Eosinophils from Lineage-Ablated \( \Delta \) dblGATA Bone Marrow Progenitors: The dblGATA Enhancer in the Promoter of GATA-1 Is Not Essential for Differentiation Ex Vivo

Kimberly D. Dyer, Meggan Czapiga, Barbara Foster, Paul S. Foster, Elizabeth M. Kang, Courtney M. Lappas, Jennifer M. Moser, Nora Naumann, Caroline M. Percopo, Steven J. Siegel, Jonathan M. Swartz, SukSee Ting-De Ravin and Helene F. Rosenberg

*J Immunol* 2007; 179:1693-1699; doi: 10.4049/jimmunol.179.3.1693

http://www.jimmunol.org/content/179/3/1693

---

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**  This article cites 30 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/179/3/1693.full#ref-list-1

**Subscription**  Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Eosinophils from Lineage-Ablated ΔdblGATA Bone Marrow Progenitors: The dblGATA Enhancer in the Promoter of GATA-1 Is Not Essential for Differentiation Ex Vivo

Kimberly D. Dyer,* Meggan Czapiga, † Barbara Foster,* Paul S. Foster, ‡ Elizabeth M. Kang, § Courtney M. Lappas, Jennifer M. Moser, Nora Naumann, Caroline M. Percopo, *, Steven J. Siegel, Jonathan M. Swartz, SukSee Ting-De Ravin, § and Helene F. Rosenberg*

A critical role for eosinophils in remodeling of allergic airways was observed in vivo upon disruption of the dblGATA enhancer that regulates expression of GATA-1, which resulted in an eosinophil-deficient phenotype in the ΔdblGATA mouse. We demonstrate here that bone marrow progenitors isolated from ΔdblGATA mice can differentiate into mature eosinophils when subjected to cytokine stimulation ex vivo. Cultured ΔdblGATA eosinophils contain cytoplasmic granules with immunoreactive major basic protein and they express surface Siglec F and transcripts encoding major basic protein, eosinophil peroxidase, and GATA-1, -2, and -3 to an extent indistinguishable from cultured wild-type eosinophils. Fibroblast coculture and bone marrow cross-transplant experiments indicate that in vivo eosinophil deficit is an intrinsic progenitor defect, and remains unaffected by interactions with stromal cells. Interestingly, and in contrast to those from the wild type, a majority of the GATA-1 transcripts from cultured ΔdblGATA progenitors express a variant GATA-1 transcript that includes a first exon (1Ea), located ~3700 bp downstream to the previously described first exon found in hemopoietic cells (1Ea), and ~42 bp upstream to another variant first exon, 1Ea. These data suggest that cultured progenitors are able to circumvent the effects of the ΔdblGATA ablation by using a second, more proximal, promoter and use this mechanism to generate quantities of GATA-1 that will support eosinophil growth and differentiation. The Journal of Immunology, 2007, 179: 1693–1699.

The he generation of eosinophil lineage-deficient mouse models has permitted direct dissection of the role of this granulocyte in allergen-challenged mice and in response to infection with helminthic parasites (1–3). The ΔdblGATA eosinophil-ablation model was engineered by deletion of a palindromic GATA-binding site (dblGATA) in the hemopoietic promoter that is believed to mediate positive autoregulation of GATA-1 expression (4). The GATA-1 transcription factor directs immature myeloid progenitors to differentiate into erythroid cells, megakaryocytes, and eosinophils (5). Initial gene-targeting studies revealed that GATA-1 is essential for normal erythropoiesis, as hemizygous male GATA-1-null mice die in utero from severe anemia (5, 6). In contrast, the results of the ΔdblGATA ablation are less dramatic, resulting instead in selective loss of the eosinophil lineage observed at homeostasis (4) as well as in pathophysiologic settings (1).

