were incubated in the presence of 50 ng/ml stem cell factor (SCF), IL-3, and GM-CSF for 5 d, then further incubated in the presence of IL-5 (50 ng/ml) only for 10 d. To exclude macrophages, cultured cells were stained with PE-conjugated CD14 (BD PharMingen), and then CD14-negative cells were collected by cell sorting. May-Grüenwald Giemsa staining revealed almost all cells collected are eosinophils.

Transduction of $CD34^+$ Cells. $CD34^+$ cells were prestimulated in IMDM (Sigma-Aldrich) supplemented with 10% FBS, 50 ng/ml SCF, thrombopoietin (TPO; kindly provided by KIRIN, Tokyo, Japan), IL-6, and Flt-3 ligand (PeproTech) for 20 h. After replating onto recombinant fibronectin fragment-coated culture dishes (Takara Shuzo) containing virus supernatant and 5 μg/ml protamine sulfate (Sigma-Aldrich), cells were centrifuged at 1,000 *g* for 30 min. Transduction was repeated with fresh virus supernatant every 12 h three times. At 60 h after the first transduction, cells were stained with PE-conjugated anti-CD34 (BD PharMingen). Cells positive for both CD34 and enhanced GFP (EGFP) were selected by cell sorting and subjected to subsequent analyses. At this time point, around 85% of the cells were still positive for CD34 (23)

In Vitro Colony Assay and Liquid Culture. $CD34^+$ cells transduced with indicated retroviruses were plated in methylcellulose medium (StemCell Technologies, Inc.) or cultured in IMDM with 10% FBS. Cytokines were supplemented to culture at the concentrations of 50 ng/ml human SCF, GM-CSF, IL-3, and IL-5 (PeproTech), and 5 U/ml human erythropoietin (kindly provided by Chugai Pharmaceutical Inc., Tokyo, Japan). The culture dishes were incubated at 37°C in a 5% CO_2 atmosphere. Colony numbers were counted at day 14. To check the development of IL-5–responsive eosinophils, cells were cultured in the presence of SCF and GM-CSF for the first 5 d. Then, cells were further incubated in the presence of IL-5 alone. Alternatively, preculture media were supplemented with IL-3 in addition to SCF and GM-CSF to facilitate development of eosinophil progenitors.

Analysis of Eosinophil Development in Mice. The fetal liver cell suspensions were prepared by dissecting E11.5 fetal livers by pipetting. The bone marrow cell suspensions were prepared from adult mice and were overlaid with sodium metrizoate solution (1.086 g/ml; Nycomed), then centrifuged at 400 *g* for 20 min. The low-density cells were harvested and stained with anti-lineage marker antibodies (Mac-1, Gr-1, B220, CD4, CD8, and TER119; BD PharMingen), then lineage marker-negative (Lin^-) cells were collected by cell sorting. The fetal liver and Lin^- bone marrow cells were plated in methylcellulose medium (StemCell Technologies, Inc.) in the presence of mouse IL-5 (50 ng/ml; Genzyme) or in the presence of mouse SCF, IL-3 (10 ng/ml), and human erythropoietin (5 U/ml). Colony numbers were counted at day 10.

Flow Cytometric Analysis. Expression of the cell surface and cytoplasmic antigens was analyzed on a FACS Vantage™. To detect cell surface antigens, cells were stained with allophycocyanin

(APC)-conjugated anti–human CD11b and PE-conjugated CD14 (BD PharMingen). To detect cytoplasmic major basic protein (MBP), cells were fixed in 4% paraformaldehyde and permeabilized in 0.3% saponin, then stained with anti–human MBP (Nichirei) followed by PE-conjugated anti–mouse immunoglobulins (BD PharMingen). Cells that marked with propidium iodide were gated out as dead cells.

