Targeted Deletion of a High-Affinity GATA-binding Site in the GATA-1 Promoter Leads to Selective Loss of the Eosinophil Lineage In Vivo

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Abstract

Transcription factor GATA-1 reprograms immature myeloid cells to three different hematopoietic lineages-erythroid cells, megakaryocytes, and eosinophils. GATA-1 is essential for maturation of erythroid and megakaryocytic precursors, as revealed by gene targeting in mice. Here we demonstrate that deletion of a high-affinity GATA-binding site in the GATA-1 promoter, an element presumed to mediate positive autoregulation of GATA-1 expression, leads to selective loss of the eosinophil lineage. These findings suggest that GATA-1 is required for specification of this lineage during hematopoietic development. Mice lacking the ability to produce eosinophils should prove useful in ascertaining the role of eosinophils in a variety of inflammatory or allergic disorders.

Key words: eosinophil • GATA-1 • gene targeting • hematopoiesis • transcription

Introduction

Transcription factor GATA-1 is expressed in four hematopoietic lineages-erythroid cells, megakaryocytes, mast cells, and eosinophils-and is competent to reprogram *myb/ets*-transformed chicken myelomonocytic cells into three of these (erythroid, megakaryocytic, and eosinophilic) (1). Intermediate levels of GATA-1 protein lead to formation of eosinophils, and higher levels to thromboblasts and probably erythroblasts. Gene targeting in mice has revealed essential roles for GATA-1 in both erythroid and megakaryocytic differentiation. In both contexts, the loss of GATA-1 arrests cellular maturation at a relatively late stage after lineage commitment. Overlapping expression of the related factor GATA-2 very likely accounts for lineage commitment in the absence of GATA-1. The extent to which GATA-1 is required for eosinophil or mast cell development or function is unresolved.

Transcription of the GATA-1 gene itself is directed by several cis-regulatory elements. In the sole nonhematopoietic site of expression an upstream promoter (IT) of murine GATA-1 directs Sertoli-cell specific expression (2). Hematopoietic cells express GATA-1 predominantly from a downstream promoter (IE) and exhibit heterogeneous transcriptional start sites (3–7). At least two DNase I hypersensitive regions neighboring, or upstream of, the IE promoter have been identified in hematopoietic cells. The more distal region (termed HSI) is hypersensitive in both erythroid and megakaryocytic cells and encompasses a potent enhancer core of 169 bp that is able to direct expression in these lineages in transgenic mice (8, 9). Deletion of HSI in the native chromosomal context by gene targeting ablates expression of GATA-1 in megakaryocytes (10). The expression of GATA-1 in erythroid cells is maintained, presumably due to compensatory cis-elements located elsewhere within the GATA-1 locus.

Other sequences neighboring the IE promoter also exhibit DNase I hypersensitivity in erythroid cells. In vivo DNA footprinting has revealed protein occupancy in a high affinity palindromic (or "double") GATA-site positioned 500-bp upstream of the heterogeneous start sites (6). This complex GATA-site binds a single molecule of GATA-1 with an affinity ~7 times that of a single GATA site (6, 11). In transient transactivation reporter assays the palindromic GATA-site is required for full promoter activity. Chromatin-immunoprecipitation (CHIP) assay demonstrates direct binding of GATA-1 protein to this site in cultured erythroid cells (unpublished data). It has been pro-

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posed that the palindromic GATA-site in the GATA-1 promoter mediates positive autoregulation of GATA-1 transcription by GATA-1 itself, and thereby ensures expression of the gene once activated (6). Indeed, transgenic experiments in zebrafish have shown that GATA-1 expression is dependent on a similar palindromic site in the zebrafish gene promoter and have been interpreted as support for this hypothesis (12).

In the studies reported here we sought to determine the in vivo relevance of the palindromic GATA-site by modifying the endogenous GATA-1 locus in mice. Remarkably, mice harboring a deletion of the double GATA-site (ΔdblGATA) are unable to produce eosinophils. Unlike GATA-1-null mice, which die in utero with profound anemia and defective megakaryocyte development, ΔdblGATA mice are viable and fertile. Platelet and mast cell development appear normal, while red cell production is only subtly impaired. Thus, the double GATA-site is not required for activation or maintenance of GATA-1 expression in erythroid cells, megakaryocytes, mast cells, or earlier hematopoietic precursors, but is necessary for eosinophil development. Taken together with evidence that GATA-1 can reprogram myeloid cells to eosinophils, our findings suggest that GATA-1 is required for specification of the eosinophil lineage.

Materials and Methods

Gene Targeting of the Murine GATA-1 Locus. Homologous recombination was used to replace a 21-bp region comprising the double-GATA site upstream of the last nucleotide of the first hematopoietic exon (IE) of the murine GATA-1 gene. Two fragments of the GATA-1 locus were amplified by PCR with the following primer pairs: DGU53, 5′-TTATGGATCCAAGGAAGAGAGGACATTAGCAT-3′ and DGU35, 5′-TATCAGCGGCCGCGGACTCGACTGTGGCTGTTGGT-3′; DGD53, 5′-TCACCAGCGGCCGCCCCAGAGCAGGCCAGAGC-3′ and DGD35, 5′-TTGTGTCGACCAGCCCTCACCCCAGGTAACT-3′. These products were cloned into pBlueScript (Stratagene), and a floxed PGK-neo cassette was introduced into the deleted site. The construct was linearized with PvuI and electroporated into CJ7 ES cells as described previously (13). The PGK-neo cassette was removed after electroporation of a vector expressing the cre recombinase. In the resulting mutant allele the double GATA-site is replaced by a single loxP site flanked by two NotI sites.