In this study, we demonstrate that bone marrow progenitors from ΔdblGATA eosinophil-ablated mice develop into cells with eosinophil-specific characteristics when subjected to cytokine stimulation ex vivo. In an attempt to understand why ΔdblGATA bone marrow progenitors can develop into eosinophils ex vivo but minimally (7) if at all in vivo, we also investigated GATA-1 promoter usage and relative expression of transcripts encoding GATA-1,-2, and -3. Finally, we explored an ex vivo cross culture model and an in vivo bone marrow transplant model to determine whether the ΔdblGATA eosinophil lineage ablation is an intrinsic defect of progenitor cells or involves dysfunctional interactions with the environment.

Materials and Methods

Mice

Four male ΔdblGATA (CD90.2) mice were obtained from Drs. A. Humbles and C. Gerard (Harvard Medical School, Boston, MA) and crossed with wild-type BALB/c mice (CD90.2; Taconic Farms) to generate our colony. Two breeding pairs of BALB/c thy1.1/Cd90.1 (used in the bone marrow transfer) were a gift from Dr. R. DiPaolo (National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, MD). Experimental protocols were reviewed by the Animal Care and Use Committee, NIAID, NIH, protocol number LAD-7E.

Isolation of bone marrow cells

Mouse bone marrow was collected and cells were counted as described previously (8). Cytospin preparations were fixed and stained using a modified Giemsa-stain (Diff Quik; Dade Behring). Ex vivo culture of eosinophils and fibroblast monolayers. Bone marrow cells were cultured at 10^6/ml in basic medium containing IMDM (Invitrogen Life Technologies) with 20% FCS (Cambrex), 100 IU/ml penicillin and 10 μg/ml streptomycin (Cellgro), 2 mM glutamine (Invitrogen Life Technologies), and supplemented with 100 ng/ml SCF (PeproTech) and 100 ng/ml FLT3-L (PeproTech). On day 4, the cells were transferred into basic medium containing 20 ng/ml

*Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases/National Institutes of Health (NIAID/NIH), Bethesda, MD 20892; † Research Technologies Branch, NIAID/NIH, Bethesda, MD 20892; 1School of Biomedical Sciences, University of Newcastle, Newcastle, New South Wales, Australia; and 2Laboratory of Host Defenses, NIAID/NIH, Bethesda, MD 20892

Received for publication February 16, 2007. Accepted for publication May 14, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institute of Allergy and Infectious Diseases Division of Intramural Research.

2 Address correspondence and reprint requests to Dr. Kimberly D. Dyer, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases/National Institutes of Health, 10 Center Drive, Building 10 Room 11C216, Bethesda, MD 20892-1883. E-mail address: kdyer@niaid.nih.gov

www.jimmunol.org
IL-3 (PeproTech), 10 ng/ml IL-5 (R&D Systems), and 10 ng/ml GM-CSF (PeproTech). On day 8, the cells were switched into basic medium containing 20 ng/ml IL-3 and 10 ng/ml IL-5 only. Fibroblast cultures (used in the ex vivo culture experiments) were derived by plating bone marrow cells in the absence of cytokines. After 24 h, the nonadherent cells were removed. The adherent cells were cultured for 4 wk in 10% mouse serum (Atlanta Biologicals) and 10% FCS (Cambrex), 100 IU/ml penicillin, and 10 µg/ml streptomycin (Cellgro), 2 mM glutamine (Invitrogen Life Technologies).

**Bone marrow transfer experiments**

In two separate experiments, a total of 14 ΔdblGATA mice (CD90.2) and 16 wild-type BALB/c mice (CD90.1) were irradiated (850 rad). These recipient mice were given water containing neomycin and transplanted 24–30 h later with bone marrow (3–6 × 10^6 cells/150 µl of HBSS) from either wild-type or ΔdblGATA mice. All surviving mice (15 BALB/c and 9 ΔdblGATA) exhibited normal peripheral blood cell values by week 4. By week 6, the wild-type CD90.1 recipient mice transplanted with ΔdblGATA (CD90.2) bone marrow exhibited 68% ± 11% CD90.2 cells (percentage of CD3^+^) and 21% ± 9% CD90.1 cells. The ΔdblGATA CD90.2 recipient mice transplanted with bone marrow from wild-type BALB/c (CD90.1) mice exhibited 78% CD90.1 cells (percentage of CD3^+^) and 2% CD90.2 cells. At week 6, both peripheral blood and bone marrow were collected and analyzed by Siglec F expression. Cytospin slides were prepared and stained with the modified Giemsa method (DiffQuik) for manual eosinophil counts.