Cytochemical Analysis. Cells were cytocentrifuged onto glass slides and were stained by May-Grüenwald Giemsa staining. To detect eosinophil-specific granules, cytospun cells were fixed in methyl alcohol, then stained with 0.2% Fast green (Sigma-Aldrich) in 70% ethyl alcohol. To detect eosinophil peroxidase (EPO) expression, cytospun cells were fixed in 4% paraformaldehyde and 60% acetone, then stained with anti–human EPO (BD PharMingen) followed by EnVision labeled polymer/alkaline phosphatase (Dako).

RT-PCR. Total RNA was isolated from sorted cells using ISOGEN-LS solution (Nippon Gene) and reverse-transcribed using ThermoScript RT-PCR system (GIBCO BRL) and oligo-dT primer. The amount of cDNA was normalized by the quantitative PCR using TaqMan rodent GAPDH control reagent (PerkinElmer/Applied Biosystem). Semi-quantitative RT-PCR reactions were then performed for 35 cycles using normalized cDNAs and recombinant Taq DNA polymerase (Takara). Cycling parameters were denaturation at 94°C for 20 s, annealing at 58°C for 20 s, and extension at 72°C for 30 s. PCR products were separated on agarose gels and visualized by ethidium bromide staining. The primer sequences were: GATA-1 sense primer 5′-TCA ATT CAG CAG CCT ATT CC-3′; antisense primer 5′-TTC GAG TCT GAA TAC CAT CC-3′, GATA-2 sense primer 5′-TGT TGT GCA AAT TGT CAG ACG-3′; antisense primer 5′-CAT AGG TGC CAT GTG TCC AGC-3′, GATA-3 sense primer 5′-AAG TGC ATG ACT CAC TGG AGG-3′; antisense primer 5′-TAG GCT TCA TGA TAC TGC TCC-3′.

Results

Expression of GATA Family Genes in Eosinophils. High levels of mRNA expression of GATA genes have been reported in human primary eosinophils (18). In this study, RT-PCR analysis also demonstrated that expression of *GATA-1* and *GATA-2* in human eosinophils is comparable to that in hematopoietic progenitor cells and/or erythroblasts. In contrast, expression of GATA-3 was not significant in eosinophils (Fig. 1 A). Next, we analyzed expression of *GATA-1* in mouse eosinophils. We previously generated transgenic mice bearing *IE3.9int*-directed GFP (20). *IE3.9int* is the GATA-1 gene regulatory region that contains 3.9 kb of 5′ sequence to the IE exon, the IE exon itself, the first intron, and a part of the second exon of the *GATA-1* gene. *IE3.9int* is capable of conferring the complete *GATA-1* expression profile to a reporter transgene in hematopoietic cells (20). IL-5 is a late-acting cytokine that specifically supports eosinophil proliferation. In the presence of IL-5 alone, bone marrow cells from transgenic mice predominantly gave rise to GFP-positive colonies (Fig. 1 B). All the GFP-positive colonies consisted of mature eosinophils, which were positive for fast green staining that detects eosinophil granules (data not shown). On the other hand, the colonies of neutrophils and/or macrophages formed in the presence of IL-3 did not express GFP at all (data not shown). These findings indicate that the transcription of *GATA-1* gene is active in mouse eosinophils.