Wild-type (wt) and mutant alleles were distinguished by PCR with the following primers: G1mutF1, 5′-CCCAATCCTCTGGACTCCCA-3′, and G1mutR, 5′-CCTACTGTGTACCAGGCTAT-3′, which yielded a 459-bp product from wt and a 509-bp product from mutant. Further genotype confirmation was performed by digestion of these PCR products with NotI; this digestion cleaves only the mutant PCR product to yield fragments of 300 and 200 bp.

Hematologic Evaluations. 200–500 μl of blood from adult mice were collected in EDTA-coated tubes (Becton Dickinson) and analyzed on a Bayer ADVIA 120 Hematology System by the Clinical Laboratories of Children's Hospital (Boston, MA). Manual leukocyte differential counts of >300 cells per sample were performed on Wright-Giemsa–stained blood smears. Cytocentrifuge preparations of the bone marrow and spleens of adult mice were also stained with Wright-Giemsa for microscopic evaluation.

Transgenic Mice Expressing IL-5. Mice heterozygous for a transgene that expresses murine IL-5 under the control of human CD2 regulatory sequences (14) were provided by Alison Humbles and Craig Gerard (Children's Hospital). The presence of the hCD2-mIL-5 transgene was verified by PCR with a forward primer from human CD2 (C1F1, 5′-ACCCATGTAGAGGCAACAGC-3′) and a reverse primer from murine IL-5 cDNA (C1R1, 5′-CTACCGGTGCAAAGTGTGTG-3′), which yield a 585-bp product.

RT-PCR Analysis of Bone Marrow Gene Expression. Bone marrow was harvested from femurs of 6-mo-old wt or hemizygous ΔdblGATA mutant male mice. Total RNA was prepared with the Trizol reagent (LifeTech) according to the manufacturer's instructions. Complementary DNA was created with the SuperScript II RT (LifeTech) according to the manufacturer's instructions. PCR was subsequently performed with the following primer pairs (all listed with 5′ end first), yielding products of the indicated sizes. CD11b: CD11BF1, GACCCAGGTTACCGTCTACTAC, and CD11BR1, TTCAGCACTGGGGTCCTTTCAAGC, 622 bp; CD34: CD34F1, GGGTATCTGCCTGGAACTAAG, and CD34R1, TTGCCCACCCAACCAAATCAC, 614 bp; myeloperoxidase: MPOF1, ATGCAGTGGGGACAGTTTCTG, and MPOR1, GTCGTTGTAGGATCGGTACTG, 695 bp; G-CSF receptor: GCSFRF1, CTCAAACCTATCCTGCCTCATG, and GCSFRR1, TCCAGGCAGAGATGAGCGAATG, 573 bp; PU.1: PU1F1, GAGTTTGAGAACTTCCCTGAG, and PU1R1, TGGTAGGTCATCTTCTTGCGG, 500 bp. Eosinophil peroxidase: EPXF1, CCTTTTGACAACCTGCATGA, and EPXR1, CCCAGATGTCAATGTTGTCG, 799 bp; eosinophil major basic protein 1: MBP1F1, GGAGCGTCTGCTCTTCATCT, and MBP1R1, ACTTCCATCAACCCATCGAA, 501 bp; CCR3: CCR3F1, TCCTGCCTCCACTGTACTCC, and CCR3R1, CGTGCTGTGAAAAGCAGAAA, 695 bp. Previously described primers were used to identify glycoprotein Ib (15) and von Willebrand factor (16). Erythropoietin receptor: EPORF, GGACACCTACTTGGTATTGG, and EPORR, GACGTTGTAGGCTGGAGTCC, 452 bp; α-globin: ALPHA3, CTTCTGATTCTGACAGACTCAG, and ALPHA4, GCATGGCCAGAAGGCAAGCC, 494 bp; β-globin: BETA1, GCTTCTGACATAGTTGTGTTG, and BETA3, GTGGTACTTGTGAGCCAAGGC, 623 bp.

Generation and Analyses of Bone Marrow–derived Mast Cells. Bone marrow was obtained from both femurs of a 6-mo-old male hemizygous ΔdblGATA mutant or a wt male littermate control. Whole marrow was cultured in IMDM (Sigma-Aldrich) containing 15% FCS (Hyclone), 2 mM glutamine (Sigma-Aldrich), 100 U/ml penicillin G/streptomycin (Sigma-Aldrich), 20 ng/ml murine recombinant SCF (R&D Systems), and 10 ng/ml recombinant murine IL-3 (R&D Systems) and passaged every 3–4 d into fresh tissue culture dishes for a total of 7 wk.

After 7 wk of culture, cells were cytocentrifuged and stained with May-Grünwald-Giemsa following standard procedures. Duplicate cytospin preparations were fixed in 100% methanol for 5 min, stained in 1% acidified toluidine blue (Sigma-Aldrich) for 5 min, and rinsed in water.