**Gene microarray analysis**

Bone marrow RNA samples from uninfected and Schistosoma mansoni-infected BALB/c and ΔdblGATA mice (n = 2–4 mice/group, t = 9 wk) were pooled and subjected to gene microarray at the Microarray Core Facility (Rochester, NY) as described previously (9). Data were generated using the M430 mouse genomic chip and analyzed with GeneSpring 7.0 software (Silicon Genetics) and analyses as previously described by Domachowskie et al. (9). The data reported in Table I reflect normalized fluorescence for genes of interest in which the gene was present in at least one of the four conditions examined.

**Quantitative RT-PCR**

Cells were suspended in RNazol B (Teltest) at 1 ml/10^6 cells and extraction proceeded as per manufacturer’s instructions. Two micrograms of RNA were subjected to DNase I treatment (Invitrogen Life Technologies) and reverse transcribed using a First Strand cDNA Synthesis kit for RT-PCR (AMV; Roche Diagnostics). One microcitor of cDNA was subjected to TaqMan (Q) PCR using custom FAM-labeled probe and primers to each indicated mouse gene. All primer probe sets were purchased from Applied Biosystems. All experiments included no reverse transcriptase and no template controls and mouse GAPDH (Applied Biosystems) was used as the endogenous control.

**Flow cytometry**

Cells were probed with either PE-conjugated rat anti-mouse Siglec F or PE-conjugated IgG2A isotype control (1 µg/10^6 cells; BD Pharmingen) for 30 min at 4°C or a combination of anti-mouse CD3e PE (BD Pharmingen), anti-rat/mouse Thy.1 Alexa Fluor 488 (Ox-7; BioLegend), and anti-mouse Thy.1 PE (BioLegend). After staining, the cells were fixed in 4% paraformaldehyde and analyzed by flow cytometry. Data were acquired with a FACSCalibur flow cytometer (BD Biosciences) and analyzed with FloJo software version 7.1 (Tree Star). Siglec F-positive cells were identified by comparison to the PE-conjugated IgG2A isotype control. Thy.1.1 (CD90.1) and Thy.1.2 (CD90.2) expression was examined within the CD3^+^ population.

**5′ RACE**

cDNA was synthesized from 2 µg of RNA from freshly isolated bone marrow cells or from bone marrow cells that had been maintained in culture for 24 days with Moloney murine leukemia virus reverse transcriptase as per the manufacturer’s instructions (SMART RACE, Clontech). RACE ready cDNAs were templates for PCR using the following oligonucleotides specific for the mouse GATA gene: primer 1826: CATCAGATTCCACAGTTTCTTTCTCT: primer 1799: TTGTTGTGATTGTGCCCTGTTGTC. One microcitor of the primary PCR amplification products was used as template in a second or nested PCR. The amplified PCR fragments were gel purified (BIO 101) and subcloned into the pCR2.1 (Invitrogen Life Technologies) and multiple colonies were sequenced. The sequences were assembled with GenBank NM_000809 using Sequencher 4.1 (GeneCodes) to determine the gene structure.