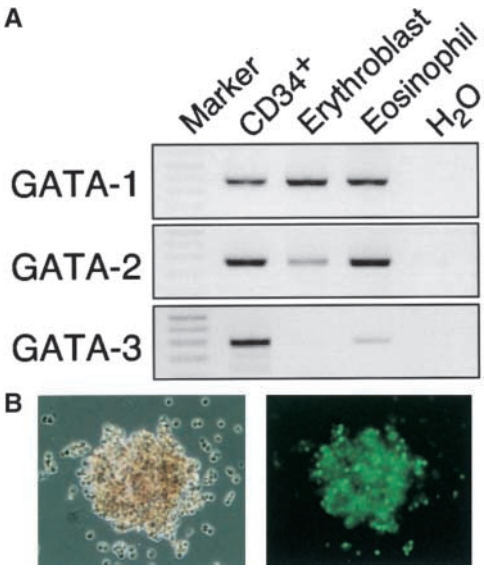

Figure 1. Expression of GATA family genes in eosinophils. (A) Expression of GATA family genes in human eosinophils. RT-PCR was performed on normalized cDNA templates from cord blood $CD34^+$ cells, glycophorin A^+ erythroblasts, and eosinophils obtained by in vitro culture (details in Materials and Methods). Lane H_2O represents the negative control without template. (B) Expression of *GATA-1* in mouse eosinophils. Eosinophil colonies were generated from bone marrow cells of transgenic mice bearing *IE3.9int*-directed GFP in the presence of IL-5 alone. Bright field micrograph (left) and fluorescence micrograph (right).

Role of GATA Factors in Eosinophil Development In Vitro. Roles of GATA factors in eosinophil development have not yet been characterized in primary hematopoietic system. By using retrovirus, we expressed *GATA-1*, *GATA-2*, and their dominant negative-type genes in human $CD34^+$ hematopoietic progenitor cells. The dominant negative GATA-1 and GATA-2 contain engrailed repression domain instead of their own NH_2-terminal activation domain (Fig. 2 A), which strongly repress GATA-dependent transcriptional activation. The retroviral vector drives expression of both GATA-related genes and EGFP from a single bicistronic message. After transduction, cells positive for both CD34 and EGFP were selected by cell sorting and subjected to in vitro assay.

To evaluate the effects of GATA-1 and GATA-2 on eosinophil commitment, we first assessed the frequency of eosinophil colony formation. In the presence of SCF and GM-CSF, $CD34^+$ cells transduced with either *GATA-1* or *GATA-2* gave rise to comparable number of colonies to the control. Surprisingly, in contrast with the control that predominantly gave rise to granulocyte/macrophage colonies, $CD34^+$ cells expressing either *GATA-1* or *GATA-2* exclusively gave rise to eosinophil colonies (Fig. 2 B). Eosinophil colonies consisted of mature eosinophils, which were positive for fast green staining that detects eosinophil

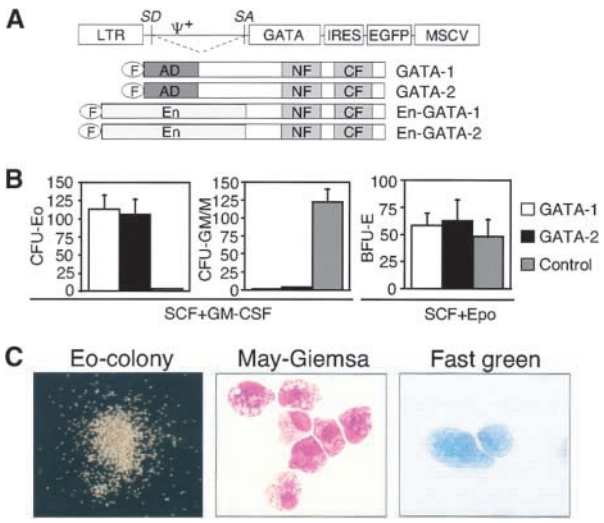

Figure 2. GATA-1 and GATA-2 promote human eosinophil development. (A) Schematic representation of the retroviral vector, GCsam-IRES-EGFP, harboring wild-type or dominant negative GATA genes linked by an internal ribosome entry site (IRES) to a cDNA encoding EGFP. ψ+, packaging signal; SD, splice donor; SA, splice acceptor; F, Flag-tag; AD, activation domain; NF, NH_2-terminal zinc finger; CF, COOH-terminal zinc finger; En, engrailed repression domain. (B) Colony formation of $CD34^+$ cells transduced with wild-type GATA genes. Human $CD34^+$ hematopoietic progenitor cells transduced with empty vector (control), *GATA-1* or *GATA-2* were cultured in methylcellulose medium. CFU-GM/M, CFU-granulocyte/macrophage and macrophage, BFU-E, erythroid burst-forming unit. Results are shown as mean ± SD per 1,000 cells of three representative experiments. (C) The morphology of an eosinophil colony generated from *GATA-1*–transduced $CD34^+$ progenitor cells. Morphology under a phase-contrast microscope (left). The eosinophil colony was lifted and cytocentrifuged onto a glass slide, then stained by May-Grüenwald Giemsa staining (middle) and Fast green staining (right).

granules (Fig. 2 C). There was no significant difference in erythroid colony formation between the control and GATA-expressing cells (Fig. 2 B; BFU-E).