FACS® analysis for expression of c-kit and the high-affinity IgE receptor was performed as previously described with few modifications (17). Briefly, bone marrow–derived mast cells were obtained as described above. Low-affinity IgE and FcγRII/III$^+$ receptors were first blocked by incubating with 10 μg/ml anti-CD23 B3B4 mAb (BD PharMingen) and 10 μg/ml anti-Fc-

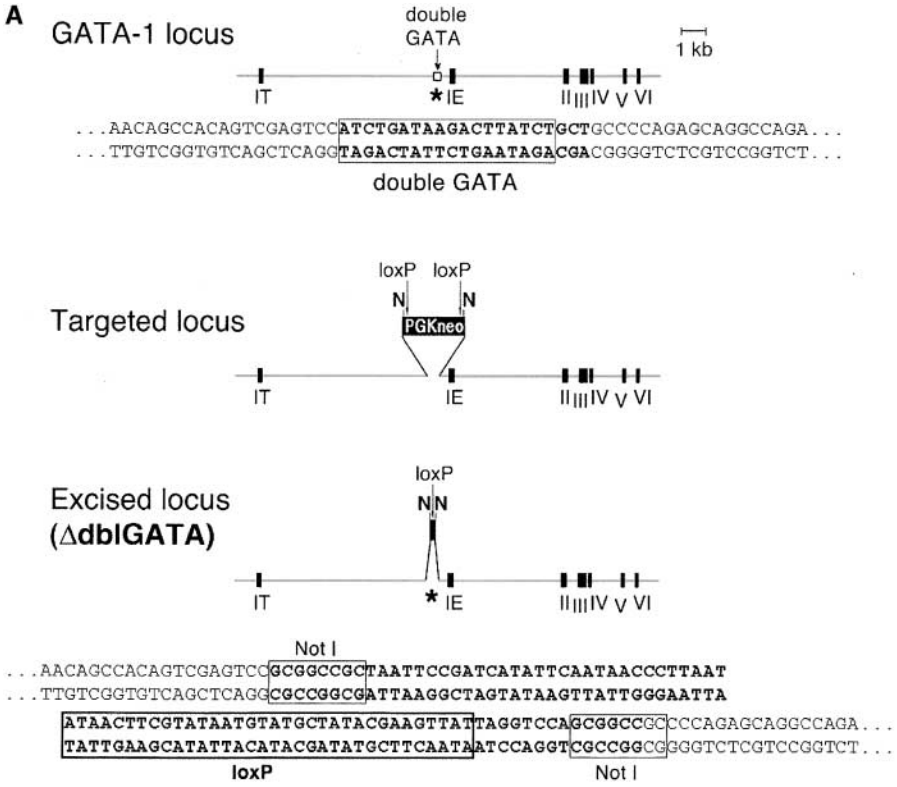
γRII/III$^+$ 2.4G2 mAb (BD PharMingen), respectively, for 15 min at 4°C in DMEM containing 2% FCS. Mouse IgE anti-dinitrophenol (DNP) mAb (Sigma-Aldrich) was then added to a final concentration of 5 μg/ml and the cells incubated for 1 h at 4°C. After washing, biotinylated anti–mouse IgE was added (5 μg/ml, final concentration) and the cells incubated for 30 min at 4°C. The cells were washed and incubated with streptavidin-APC (BD PharMingen; final concentration, 2 μg/ml) and PE-conjugated anti-CD117 (c-kit) (BD PharMingen; final concentration, 2 μg/ml) for 30 min at 4°C. The cells were washed and analyzed on a FACSCalibur™ flow cytometer (Becton Dickinson). Negative controls were performed using an isotype-matched PE-conjugated control antibody (c-kit) and omission of primary IgE antibody (IgE receptor).

Results and Discussion

Targeted Deletion of the Palindromic GATA-Site. Sequences at −691 to −671 bp upstream of the last nucleotide of the first hematopoietic exon (IE) of the GATA-1 gene were deleted as outlined in Fig. 1 A. The targeted and excised (henceforth ΔdblGATA) alleles were distinguished from the wt GATA-1 allele in mice by PCR with primers flanking the deleted region and subsequent digestion with NotI (Fig. 1 B). Mice harboring only the ΔdblGATA allele (i.e., homozygous mutant females and hemizygous mutant males, since GATA-1 is an X-chromosome gene) were viable and fertile and demonstrated no gross abnormalities. Test crosses between heterozygous mutant females and hemizygous mutant males yielded expected ratios of progeny of different genotypes (data not shown).

Erythro- and Myelopoiesis of ΔdblGATA Mice. Automated analysis of peripheral blood from adult mice revealed no significant differences between wt and ΔdblGATA mice with respect to white cell or platelet counts (Table I and data not shown). Erythroid development, however, was subtly affected in hemizygous mutant males. Erythrocyte number was diminished by 27% from 9.57 ± 0.29 × 10^6/μl (mean ± SEM) in wt to 6.97 ± 0.49 × 10^6/μl in mutant mice ($P = 0.004$), with a reduction in hematocrit from 49.6 ± 1.1% (wt) to 37.8 ± 3.5% (mutant; $P = 0.018$). The hemoglobin level was also reduced in proportion to the reduction in erythrocyte number (14.55 ± 0.34 g/dL in wt to 11.00 ± 0.94 g/dL in mutant, $P = 0.012$). Apart from a small elevation in the reticulocyte count in several mutant samples, all other red cell parameters appeared normal. Inspection of peripheral blood smears revealed normocytic, normochromic erythrocytes, and platelets of normal size (Fig. 2). Modest splenomegaly was observed in mutant animals (data not shown). Together these observations are consistent with an appropriate in vivo response to slight impairment in red cell production.