**Immunostaining and observation under confocal microscopy**

One million cells were washed and then fixed in 4% paraformaldehyde followed by permeabilization in ice-cold methanol. Rabbit anti-major basic protein (MBP) (no. 509, a gift from Drs. N. A. Lee and J. J. Lee, Mayo Clinic, Scottsdale, AZ) was used at a 1:5,000 dilution for 1 h at 4°C; the cells were washed and goat anti-rabbit IgG-Alexa 488 (Molecular Probes) was applied at a 1:100 dilution. After 1 h of incubation, the cells were washed and incubated with the nuclear stain, 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI; Molecular Probes). Images were collected as previously described (10) and imaging fields were selected “blinded” in that the microscope was centered on the DAPI-stained nuclei and that field was imaged regardless of the presence or absence of myelin basic protein-Alexa Fluor 647-positive cells, as Alexa Fluor 647 is far red and not visible to the eye.

**Results**

**Detection of eosinophils in the bone marrow of wild-type and ΔdblGATA mice in response to prolonged Th2 stimulation**

We (3) and others (1, 4) did not detect eosinophils in bone marrow of eosinophil-deficient ΔdblGATA mice at homeostasis or in response to prolonged stimulation with IL-5. Microarray analysis of bone marrow progenitors from uninfected and S. mansoni-infected wild-type and ΔdblGATA mice reveals differential expression of eosinophil-associated gene transcripts. The transcripts encoding two prototypical eosinophil markers, MBP and eosinophil peroxidase (EPO), exhibit 56- and 36-fold reduced expression, respectively, in ΔdblGATA compared with wild-type bone marrow; this increased to 74- and 209-fold differences when comparing S. mansoni-infected ΔdblGATA and wild-type mice.

---

Table I. Differential expression of eosinophil-related transcripts

<table>
<thead>
<tr>
<th>Eosinophil-Related Transcripts</th>
<th>GenBank Acc. No.</th>
<th>Uninfected BALB/c</th>
<th>Uninfected ΔdblGATA</th>
<th>Fold Difference</th>
<th>S. mansoni-Infected BALB/c</th>
<th>S. mansoni-Infected ΔdblGATA</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP</td>
<td>NM_008920</td>
<td>3.35</td>
<td>0.06</td>
<td>55.8</td>
<td>5.87</td>
<td>0.08</td>
<td>73.8</td>
</tr>
<tr>
<td>Ear1</td>
<td>NM_007894</td>
<td>2.38</td>
<td>0.04</td>
<td>59.5</td>
<td>4.73</td>
<td>0.02</td>
<td>236.5</td>
</tr>
<tr>
<td>EPO</td>
<td>NM_007946</td>
<td>1.79</td>
<td>0.05</td>
<td>35.8</td>
<td>8.34</td>
<td>0.04</td>
<td>208.5</td>
</tr>
<tr>
<td>Siglec F</td>
<td>AF293371</td>
<td>1.19</td>
<td>0.46</td>
<td>2.6</td>
<td>2.32</td>
<td>0.64</td>
<td>3.6</td>
</tr>
<tr>
<td>PAI-2</td>
<td>NM_011111</td>
<td>0.96</td>
<td>1.53</td>
<td>0.6</td>
<td>4.06</td>
<td>0.76</td>
<td>5.3</td>
</tr>
<tr>
<td>IL-5Rα</td>
<td>NM_008370</td>
<td>0.66</td>
<td>1.13</td>
<td>0.6</td>
<td>1.83</td>
<td>1.18</td>
<td>1.6</td>
</tr>
<tr>
<td>GATA-1</td>
<td>NM_008089</td>
<td>1.417</td>
<td>0.717</td>
<td>2.0</td>
<td>1.185</td>
<td>0.43</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Differential expression of eosinophil-related transcripts in bone marrow from wild-type BALB/c and ΔdblGATA eosinophil-ablated mice as determined by gene microarray. MBP, major basic protein; Ear1, eosinophil-associated ribonuclease 1; PAI-2, plasminogen activator inhibitor-2 (30). Values are normalized fluorescence intensities (see Materials and Methods) and fold difference is wild-type/ΔdblGATA.