In liquid culture supplemented with SCF and GM-CSF that allows myeloid cell development, cells transduced with either *GATA-1* or *GATA-2* showed slightly decreased cell growth compared with the control, and cells transduced with dominant negative GATA genes showed even lower cell growth rate (Fig. 3 A). To check the development of IL-5–responsive eosinophil progenitors, the transduced cells were cultured in the presence of SCF and GM-CSF for the first 5 d, and then cytokines were replaced with IL-5, the cytokine that specifically supports eosinophil proliferation. Only cells transduced with *GATA-1* or *GATA-2* proliferated in an IL-5-dependent manner (Fig. 3 A). Next, we supplemented culture medium with IL-3 in addition to SCF and GM-CSF. Under this cytokine condition, eosinophil development is further promoted and even the control cells showed IL-5–dependent cell growth. However, the cells transduced with dominant negative GATA genes did not respond to IL-5 at all (Fig. 3 A), indicating an crucial role of GATA factors for the development of eosinophil progenitors in vitro. Flow cytometric analysis revealed that cells expressing GATA genes failed to develop $CD14^+$ macrophages, but gave rise to MBP-positive eosinophils (Fig. 3 B). Cytological analyses again demonstrated the eosinophilic features of transduced cells, which were positive for both EPO and fast green staining (Fig. 3 C). These data clearly show that GATA-1 and GATA-2 have a strong instructive ability to render myeloid progenitor cells to commit into eosinophils and to support their terminal differentiation.