Absence of Eosinophils in ΔdblGATA Mice. In considering other possible effects of the targeted mutation on hematopoiesis, we examined the production of cells of the other lineages in which GATA-1 is expressed. Eosinophils are present in low numbers in normal mice. Manual differential counts of eosinophil numbers in blood smears were performed by a blinded observer. As shown in Table I, eosinophils were absent in homozygous ΔdblGATA females and ΔdblGATA mutant males, whereas they com-



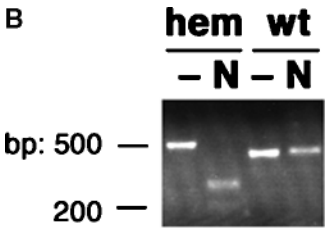


Figure 1. Targeting of the GATA-1 locus. (A, top) The murine GATA-1 locus contains a double GATA-site upstream of the hematopoietic exon IE. Other exons are designated with Roman numerals, including testis exon IT. The double-stranded sequence at the asterisked region is given below the schematic, with nucleotides to be deleted marked in boldface type. (Middle) After targeting, the double GATA-site is deleted, replaced by a floxed PGK-neo cassette. (Bottom) After cre recombinase-mediated excision, the "excised locus" has a minimal sequence consisting primarily of a single loxP site surrounded by two Not I sites. *N*, Not I site. The double-stranded sequence at the asterisked region is given below the schematic. (B) PCR genotyping of male mice carrying the wt or ΔdblGATA mutant (hem) allele. PCR products before (–) and after (N) digestion with Not I.

Table I. *Hematologic Parameters in wt and ΔdblGATA Mice*

Sex	dblGATA Genotype	WBC	RBC	HGB	HCT	MCV	PLT	MPV	Manual EO
		$10^3/\mu l$	*$10^6/\mu l$*	*g/dL*	*%*	*fL*	*$10^3/\mu l$*	*fL*	*%*
F	**het**	6.60	8.75	14.0	44.5	50.9	1885	14.6	1–2
F	**hom**	4.10	8.25	14.5	44.0	53.6	1090	21.7	**0**
F	**hom**	8.20	8.25	14.0	44.5	54.1	1635	16.5	**0**
M	**wt**	5.60	8.95	14.5	44.0	49.2	1460	21.7	1–2
M	**wt**	12.65	9.50	14.5	45.0	47.6	2205	17.1	1–2
M	**hem**	7.50	7.30	11.0	36.0	49.0	1950	15.9	**0**
M	**hem**	8.15	8.15	13.5	43.5	53.2	1095	18.3	**0**

Automated measurement of blood from adult wt and ΔdblGATA mutant mouse littermates from a cross between heterozygous mutant female and a hemizygous mutant male. The dblGATA genotype is designated as follows: het, heterozygous (ΔdblGATA/+) mutant female; hom, homozygous (ΔdblGATA/ΔdblGATA) mutant female; wt, wild-type (+/Y) male; and hem, hemizygous (ΔdblGATA/Y) mutant male. Eosinophils are not observed in homozygous ΔdblGATA mutant females or hemizygous ΔdblGATA mutant males. HCT, hematocrit; HGB, hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; manual EO, manual differential count of eosinophils by a blinded observer; PLT; platelet count; RBC, erythrocyte count; WBC, leukocyte count.

prised 1–2% of nucleated cells in the peripheral blood of controls.

To assess more stringently the potential of ΔdblGATA mice to produce eosinophils, we introduced an IL-5–expressing transgene into wt and mutant backgrounds. The IL-5 transgenic mice we employed express a murine IL-5 cDNA under the control of human CD2 regulatory sequences. In this context, T-lymphocytes constitutively express IL-5. Prolonged release of IL-5 leads to chronic eosinophilia, resembling that seen in mice after inoculation with helminthic parasites (14). The hematological effects of IL-5 expression are shown in Table II. Eosinophil numbers were increased in wild-type or heterozygous ΔdblGATA female mice with the transgene. Nonetheless, eosinophils were absent in hemizygous male ΔdblGATA mice. Hence, even under strenuous cytokine stimulation of eosinophil production, ΔdblGATA mice are unable to produce identifiable eosinophils.