---

3 Abbreviations used in this paper: MBP, major basic protein; DAPI, 4′, 6-diamidino-2-phenylindole dihydrochloride; EPO, eosinophil peroxidase; Fpr1, formyl peptide receptor 1.
results from the single gene microarray experiment were examined further by quantitative RT-PCR (Fig. 1). Expression of transcripts encoding EPO, MBP, and GATA-1 was significantly diminished in bone marrow of ΔdblGATA mice, as was the expression of IL-5Rα. Interestingly, and consistent with previous findings (4), we observed no differential expression of CCR3, considered to a unique marker of mouse eosinophils (11).

**Generation and quantitative analysis of eosinophils in bone marrow culture**

We used an ex vivo culture system providing cytokines that promote stem cell growth and eosinophil differentiation (see Materials and Methods). Confocal microscopic analysis of cultured ΔdblGATA bone marrow progenitors revealed immunoreactive MBP within cytoplasmic granules and rudimentary bilobed nuclei, indistinguishable from those detected in cultured wild-type progenitors (Fig. 2). Eosinophils in culture were detected and enumerated by cell surface expression of Siglec F (12, 13). In a previous study, we found no Siglec F⁺ eosinophils in the bone marrow of ΔdblGATA mice when gating on the side scatter-high mature granulocyte population (8). Data presented in Fig. 2D are not gated and represent the percent Siglec F⁺ cells in comparison to the isotype control only. At the initiation of culture (day 0), there are more Siglec F⁺ cells in the wild-type than in the ΔdblGATA culture (6 ± 0.6% vs 0.8 ± 0.03%, p < 0.0001), reflecting at most a minimal number of eosinophil progenitors in bone marrow from ΔdblGATA mice. As the half-life of mature eosinophils in culture without cytokine support (IL-5, GM-CSF) is 24–48 h (14, 15), the original mature eosinophil population will be substantially diminished before addition of cytokines on day 4. This is seen at day 8, as the percentage of Siglec F⁺ cells in the wild-type culture is reduced to 1.8 ± 0.08%; this reduction was confirmed by direct observation of stained cytospin preparations. At day 14, the eosinophil population in the wild-type cultures expanded to 5 ± 1% Siglec F⁺ cells, and in the ΔdblGATA cultures, 7 ± 1% Siglec F⁺ cells (a 3- to 4-fold increase over that observed at day 8, p < 0.01). No significant difference in percentage of Siglec F⁺ cells was observed between the wild-type and ΔdblGATA cultures at day 14 (p = 0.18). Interestingly, we were unable to detect eosinophils in the ΔdblGATA cultures with the modified Wright-Giemsa stain.

**Expression of eosinophil-associated transcripts in bone marrow culture**

At day 0 (at isolation), we detect differential expression of eosinophil-associated transcripts MBP, EPO, and GATA-1, as anticipated from the characterized eosinophil ablation of the ΔdblGATA mouse strain (Fig. 3). Eosinophilopoietic cytokines are added to the cultures on day 4, and beginning on day 8, we observe no further differential expression of MBP, EPO, or GATA-1 until day 14. In contrast, maximum expression of formyl peptide receptor 1 (Fpr1), a neutrophil-associated transcript, is observed on day 0, with no differential expression detected at all throughout the course of the experiment. No differential transcription of GATA-2 or -3 was observed either at isolation (day 0) or in response to cytokine stimulation.

**Sequence analysis of GATA-1 transcripts**

Transcription of GATA-1 has been traditionally understood as driven by one of two promoters, with the distal promoter, the region 5’ to exon 1T, primarily active in the testis (17) and the proximal promoter, 5’ to exon 1E (Fig. 4A), which includes the ΔdblGATA ablation, primarily active in hemopoietic cells (18,
19). Included in the schematic shown here is exon E1B, identified by Seshasayee et al. (20) in mouse bone marrow culture, and located ~3700 bp proximal to 1E_A. Using the 5′ RACE procedure, we amplified two transcript variants from testis tissue that included either exons 1T or 1EA, with no significant differences in distribution when comparing wild type to ΔdblGATA mice (data not shown). Two transcript variants were isolated from bone marrow progenitors. The predominant variant (representing 70–90% of the total transcripts amplified) included exon 1EA and a minor population contained the aforementioned exon 1EB. No significant differences in distribution were observed when comparing transcripts amplified from freshly isolated wild-type vs ΔdblGATA bone marrow (Fig. 4B).