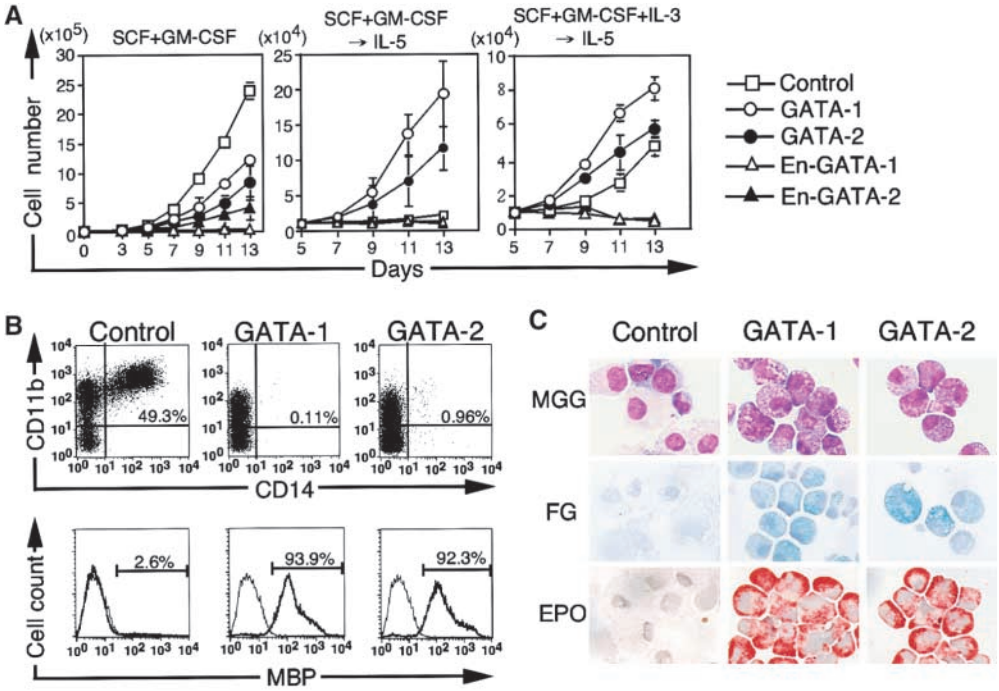

Figure 3. Effect of GATA genes on eosinophil development. (A) Cell growth and differentiation of $CD34^+$ progenitor cells transduced with either wild-type GATA or dominant negative GATA. Cytokine-dependent growth of transduced cells was evaluated by liquid culture. To evaluate IL-5–dependent cell growth, cells were cultured in the presence of SCF and GM-CSF for the first 5 d. Then, cytokines were replaced to IL-5 alone (middle). Alternatively, preculture media were supplemented with IL-3 in addition to SCF and GM-CSF to facilitate development of eosinophil progenitors (right). Results are shown as mean ± SD of three representative experiments. (B) Flow cytometric profiles of transduced cells cultured for 10 d in the presence of SCF and GM-CSF. The percentages of $CD11b^+CD14^+$ macrophages and MBP^+ eosinophils are indicated. (C) Eosinophilic features of *GATA*-expressing cells. $CD34^+$ progenitor cells transduced with wild-type GATA genes were cultured for 10 d in the presence of SCF and GM-CSF, then analyzed by May-Grüenwald Giemsa staining (MGG), Fast Green staining (FG), and immunostaining for intracytoplasmic EPO.

GATA-1 Functional Domains Responsible for Eosinophil Development. To clarify the requirement for GATA-1 functional domains during eosinophil development, we introduced a series of GATA-1 mutants into $CD34^+$ progenitor cells. The GATA-1 mutants used are an NH_2-terminal deletion (ΔNT), an NH_2-terminal zinc finger deletion (ΔNF), a COOH-terminal zinc finger deletion (ΔCF), and a mutant that lacks both the NH_2 terminus and the NH_2-terminal zinc finger (ΔNTNF; reference 21). We further generated an additional mutant with a single amino acid substitution of valine 205 into glycine (V205G) that abolishes interaction with FOG-1 (V205G; Fig. 4 A). In the colony assay supplemented with SCF and GM-CSF, $CD34^+$ progenitor cells transduced with wild-type GATA-1, ΔNF, or V205G gave rise to comparable number of eosinophil colonies (Fig. 4 B). Notably, $CD34^+$ cells transduced with ΔNT gave rise to a higher number of eosinophil colonies. $CD34^+$ cells transduced with ΔNTNF formed slightly decreased number of eosinophil colonies. Cells with ΔCF were even more inefficient in forming eosinophil colonies and formed a significant number of granulocyte/macrophage colonies. Importantly, the eosinophil colonies generated from cells transduced with ΔNTNF or ΔCF were much smaller in size than the others (data not shown).

In liquid culture supplemented with SCF and GM-CSF, cells transduced with ΔNT and ΔNTNF showed comparable cell growth with the control while the others showed retarded cell growth (Fig. 4 C, left panel). EPO staining revealed that ΔNT, ΔNF, and V205G efficiently