These morphological observations were confirmed by automated leukocyte differential analysis (Fig. 3 A–D). Fig. 3 B demonstrates a profile of a female heterozygous for the ΔdblGATA allele but lacking the IL-5 transgene. Fig. 3 C shows a marked increase in both the number of eosinophils and the number of "large unclassified cells" (LUC) in wild-type male mice with the IL-5 transgene. In Fig. 3 D, an increase in LUC but the absence of eosinophils is evident in a male hemizygous for the ΔdblGATA allele with the IL-5 transgene. Flow cytometric analysis of cell surface markers

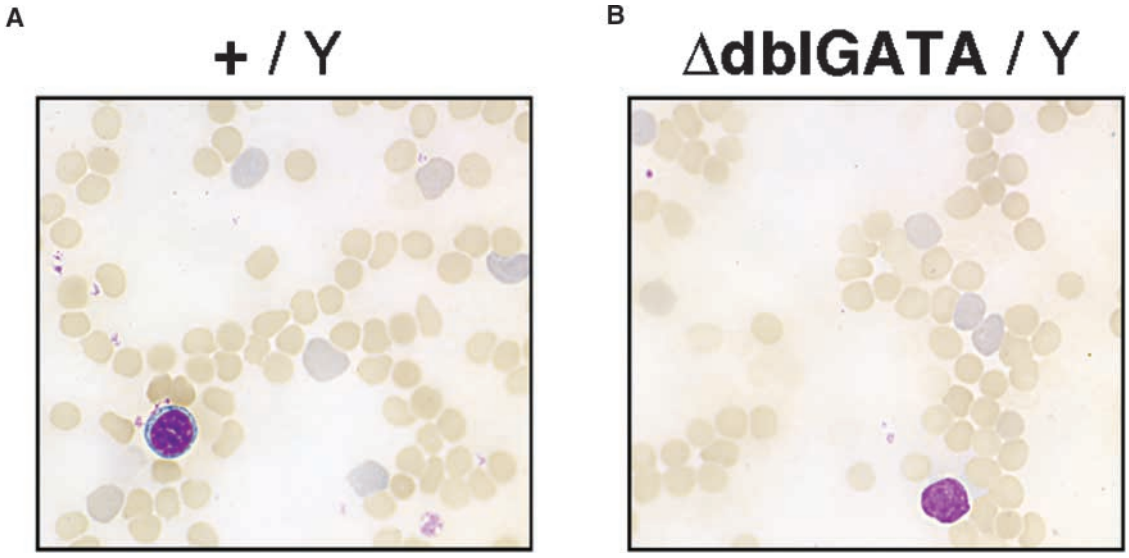


Figure 2. Peripheral blood from a wt male and a ΔdblGATA mutant male mouse. Wright-Giemsa staining of blood smears from a wild-type male (A) and hemizygous ΔdblGATA mutant (B) male. Red cells and platelets appear normal in the ΔdblGATA mutant male. Original magnification: 1,000×.

Table II. *Hematologic Parameters in wt and ΔdblGATA Mice With or Without an IL-5 Transgene*

Sex	dblGATA Genotype	IL-5 Tg	WBC	Manual EO
			$10^3/\mu l$	%
F	het	−	5.76	1
F	het	+	43.34	3
F	het	+	24.83	10
M	wt	+	29.4	10
M	wt	+	75.69	9
M	hem	−	8.31	0
M	hem	+	50.35	0
M	hem	+	46.65	0

Automated measurement of blood from adult wt and ΔdblGATA mutant mouse littermates from a cross between heterozygous ΔdblGATA mutant female and a male heterozygous for an IL-5 transgene (see text for details). For an explanation of abbreviations, see Table I. The IL-5 transgene stimulates eosinophilia in heterozygous females and wt males but not in male mice hemizygous for the ΔdblGATA mutant allele. Manual EO, manual differential count of eosinophils by a blinded observer; WBC, leukocyte count.

on LUC from both wt and hemizygous ΔdblGATA mice showed that these are predominantly B cells, which, like eosinophils and basophils, express the IL-5 receptor α chain (reference 18 and data not shown).

Finally, Wright-Giemsa staining of bone marrow from IL-5 transgenic mice revealed an abundance of eosinophils in a male with a wt GATA-1 allele (Fig. 3 E); no eosinophils are seen in the bone marrow of hemizygous ΔdblGATA mutant male (Fig. 3 F). Examination of splenocytes reveals similar findings (data not shown). Together these findings illustrate that eosinophils are not produced in ΔdblGATA mutant mice, even under conditions that normally stimulate eosinophilia.

If the targeted deletion of the double GATA-site impairs activation or maintenance of GATA-1 expression necessary for eosinophil specification, one would anticipate that loss of GATA-1 through conventional gene targeting would also prevent eosinophil development. Because GATA-1^- embryos die at E10–11 due to profound anemia (13), we resorted to analysis of heterozygous female GATA-$1^{+/-}$ mice. Bone marrow cells of wt and heterozygous mice were cultured in media containing IL-5 with or without G418. As the targeted GATA-1 allele contains a neomycin-resistance gene, growth in G418 reflects selection for

