Expression of c-MYC under the Control of GATA-1 Regulatory Sequences Causes Erythroleukemia in Transgenic Mice

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Summary

To study oncogenesis in the erythroid lineage, we have generated transgenic mice carrying the human c-MYC proto-oncogene under the control of mouse GATA-1 regulatory sequences. Six transgenic lines expressed the transgene and displayed a clear oncogenic phenotype. Of these, five developed an early onset, rapidly progressive erythroleukemia that resulted in death of the founder animals 30–50 d after birth. Transgenic progeny of the sixth founder, while also expressing the transgene, remained asymptomatic for more than 8 mo, whereupon members of this line began to develop late onset erythroleukemia. The primary leukemic cells were transplantable into nude mice and syngeneic hosts. Cell lines were established from five of the six leukemic animals and these lines, designated erythroleukemia/c-MYC (EMY), displayed proerythroblast morphology and expressed markers characteristic of the erythroid lineage, including the erythropoietin receptor and β-globin. Moreover, they also manifested a limited potential to differentiate in response to erythropoietin. Studies in the surviving transgenic line indicated that, contrary to our expectations, the transgene was not expressed in the mast cell lineage. That, coupled with the exclusive occurrence of erythroleukemia in all the transgenic lines, suggests that the GATA-1 promoter construct we have used includes regulatory sequences necessary for in vivo erythroid expression only. Additional sequences would appear to be required for expression in mast cells. Further, our results show that c-MYC can efficiently transform erythroid precursors if expressed at a vulnerable stage of their development.

One of the genes controlling the differentiation of immature hematopoietic progenitor cells encodes a zinc finger transcription factor called GATA-1 (1). Disruption of the GATA-1 gene through homologous recombination in embryonic stem cells prevents normal erythroid development (2, 3). Consistent with its proposed role as a key regulator in erythropoiesis, GATA-1 mRNA is present in the earliest identifiable blood island cells in the yolk sac and is later found in fetal liver and in adult erythroid cells (4). In addition, GATA-1 mRNA is also expressed in adult hematopoietic cells of megakaryocytic, eosinophilic, and mast cell origin (5–8). This expression pattern suggests that GATA-1 might also be present in progenitor cells committed to the erythroid, megakaryocytic, eosinophilic, and mast cell lineage. Therefore, the regulatory sequences that control expression of the GATA-1 gene might provide the opportunity to direct expression of transgenes to hematopoietic progenitors with multilineage potential. Nonhematopoietic cells, with the exception of the testis, do not express GATA-1. The testis form is transcribed from a separate promoter located 5′ to the erythroid first exon (9).

Deregulated expression of the c-myc proto-oncogene in vivo can immortalize a variety of hematopoietic lineages including lymphoid (10, 11) and myelo-monocytic cells (12, 13). c-myc is ubiquitously expressed and plays a central role in the control of cell proliferation (14). For oncogenic activity the myc protein must form a heterodimer with max (15), a ubiquitously expressed member of the helix-loop-helix family (16). Despite its promiscuous properties as an oncogene, its potential in the transformation of erythroid cells has yet to be defined.

To derive an in vivo model and to immortalize cell lines that represent stages in hematopoietic development dependent on GATA-1, we used regulatory sequences of the murine GATA-1 gene to express the human c-MYC proto-oncogene in transgenic mice. Several such independently derived transgenic mice developed early onset erythroleukemia and although these mice died before reaching sexual maturity, we were able to derive and characterize cell lines from these animals. One
transgenic line, by contrast, developed late onset erythroleukemia and survived to provide an in vivo model of this disease. This surviving line also allowed us to assess the expression pattern of the transgene directed by the GATA-1 regulatory sequences used in these studies.

Materials and Methods

Construction of the GATA-1/c-MYC Transgene and Generation of Transgenic Mice. A 4.3-kb XbaI-Smal genomic fragment, including 2.7 kb of 5' flanking region, the nontranslated exon 1 (80 bp), and 1.5 kb of intron 1 of the mouse GATA-1 gene, was ligated to a 3.8-kb Smal-XbaI genomic fragment, comprising the 3' part of the first intron as well as exon 2, intron 2, and exon 3 of the human c-MYC gene (17). The resulting 8.1-kb GATA-1/c-MYC fragment, which contains no vector sequences, was used for oocyte microinjection. We have generated 13 transgenic founder mice in the FVB/N inbred strain (Taconic Farms, Inc., Germantown, NY) by standard oocyte injection methods (18). The animals were maintained under specific pathogen-free conditions in microisolator cages.