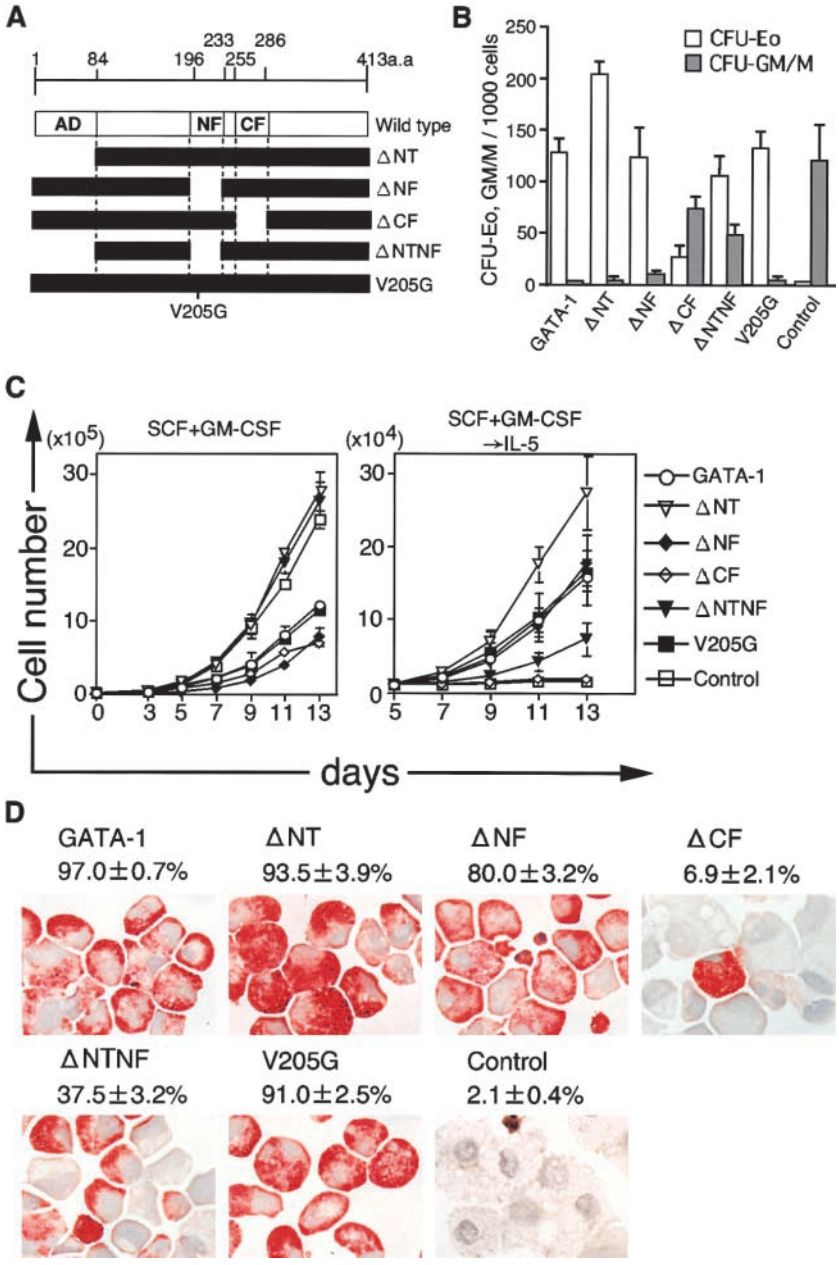

Figure 4. GATA-1 functional domains responsible for eosinophil development. (A) Schematic representation of GATA-1 mutants. (B and C) Growth and differentiation of $CD34^+$ cells transduced with GATA mutants. (B) $CD34^+$ cells transduced with empty vector (control), wild-type GATA-1, or GATA-1 mutants were cultured for 14 d in methylcellulose medium supplemented with SCF and GM-CSF. (C) Cytokine-dependent cell growth was also evaluated by liquid culture as in Fig. 3 A. Results are shown as mean ± SD of three representative experiments. (D) Effects of GATA-1 mutants on eosinophil development. Transduced $CD34^+$ progenitor cells were cultured for 10 d in the presence of SCF and GM-CSF, then analyzed by immunostaining for intracytoplasmic EPO. The percentages of EPO^+ eosinophils are indicated as mean ± SD of three representative experiments.

drive eosinophil development while ΔNTNF is less efficient and ΔCF barely induces eosinophil development (Fig. 4 D). As expected from these data, IL-5–responsive cell growth was promoted with cells transduced with wild-type GATA-1, ΔNT, ΔNF, or V205G, and was most evident with ΔNT-transduced cells. In contrast, cells with ΔNTNF poorly responded to IL-5 and ΔCF did not respond at all (Fig. 4 C, right panel). All these data indicate that both NT and NF domains are dispensable in promoting eosinophil development, and the CF domain is absolutely required in this process.