In contrast, three transcript variants were amplified from cultured bone marrow progenitors (Fig. 4B). Exon 1E_A was included in 83% of the transcripts in wild-type culture, but in only 45% of the transcripts in the ΔdblGATA culture. Other variants included transcripts with exon 1E_B (10% in wild type vs 52% in ΔdblGATA progenitor culture) and a novel minor transcript, which included a new exon 1E_C, located only ~42 bp 3′ to exon 1E_B. The distribution

FIGURE 3. Differential expression of eosinophil-associated transcripts in wild-type and ΔdblGATA bone marrow cells before and during culture ex vivo. RNA was extracted from bone marrow progenitors from wild-type BALB/c and ΔdblGATA mice and subjected to quantitative RT-PCR to determine the relative expression of the transcripts indicated. ■, Wild-type BALB/c; □, ΔdblGATA. A, MBP; B, EPO; C, Fpr1; D, GATA-1; E, GATA-2; F, GATA-3. All Ct values were normalized to GAPDH and transcription is expressed as fold increase over a selected transcript from ΔdblGATA bone marrow on day 4 which was normalized to 1.0. Cytokines present in culture are indicated by arrows along x-axis. Statistical significance was determined by using the two-tailed t test assuming equal variance; *, p < 0.05.
of the transcripts in the wild-type vs ΔdblGATA cultures differed markedly from one another (Fisher’s exact test, \( p < 0.001 \)), with pronounced differential transcription of variants containing exons \( 1E_A, 1E_B \), and \( 1E_C \). Additionally, the distribution of transcript variants is significantly different in ex vivo-cultured ΔdblGATA than in freshly isolated bone marrow cells (\( p < 0.001 \)) while the distribution of transcripts from BALB/c bone marrow is not altered significantly in response to culture conditions.

Cross-culture and bone marrow transfer experiments

Bone marrow coculture experiments were performed as described in the Materials and Methods. As shown in Fig. 5A, fibroblasts from ΔdblGATA mice had no detrimental effect on the development of eosinophils from wild-type progenitors when measured at either days 7 or 14 of growth in culture; likewise, wild-type BALB/c fibroblasts had no impact on the growth and differentiation of eosinophils from ΔdblGATA progenitors.

In Fig. 5B, we present the results of experiments in which eosinophil hemopoiesis was monitored in wild-type BALB/c (CD90.1) and ΔdblGATA (CD90.2) mice that received myelosuppressive irradiation and were then transplanted with matched or heterologous bone marrow progenitors. Similar to our findings with fibroblast cross-cultures, wild-type BALB/c bone marrow transplanted into irradiated ΔdblGATA-recipient mice (heterologous) develop into Siglec F\(^+\) eosinophils with equivalent efficiency to the those transplanted into irradiated wild-type mice (matched). Likewise, few (wild-type recipients) to no (ΔdblGATA recipients) Siglec F\(^+\) or Giemsa-stained eosinophils develop in mice receiving ΔdblGATA marrow progenitors.