RNA Isolation, RNase Protection Assay, and Northern Analysis. RNA samples were prepared in 4 M guanidium isothiocyanate, followed by ultracentrifugation on a 5.7 M CsCl cushion (19). For RNase protection analysis, T3 or T7 antisense probes were synthesized and hybridized to total RNA samples as described (20). Protected fragments were separated on 6% polyacrylamide/8 M urea sequencing gels, which were then dried and exposed for autoradiography using Kodak XAR-5 film and an intensifying screen.

Human c-MYC mRNA was detected with a riboprobe transcribed from the plasmid pFcRVS (a gift from David Beier, Harvard Medical School, Boston, MA). pFcRVS consists of a 360-bp EcoRV-SstII fragment from exon 2 of the human c-MYC gene cloned into pBluescriptII vector.

Antisense RNA transcribed from pFcRVS protects a 360-bp fragment of human c-MYC and fragments of 220 and 160 bp of mouse c-myc corresponding to conserved regions between human and mouse. We synthesized ribosomal protein L32 riboprobes (gift from Michael M. Shen, Harvard Medical School) at one-tenth the specific activity of MYC probes (21) as an internal standard. The fragment protected by this riboprobe comigrates with the 220-bp mouse c-myc band protected by pFcRVS. Mouse GATA-1 mRNA was probed with a riboprobe which protects a 240-nucleotide (nt) region extending from a HinfI site in exon 2 to an EcoRI site in exon 3 of the GATA-1 gene. Mouse α-globin riboprobe (gift from Aya Leder, Harvard Medical School) consisted of a 210-bp PstI-BamHI fragment from the mouse α-globin gene (22).

For Northern analysis, total RNA (10 µg) was electrophoresed on a 1% agarose gel with formaldehyde and transferred to a positively charged nylon membrane (Nytran; Schleicher & Schuell, Inc., Keene, NH). The filters were sequentially hybridized and stripped to the following 32P-labeled double stranded probes: XhoI fragment of the murine EPO-R cDNA (gift from Alan D'Andrea, Dana Farber Cancer Institute, Boston, MA) (23); mouse β-globin (gift from Frederick Lee, Harvard Medical School) (24); SacI fragment from the Spi-1 cDNA (25, 26), Fli-1 cDNA (27); mouse GATA-1 cDNA (28); human c-MYC cDNA (17); and ribosomal protein L32 pseudogene (21, 29).

Blood and Tissue Analysis. Blood was obtained by phlebotomy of the tail vein or by cardiac puncture. Blood smears were stained with May-Grünwald-Giemsa. Bone marrow or spleen cells were resuspended in 50–100 µl PBS and concentrated on microscopic slides using a Cytospin 3 centrifuge (Shandon, Ostmore, UK).

Automated blood counts were performed with a cell counter (model H-4; Tecnicon Co., Tarrytown, NY). For histology, freshly dissected tissues were fixed in Optimem-1 Fix (American Histology Reagent Co., Stockton, CA). Fixed specimens were embedded in paraffin, sectioned, and stained by the Transgenic Pathology Laboratory at the University of California at Davis.

Cell Culture and Colony Assays. Cell lines were established by plating suspensions of cells from bone marrow or spleen in RPMI media supplemented with 10% bovine calf serum, glutamine, and antibiotics. After a few days, cells were growing rapidly in suspension and after a few weeks the cells appeared morphologically homogeneous. The cell lines required fresh media every 2 d. Subclones were derived by placing single cells into 96-well plates lined with NIH 3T3 cells that had been treated with mitomycin C to irreversibly prevent cell division.

We tested the differentiative potential of EMY-1 and EMY-2 cells by growing them in media containing mouse erythropoietin (sp act: 350,000 U/mg) (a gift from the Genetics Institute, Cambridge, MA) at a final concentration of 0.1 µg/ml (∼35 U/ml). After 10 d, total RNA was prepared for Northern analysis.