Essential Role of GATA Factors in Eosinophil Development In Vivo. Analyses of definitive hematopoiesis in the GATA-1–deficient fetal liver thus far have demonstrated defects in differentiation specific to erythroid and megakaryocytic lineages (13–15). Eosinophil development occurs in the fetal liver from the definitive hematopoietic stem cells. To determine the role of GATA-1 in eosinophil development in vivo, we analyzed the development of eosinophil progenitor cells in the *GATA-1*–deficient fetal livers (Table I). We previously generated mice with an erythroid promoter-specific mutant allele of the *GATA-1* gene (*GATA-1.05* mice), in which *GATA-1* was expressed at ~5% of the wild-type level (14). Because *GATA-1* is located on the X chromosome, all male embryos hemizygous for the mutation show GATA-1–deficient phenotypes. As previously observed, the mutant fetal livers showed defective erythropoiesis and contained much less nucleated cells than the wild-type livers. Nonetheless, they contained similar numbers of colony forming units to the wild-type livers. Notably, IL-5–responsive eosinophil colonies were formed from wild-type fetal liver cells but not from mutant fetal liver cells, indicating that eosinophil progenitor cells do not develop in the absence of GATA-1. We next analyzed if transgenic expression of GATA factor genes could rescue deficient eosinophil development in *GATA-1.05* mice. We previously succeeded to rescue the embryonic lethal phenotype of *GATA-1.05* mice by GATA factor transgenes under the control of the *GATA-1 IE3.9int* gene (20). Although rescued mice by GATA-2 but not by GATA-1 transgene develop anemia after birth, they survive and were subjected to analysis for eosinophil development. As expected from in vitro data in Figs. 2 and 3, GATA-2 as well as GATA-1 transgenes efficiently compensated for GATA-1 deficiency in terms of eosinophil development in vivo (Table II).

Table I. *Eosinophil Progenitors in the Fetal Liver*

			No. of CFU			
			per liver		per 10^5 cells	
Genotype	Embryo no.	Total cell no. ($\times 10^5$)	Eosino	GM/Mix	Eosino	GM/Mix
male (y/−)	1	0.30	0.00	950	0.00	3,167.0
	2	0.20	0.00	1,560	0.00	7,800.0
	3	0.15	0.00	1,230	0.00	8,200.0
	Avg. (n = 3)	0.22	0.00	1,247	0.00	6,389.0
female (+/−)	4 (n = 1)	2.00	26.64	4,130	13.32	2,065.0
male (y/+)	5	1.60	25.53	460	15.96	287.5
	6	2.40	45.51	1,290	18.96	537.5
	7	3.60	16.65	2,160	4.59	595.0
	8	3.00	42.18	1,580	14.06	526.7
	Avg. (n = 4)	2.40	32.47	1,373	13.39	486.7
female (+/+)	9	2.70	21.09	1,000	7.81	370.4
	10	3.50	62.16	2,440	17.76	697.1
	11	4.00	34.41	2,060	8.60	515.0
	12	4.78	57.72	2,320	12.15	488.4
	13	4.25	56.61	2,440	13.32	574.1
	Avg. (n = 5)	3.84	46.40	2,052	11.93	529.0

E11.5 fetal liver cells were plated in methylcellulose medium supplemented with IL-5 alone (for CFU-Eo) or SCF, IL-3, and erythropoietin (for CFU-GM/Mix). Colony numbers were counted at day 10. CFU/Mix, mixed-CFU. Numbers of nucleated cells recovered from the fetal liver, CFU per liver and CFU per 10^5 nucleated cells are presented.

Discussion

Eosinophils develop from myeloid progenitor cells along with neutrophils and macrophages. In this study, we demonstrated that GATA-1 strongly promotes eosinophil commitment of primary myeloid progenitor cells and supports their terminal differentiation. This effect was much more drastic than that of GATA-1 to reprogram chicken transformed myeloblasts into eosinophils. In addition, we clearly showed an essential role of GATA-1 in eosinophil development in vivo. These data establish GATA-1 as an essential regulator for eosinophil development.