Discussion

Eosinophils are among the most enigmatic of cells, as the precise nature of their role in host defense against parasitic infection and/or in the pathogenesis of allergic disease remains a subject of profound controversy (21). Although several eosinophil-specific gene promoters have been characterized (22–25), no one has succeeded in identifying transcription factors or events that are clearly unique to this lineage. The serendipitous finding of Yu et al. (4) that deletion of the palindromic ΔdblGATA consensus binding site destroys a potential positive feedback loop (19). Interestingly, we

![FIGURE 4. Quantitative analysis of transcripts encoding GATA-1 in wild-type and ΔdblGATA cultured bone marrow progenitors. A, Schematic of the structure of the gene encoding GATA-1, modified from that proposed by Ito et al. Exons 1 include IT, IE(A), IE(B), and the newly identified IE(C). Also shown are the relative positions of the palindromic ΔdblGATA and tandem GATA\(_3\) consensus sites (20). B, Distribution of transcripts encoding GATA-1 identified in freshly isolated bone marrow and cultured bone marrow progenitors from wild-type BALB/c and ΔdblGATA mice. Values denote percentage of transcripts isolated that use the exon 1 variant (1E_A, 1E_B, or 1E_C) as indicated. Statistical significance determined by Fisher’s exact test: * , \( p < 0.001 \) between groups as shown.](http://www.jimmunol.org/)

![FIGURE 5. Quantitative assessment of progenitor-stromal interactions. A, Fibroblast coculture ex vivo. Data are shown as a ratio of percent of Siglec F\(^+\) cells detected in cross-culture divided by the percent Siglec F\(^+\) progenitors in the parallel control culture (in triplicate) at days 7 and 14 under eosinophil-promoting culture conditions (see Materials and Methods). B, In vivo bone marrow transplantation. Recipient wild-type BALB/c (CD90.1, WT) and ΔdblGATA (CD90.2) mice received myelosuppressive irradiation and were transplanted with matched or heterologous bone marrow progenitors. Data were collected from two independent experiments from recipient (recip.)/donor WT/WT (\( n = 7 \)), WT/ΔdblGATA (\( n = 8 \)), ΔdblGATA/WT (\( n = 4 \)), and ΔdblGATA/ΔdblGATA (\( n = 5 \)). Statistical significance was determined by using the two-tailed t test assuming equal variance: * , \( p < 0.03 \) comparing WT vs ΔdblGATA donors.](http://www.jimmunol.org/)
show here for the first time that there is in fact diminished expression of GATA-1 in bone marrow progenitors isolated from ΔdblGATA mice (Figs. 1 and 3), with no compensatory increased expression of GATA-2 or GATA-3. The functional consequences of this finding remain to be explored.