Methylycellulose cultures of bone marrow cells were plated as triplicates at two densities (5 × 104 and 1 × 105 cells/ml) in IMDM containing 4% FCS, erythropoietin (EPO) (0.08 U/ml), hIL-1 (2 ng/ml), hIL-3 (100 ng/ml), kag ligand (2% COS cell supernatant), BSA fraction V, transferrin, and lipids according to Isacove (30). Colony assays of EMY cell lines were performed in IMDM/methylycellulose supplemented with 10% FCS without addition of growth factors.

Figure 1. The GATA-1/c-MYC transgene. A 4.3-kb EcoRI-Smal fragment including 2.7 kb of the 5' flanking region, the nontranslated first exon (solid box), and 1.5 kb of the GATA-1 first intron of the mouse GATA-1 gene was ligated to a Smal-EcoRI genomic human c-MYC fragment. This fragment includes the 3' part of the first intron, the two translated exons (hatched box) and the 3' RNA processing signal from the human c-MYC gene. The position of the initiator ATG is indicated.

1 Abbreviations used in this paper: EMY, erythroleukemia/c-MYC; EPO, erythropoietin; MCFV, mink cell focus viruses.

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### Table 1. Summary of GATA-1/c-MYC Transgenic Mice

| Transgenic mouse | Transgene expression | Life span | Spleen weight | Cell line established | Fertility |
|------------------|----------------------|-----------|---------------|----------------------|-----------|
| TG.EMY-1         | +                    | 45        | ND*           | +                    | -         |
| TG.EMY-2         | +                    | 50        | 770           | +                    | -         |
| TG.EMY-3         | +                    | 35        | 830           | +                    | -         |
| TG.EMY-4         | +                    | 30        | 700           | +                    | -         |
| TG.EMY-5 #1      | +                    | 240       | 1,600         | +                    | +         |
| #2               |                      | 260       | 1,300         | -                    | +         |
| TG.EMY-6         | +                    | 30        | 520           | -                    | -         |
| TG.EMY-7         | +                    | Normal    | 110           | -                    | +         |
| TG.EMY-8         | +                    | Normal    | 90            | -                    | +         |
| TG.EMY-9         | +                    | Normal    | 105           | -                    | -         |
| TG.EMY-10        | -                    | Normal    | 90            | -                    | +         |
| TG.EMY-11        | -                    | Normal    | 95            | -                    | +         |
| TG.EMY-12        | -                    | Normal    | 100           | -                    | -         |
| TG.EMY-13        | -                    | Normal    | 95            | -                    | -         |

* Spleen enlarged but weight not determined. Transgenic F1 offspring or infertile transgenic founder animals were analyzed. Spleen weights in age-matched wild-type controls ranged between 80 and 110 mg (not shown).

#### Figure 2. Expression of the transgene in tissues and cell lines from transgenic mice. RNase protection assay with the pFcRVS riboprobe for exon 2 of human c-MYC. As indicated by arrows, this riboprobe generates a protected fragment corresponding to the human c-MYC transcripts, as well as smaller fragments that represent mouse c-myc transcripts and correspond to conserved regions between man and mouse. (RP) Ribosomal protein L32 mRNA as an internal control (left and middle only). (Left) 6-wk-old healthy TG.EMY-5 animal; (middle) 34-wk-old leukemic TG.EMY-5 mouse; (right) cell lines EMY-3 and EMY-4. Bone marrow (bm), spleen (sp), liver (li), kidney (kd), lung (lu), brain (br), heart (ht), thymus (ty), lymph node (ln), and testes (te).
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Mast cells from TG.EMY-2 and TG.EMY-5 were derived by first culturing bone marrow cells for 3 d in Dexter media and then in RPMI media with 10% bovine calf serum and 10% WEHI-3 conditioned media as a source for IL-3. Leukemic cells died in Dexter media and a homogeneous population of IL-3–dependent cells with the typical morphology of mast cells emerged. After 3 wk of culture, cells were analyzed morphologically using Cytospin preparations stained with May-Grünwald-Giemsa or toluidine blue.