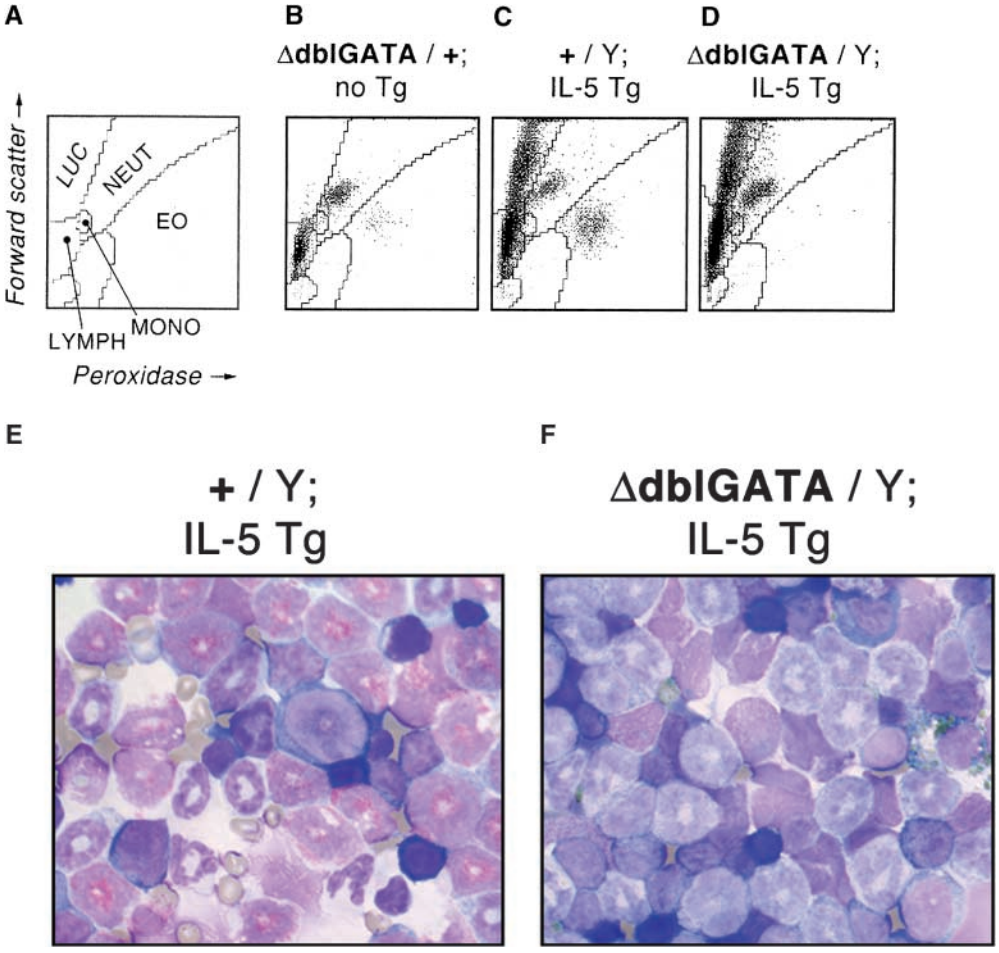


Figure 3. Eosinophilia stimulated by an IL-5 transgene is not observed in ΔdblGATA mutant mice. (A) Schematic of cell populations in automated differential analysis of mouse blood. A heterozygous ΔdblGATA mutant female displays a normal-appearing differential plot (B). The IL-5 transgene imparts an increase in the number of eosinophils and large unclassified cells (C) in a male with a wt GATA-1 locus. This increase is not observed in a hemizygous ΔdblGATA mutant male harboring the same transgene (D). (E and F) Wright-Giemsa staining of bone marrow from mice harboring the IL-5 transgene. Numerous eosinophils with bright red granules are evident in a mouse with a wt GATA-1 locus (E) but not in a hemizygous ΔdblGATA mutant male (F). Original magnification: 600×.

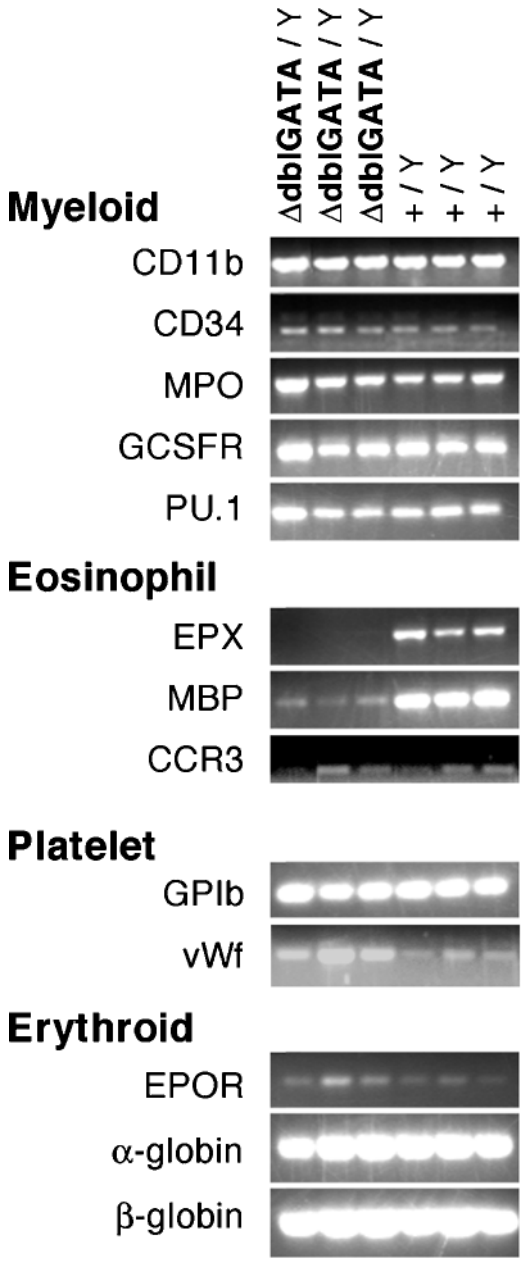


Figure 4. RT-PCR analysis of gene expression in wt and ΔdblGATA mutant male mice. RT-PCR analysis was used to examine gene expression in the bone marrows of hemizygous ΔdblGATA mutant ($n = 3$) and wt ($n = 3$) male mice. Control reactions were performed with both wt and mutant samples with all primer pair combinations, either without RT or without cDNA template; in all these cases, no PCR product was generated (data not shown). EPOR, erythropoietin receptor; EPX, eosinophil peroxidase; GCSRFR, granulocyte colony stimulating factor receptor; GPIb, glycoprotein Ib; MBP, major basic protein; MPO, myeloperoxidase; vWf, von Willebrand factor.