Thus far, C/EBPα is known to be essential for eosinophil development (11). However, C/EBPα is also crucial for neutrophil development (11). Enforced expression of C/EBPα in human $CD34^+$ myeloid progenitor cells facilitates both eosinophil and neutrophil development (12). In contrast, GATA-1 exclusively promoted eosinophil development in this study. With respect to induction of eosinophil development, GATA-1 is more specific and efficient than C/EBPα. These data indicate that the expression of GATA-1 in combination with C/EBPα could be the critical determinant for eosinophil lineage commitment. On the other hand, GATA-2 showed instructive capacity comparable to GATA-1 in vitro and efficiently compensated for GATA-1 deficiency in terms of eosinophil development in vivo, indicating that proper accumulation of GATA factors is essential for eosinophil development. However, endogenous GATA-2 did not compensate for the loss of GATA-1 function in vivo as has been observed in erythropoiesis (13, 14). These data may also indicate functional distinctions between GATA-1 and GATA-2. The role of GATA-2 in eosinophil development in vivo remains to be determined.

Structural analysis of GATA-1 in eosinophil development revealed that the NT and NF domains are dispensable in promoting eosinophil development. In addition, interaction with FOG-1 was not vital. These profiles of domain requirement are in good agreement with those of GATA-1 in the chicken eosinophil development that does not require the NF domain (10), but contrast with those of GATA-3 in Th2 development, in which each of the functional domains is required (24). Although we cannot directly compare these data with those of in vivo rescue experiments in *GATA-1.05* mutants, we previously showed that either NT or NF domain is dispensable during primitive but not definitive erythropoiesis (21). As CF encompasses the DNA-binding domain, these data indicate that the site-specific DNA binding is sufficient for the initiation of eosinophil development from primary myeloid progenitor cells that retain multipotency in differentiation. Interestingly, ΔNT was most efficient in promoting eosinophil development, indicating that the NT domain mediates some negative regulations for eosinophil commitment or proliferation. The NT domain could play distinct functions in eosinophils from those in other cell lineages. Although ΔCF do not bind to DNA, it slightly promoted eosinophil development. This effect could be mediated by indirect mechanism, for example, by sequestrating GATA-interacting factors such as FOG-1, which could negatively regulate GATA-1 in eosinophils (10), thereby enhancing endogenous GATA-1 function.

To inhibit Th2 lymphocyte–mediated allergic inflammation, many approaches have been taken that target GATA-3, such as local delivery of GATA-3 antisense oligonucleotides (25). Our data clearly implicated GATA-1 and GATA-2 in the regulation of eosinophil development and function. In addition, we have previously demonstrated that GATA-1 and GATA-2 play differential but important roles in mast cell differentiation and function (19). Because eosinophils and mast cells are largely responsible for development and progression of allergic inflammation, and GATA-1 and GATA-2 are predominantly expressed in eosinophils and mast cells in the site of allergic inflammation, GATA-1 and GATA-2 could be new therapeutic targets for the local treatment of allergy.

Table II. *Transgenic Rescue of GATA-1.05 Mutant Mice*

Genotype	Mouse no.	CFU per 10^5 Lin^- cells	
		Eosino	GM/Mix
Male (y/+)	1	21.8	2,740
	2	28.1	3,880
	3	22.0	3,150
	Avg. (n = 3)	23.9	3,257
Male (y/−):: GATA-1 transgene	4 (n = 1)	13.0	2,720
Male (y/−):: GATA-2 transgene	5	13.8	4,370
	6	25.6	4,130
	Avg. (n = 2)	19.7	4,250

Lineage marker–negative (Lin^-) bone marrow cells that did not react with anti-lineage marker antibodies (Mac-1, Gr-1, B220, CD4, CD8, and TER119) were collected by cell sorting and plated in methylcellulose medium supplemented with IL-5 alone (for CFU-Eo) or SCF, IL-3, and erythropoietin (for CFU-GM/Mix). Colony numbers were counted at day 10. Numbers of CFU per 10^5 Lin^- cells are presented.

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