Immunoprecipitations. Cell lines (107 cells each) were labeled with 0.25 mCi/ml [35S]-methionine for 6 h at 37°C. The cells were lysed on ice in 0.9 ml TBS (150 mM NaC1, 50 mM Tris, pH 8) with 1% Triton X-100 and protease inhibitors. The lysates were spun for 30 min at 10,000 g. Supernatants were adjusted to 0.2% SDS, 0.5% NP40, and 0.5% deoxycholate final concentration and incubated with a goat anti-Rauscher MuLV gp70 polyclonal antiserum that cross-reacts with SFFV gp55 (31) at a 1:200 dilution for 12 h at 4°C. Protein A-Sepharose beads (Pharmacia, Piscataway, NJ) were added, incubated for 2 h and washed four times in RIPA buffer (0.1% SDS, 0.5% NP40, and 0.5% deoxycholate in TBS) and twice with TBS. The immune complexes were eluted with SDS-PAGE loading buffer.

Results

Generation of Transgenic Mice. To direct expression of an immortalizing oncogene to erythroid or perhaps to multipotent hematopoietic precursors, we placed the human c-MYC gene under the control of mouse GATA-1 regulatory sequences (Fig. 1). To preserve the configuration of the GATA-1 regulatory region (32), we chose to use 2.7 kb of the 5′ flanking region, the noncoding first exon, and 1.5 kb of intron 1 from the GATA-1 gene. The first exon of c-MYC was excluded in this construct, as it represents a noncoding sequence that is not required for transformation (17). Thus, this fusion gene is composed of GATA-1 sequences at the 5′ portion of the first intron and of c-MYC sequences at the 3′ portion (Fig. 1).

13 founder mice carrying the GATA-1/c-MYC transgene were generated (Table 1). We have observed a phenotype in 6 of the 13 transgenics. Five transgenic founders (TG.EMY-1, TG.EMY-2, TG.EMY-3, TG.EMY-4, and TG.EMY-6) developed a rapidly progressive disease with signs of respiratory distress and anemia, and died between days 30 and 50 (Table 1). These founders were unable to generate offspring. A sixth founder, TG.EMY-5, remained healthy, allowing us to establish a breeding transgenic line. Thus far, two transgenic offspring from the TG.EMY-5 founder displayed a similar phenotype, but with a late onset, resulting in death at days 240 and 260 (Table 1). The disease is variably penetrant in this line since several transgenic mice have remained healthy past the age of 18 mo.

Analysis of the Tissue Specificity of Transgene Expression Confirmed by the GATA-1 Regulatory Sequences. To assess the transgene expression we performed an RNase protection assay with a human c-MYC riboprobe that can distinguish between transcripts originating from the transgene and from the endogenous mouse c-myc gene. We examined the surviving TG.EMY-5 line and compared the tissue distribution of transgene expression in a healthy young TG.EMY-5 animal to that in a leukemic TG.EMY-5 mouse (Fig. 2). The transgene in the healthy animal was expressed in bone marrow and, at low levels, in the spleen. This distribution agrees with that of the endogenous GATA-1 gene. In the leukemic TG.EMY-5 animal the transgene RNA was expressed at much higher levels and in several additional organs, including spleen, liver, lung, and thymus, and at low levels also in kidney, brain, and heart. Histology of the tissues from this mouse revealed leukemic infiltration of the spleen, liver (Fig. 3 D), lung, and thymus. The kidney, brain, heart, lymph node, and testis were histologically less affected or normal (not shown). This suggests that the transgene expression in these organs originated from the tumor cells. The endogenous mouse c-myc transcripts detected in the organs of the leukemic mouse seem to be derived from RNA from nonleukemic tissue, since endogenous mouse c-myc RNA was not detectable in cell lines derived from leukemic TG.EMY mice (Fig. 2). Suppression of endogenous c-myc transcripts by expression of an exogenous m cyt gene is observed regularly and appears to occur at the transcriptional level (33, 34).

In the five founders with early onset of leukemia, TG.EMY-1, TG.EMY-2, TG.EMY-3, TG.EMY-4, and TG.EMY-6, we observed a similar expression pattern of the transgene as in the leukemic TG.EMY-5 mouse and the same histopathological findings (not shown). In some cases, the lymph nodes and brain were affected as well. Three additional lines (TG.EMY-7, TG.EMY-8, and TG.EMY-9) showed expression of the transgene in bone marrow and spleen (not shown), similar to that of the healthy TG.EMY-5. We have not studied these lines in detail, although in the transgenic offspring of one of these strains a mouse was found dead that displayed splenomegaly. Offspring of the other two strains remained healthy for up to 20 mo.