precursor cells in which the GATA-1$^-$ allele is not X-inactivated. Eosinophils were observed in IL-5–containing cultures only in the absence of G418, whereas control cultures in IL-3 and G418 yielded viable myeloid cells (data not shown). These results independently confirm a requirement of GATA-1 in eosinophil formation.

Loss of Eosinophil-specific Transcripts in ΔdblGATA Mice. We examined the expression of a variety of hematopoietic-marker RNA transcripts in bone marrow cells by RT-PCR (Fig. 4). Expression of myeloid (CD11b, CD34, myeloperoxidase, granulocyte colony stimulating factor receptor, PU.1), erythroid (erythropoietin receptor, α- and β-globins), and megakaryocyte/platelet markers (platelet glycoprotein Ib, von Willebrand factor) was similar between wild-type and ΔdblGATA mice, as anticipated from hematological and morphological data. Of note, eosinophil peroxidase (EPX) was present in wild-type mice but absent in ΔdblGATA mice. Eosinophil major basic protein-1, which is highly expressed in eosinophils but not restricted to the lineage, was markedly reduced in mutant mice, where CCR3 was expressed at low level in both wt and mutant mice (19, 20). These expression data are consistent with our cellular and morphological findings regarding the deficiency of eosinophils in ΔdblGATA mice, and further suggest that eosinophil precursors are also absent.

Mast Cell Production in ΔdblGATA Mice. To assess mast cell production in ΔdblGATA mice, we cultured wt or hemizygous ΔdblGATA bone marrow cells in medium containing stem cell factor and IL-3 to obtain mast cells (17). Wt and mutant bone marrow-derived populations behaved similarly. After culture for 7 wk, mast cells were present in both, as shown by morphology (Fig. 5 A and B), the presence of toluidine blue staining granules (Fig. 5 C and D) and flow cytometric analysis (Fig. 5 E and F) for expression of c-kit and the high-affinity IgE receptor (FcεRI), a specific marker of mast cells (21). Thus, the double GATA-site is dispensable for the generation of bone marrow–derived mast cells.

Implications of Findings for GATA-1 and Eosinophil Development. Here we have demonstrated that deletion of a high-affinity GATA-site in the GATA-1 promoter specifically ablates eosinophil production, while the development of the other GATA-1–expressing lineages (erythroid, megakaryocytic, mast) is unaffected or only subtly perturbed. It appears, therefore, that GATA-1 is required in a nonredundant manner in the early phase of eosinophil development, very likely in lineage specification.

Dependence of the eosinophil lineage on GATA-1 shown here is consistent with, and extends, prior evidence supporting a role for GATA-1 in eosinophil gene expression and development. Different levels of enforced GATA-1 expression reprogram lineage determination in *myb/ets*-transformed chicken myelomonocytic cells (1); intermediate levels result in eosinophils, and higher levels in thromboblasts and probably erythroblasts. The eosinophil-specific requirement of the double GATA-site, a site with increased affinity for GATA-1 vis-à-vis single consensus GATA-site, is compatible with the concentration-dependent outcome of reprogramming. Intermediate levels of GATA-1 would favor occupancy at high-affinity GATA-sites, while high levels would allow binding at both high- and low-affinity GATA-sites.

Transcriptional mechanisms using GATA-1, and acting on the GATA-1 gene itself, differ between erythroid cells