Most intriguing is our observation that, when grown ex vivo in the presence of cytokines that promote eosinophil differentiation, the inhibitory effect of the ΔdblGATA deletion was virtually eliminated. Both wild-type and ΔdblGATA bone marrow progenitors differentiate into cells with condensed nuclei and large cytoplasmic granules that stain positively for MBP. The differentiated eosinophils from both wild-type and ΔdblGATA progenitors can also be detected by cell surface expression of Siglec F, and progenitor cultures demonstrate augmented expression of eosinophil transcripts EPO, MBP, and GATA-1. Given that the eosinophil ablation observed in vivo is related (directly or indirectly) to the disruption of a functional enhancer element in what is known as the proximal, or hemopoietic, promoter of GATA-1, we focused our attention on the nature and distribution of GATA-1 transcripts found in bone marrow and in progenitor cultures. Among our findings, we demonstrate that GATA-1 transcripts from wild-type BALB/c mice contain one of four distinct potent exons 1 (Fig. 4). The distal exon 1T was found only in transcripts amplified from testis tissue, although transcripts with the more proximal hemopoietic exon 1E (here, called 1E1p) can also be detected in testis tissue. No transcripts with exon 1T were amplified from freshly isolated bone marrow; the majority of the transcripts from wild-type bone marrow contain exon 1Eα. However, we also amplified transcripts that contain an even more proximal exon 1Eβi, located ~3700 bp from 1Eα. Seshasayee et al. (20) have previously described the 1Eβi exon within a minor population of transcripts from cultured mouse bone marrow progenitors. In our bone marrow progenitor cultures, we detect three distinct transcripts, those including the aforementioned 1Eα and 1Eβ, and also, a third population, including an even more proximal exon 1Ec. In testis and freshly isolated bone marrow, we observe no significant differential exon usage when comparing transcript distribution in both wild-type and ΔdblGATA mouse strains. However, the distribution observed in the wild-type bone marrow progenitor cultures (83% 1Eα, 10% 1Eβ, 7% 1Eβi) differs substantially from that detected in the ΔdblGATA (45% 1Eα,52%1Eβ, 3% 1Eβ; p < 0.001 by Fisher’s exact test); specifically, we observe dramatically reduced usage of exon 1Eα and increased usage of exon 1Eβ in the ΔdblGATA cultures as compared with the wild type. Of note, exon 1Eβi is immediately proximal to, and may be under more direct control of the tandem [GATA]2 enhancer, an active element of GATA-1 expression in erythroid cells and cell lines (20). Taken together, our findings indicate that the effects of the dblGATA deletion and eosinophil ablative effects may be overcome ex vivo by differential promoter usage. Likewise, our results demonstrate the plasticity of eosinophil differentiation and the existence of an important compensation mechanism supporting GATA-1 gene transcription in response to dblGATA deletion, and perhaps under other, as yet-to-be-identified pathophysiologic states. As such, it would be interesting to explore the role of GATA-1 transcription and alternate promoter usage in the pathogenesis of idiopathic hypereosinophilic syndromes. It is also intriguing to consider the possibility that prolonged, ultra-high-level cytokine stimulation, such as that achieved pharmacologically, might direct differential expression of variant GATA-1 transcripts. Finally, we considered the possibility that development of eosinophils in the ΔdblGATA mice was in some way inhibited by progenitor-stromal interactions. This would be similar to observations made regarding SLP/SMT and tgs/tg mast-cell deficient mice in which the progenitors do not differentiate due to the absence of appropriate signals from the environment (28, 29). In our two experiments, the cross-culture on bone marrow fibroblasts and the cross-bone marrow transplants, we demonstrated that the donor’s stromal contribution had no impact on the capacity of the wild-type or ΔdblGATA progenitors to generate eosinophils. We conclude that the ability (or not) to develop into eosinophils rests solely within the nature of the progenitor cells and that the ΔdblGATA mice’s inability to generate eosinophils is due to an intrinsic defect of the progenitor cells.

In summary, the ΔdblGATA mouse strain, in prominent use for studies of allergy and asthma pathogenesis (1, 3), contains an ablation of a palindromic dblGATA-binding site in the hemopoietic promoter of GATA-1 and does not support normal differentiation of the eosinophil lineage in vivo. However, bone marrow progenitors from ΔdblGATA mice respond to ex vivo culture conditions by differentiating into eosinophils in a manner indistinguishable from the wild type. Expression of GATA-1 in wild-type cultured progenitors occurs via transcripts that contain one of three distinct hemopoietic exons 1 (1E1α, 1E1β, and a novel 1E1c) in proportions that differ dramatically from those detected in ΔdblGATA progenitor cultures. Our data suggest that the ΔdblGATA progenitors may circumvent the ΔdblGATA ablation by overuse of a second, more proximal promoter that includes the [GATA]2 motif (20, 26), thereby generating quantities of GATA-1 sufficient to support eosinophilopoiesis. It remains to be seen what role is played by differential transcription of GATA-1 under pathophysiological conditions.

Acknowledgments

We thank Drs. Nancy A. Lee and James J. Lee, Mayo Clinic, Scottsdale, AZ, for their generous gift of the rabbit polyclonal anti-MBP Ab; Drs. Alison Humbles and Craig Gerhard (Harvard Medical School, Boston, MA) for the ΔdblGATA mouse and genotyping protocols; Dr. Richard DiPaolo (NIAID, NIH) for the CD90.1 BALB/c mice; and Dr. Tom Wynn (NIAID, NIH) for his ongoing assistance with the S. mansoni infection model.

Disclosures

The authors have no financial conflict of interest.

References