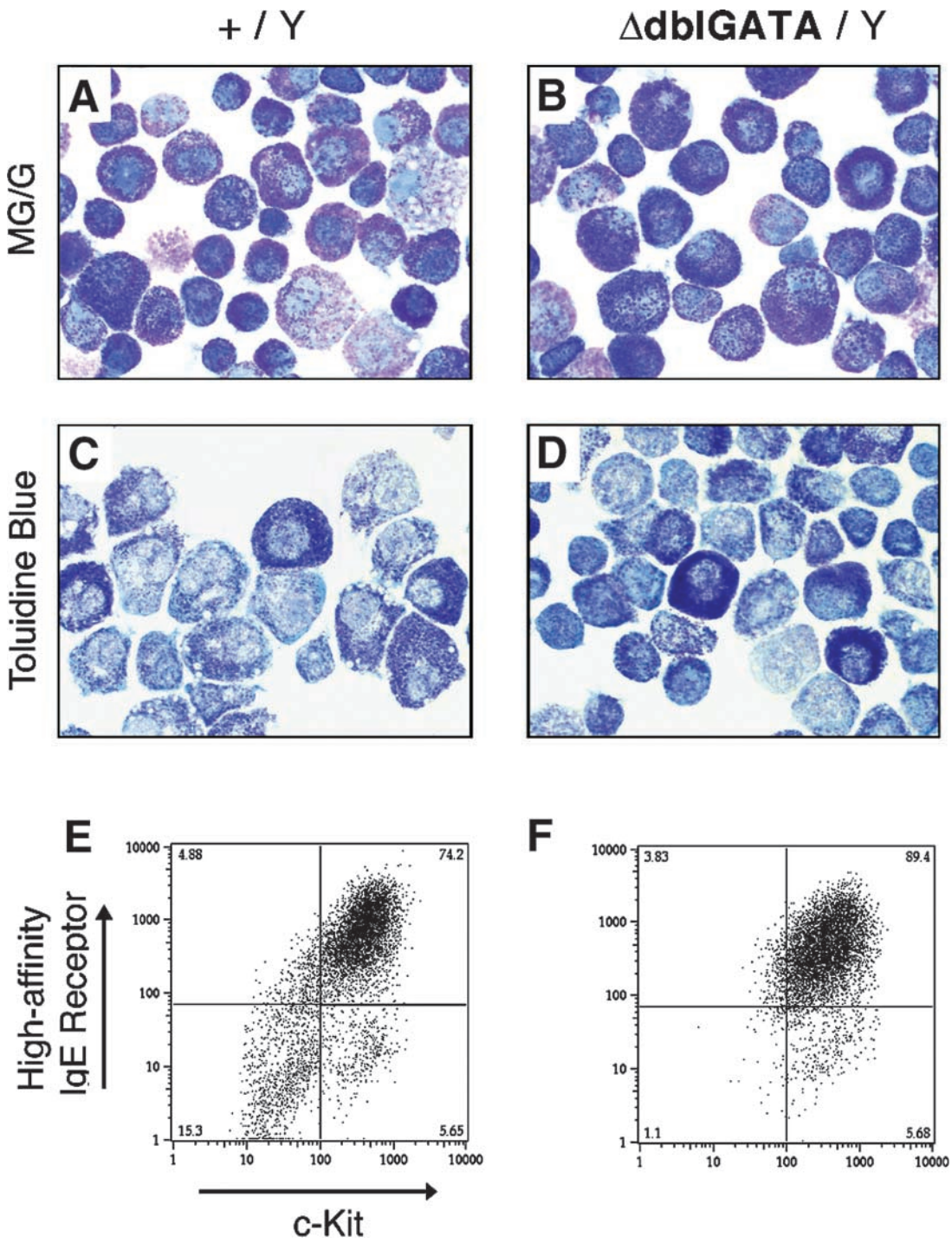


Figure 5. Production of mast cells from wt and ΔdblGATA mutant male mice. May-Grünwald-Giemsa (A and B) and toluidine blue stains (C and D) of cytospin preparations of bone marrow–derived mast cells, from wt (A and C) or ΔdblGATA mutant (B and D) male mice (original magnification: 1,000×). Toluidine blue-positive cells contain dark blue cytoplasmic granules. (E and F) Two-color FACS® analysis of c-kit (x-axis) and high-affinity IgE receptor (y-axis) expression of bone marrow–derived mast cells from wt (E) or ΔdblGATA mutant (F) male mice. Mast cells are observed in both wt and mutant mice.

and eosinophils. In erythroid cells substantial function of GATA-1 is dependent on the presence of the transcriptional cofactor FOG-1. Moreover, whereas both HSI upstream of the GATA-1 gene and the double GATA-site–containing promoter of the gene are highly active in erythroid transcription in test systems, neither region is strictly required for GATA-1 expression in the normal chromosomal context. In eosinophils, on the other hand, the function of GATA-1 is independent of FOG-1. Indeed, FOG-1 expression is antagonistic to eosinophil develop-

ment and downregulation of FOG-1 under the aegis of C/EBP is a prerequisite for eosinophil differentiation to ensue (22–24). FOG-1 independence of GATA-1 function in eosinophils may suggest that the NH_2-terminal activation domain of GATA-1, which is not absolutely required for transcription in erythroid and megakaryocyte lineages, is likely to be required in eosinophils. In addition, as shown here, removal of a limited region of the GATA-1 promoter, that containing the double GATA-site, ablates eosinophil development, presumably by interfering with activation or maintenance of GATA-1 expression in this lineage, but spares erythroid and megakaryocytic development. We speculate that the double GATA-site serves as a critical docking site for the assembly of a protein complex containing GATA-1 and other eosinophil-required transcription factors, such as C/EBPα. As an alternative explanation for the absence of eosinophils on removal of this site, it could be suggested that GATA-1 transcripts in eosinophils initiate upstream of the start positions employed in erythroid cells. If this were the case, erythroid and eosinophil GATA-1 mRNAs would differ in size. Arguing against this speculation are Northern blot analyses we have performed on RNA prepared from eosinophil-enriched populations grown from bone marrow of wild-type mice harboring the IL-5 transgene. GATA-1 transcripts of erythroid and eosinophil cells are not distinguishable in size (data not shown).

ΔdblGATA mice are unique to our knowledge in being the first mouse strain with a specific deficiency of the eosinophil lineage. These animals should prove useful in the study of eosinophil function and eosinophil-related pathologies. It should be possible, for example, to ascertain whether eosinophils represent a primary etiologic agent or a secondary inflammatory bystander in the pathogenesis of asthma or other allergic disorders.

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