As the endogenous GATA-1 gene is expressed in mast cells (5, 6), and regulates the promoters of some mast cell–specific proteases (7), we assessed expression of the GATA-1/c-MYC transgene in a homogeneous population of mast cells derived from a leukemic TG.EMY-5 animal (Fig. 4). Had the regulatory sequences used in our transgene construct been complete, we would have expected the transgene to be expressed in all cells that express the endogenous GATA-1 gene. Surprisingly, the c-MYC transgene was not expressed in mast cells from this TG.EMY-5 animal, despite expression of the endogenous GATA-1 gene. Although there was less RNA loaded in the lanes from both mast cells samples (as assessed by ribosomal protein L32 probe), mRNA for GATA-1 and Fli-1 was easily detectable. Southern analysis of DNA from these mast cells confirmed that they had not lost the transgene (not shown). Similarly, mast cells derived from the TG.EMY-1 transgenic founder did not express the transgene

Figure 3. Morphology of EMY-5 tumor cells. May-Grünwald-Giemsa stain of blood smear (A). Cytospin preparation from spleen (B) and from the EMY-5 leukemia cell line (C). Hematoxylin-eosin–stained section of the liver (D) from the leukemic TG.EMY-5 mouse.

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by the RNase protection assay (not shown). Thus, the GATA-1 sequences used in this construct are insufficient to direct expression of the transgene to the mast cell lineage.

Characterization of Primary Tumors and Derivation of Erythroleukemia Cell Lines. The peripheral blood of all leukemic animals contained proerythroblasts and erythroblasts, as shown for a leukemic TG.EMY-5 mouse (Fig. 3 A). Automated blood counts were performed on two leukemic animals and compared to healthy transgenic and wild type mice (Table 2). Leukocytosis was found in the leukemic TG.EMY-5 mouse, but not in TG.EMY-1, reflecting variability in the numbers of circulating leukemic cells. Both animals displayed severe anemia and moderate thrombocytopenia. The spleens of all leukemic mice were found to be largely replaced with leukemic cells (Fig. 3 B). Primary tumor cells from spleens of TG.EMY-1 and TG.EMY-2 animals were clonogenic in methylcellulose without addition of growth factors and were also transplantable into nu/nu mice or syngeneic FVB. Transplanted animals developed the same pathology within 3–4 wk and the leukemic cells displayed the same proerythroblast morphology (not shown).

We were able to derive cell lines from the bone marrow or spleen of leukemic animals TG.EMY-1, TG.EMY-2, TG.EMY-3, TG.EMY-4, and TG.EMY-5. All cell lines grew rapidly in suspension culture without the addition of growth factors. After a few weeks in culture they appeared homogeneous and displayed predominantly a proerythroblast morphology (Fig. 3 C). These cell lines formed colonies in methylcellulose without the addition of growth factors and 5–15% of EMY cells were clonogenic (Table 3).

The EMY cells were negative for the megakaryocytic marker acetylcholinesterase (35, 36) and the specific toluidine blue staining of mast cell granules (37). To confirm the erythroid origin of the cell lines, we performed Northern analyses with probes for erythroid-specific markers (Fig. 5 A). Both β-globin and erythropoietin receptor (EPO-R) mRNAs were expressed in all EMY cell lines, consistent with representing cells of the erythroid lineage. Treatment with recombinant mouse EPO led to upregulation of β-globin RNA (Fig. 5 B) but did not induce any morphological changes associated with maturation. We were also unable to induce the appearance of benzidine-positive cells in EMY cell lines treated with 1.2% DMSO, indicating that no significant amounts of hemoglobin were formed. Thus, erythroleukemia cells immortalized by the c-MYC proto-oncogene are partially blocked in their differentiation, similar to MEL cells transfected with c-myc expression vectors (38–40).

Since leukemia in TG.EMY-5 mice occurs stochastically after a latency of more than 30 wk, we examined whether expression of the c-MYC transgene has an effect on erythropoiesis in younger healthy transgenics. We found no difference in blood counts or red blood cell parameters between healthy 6–8-wk-old TG.EMY-5 mice and the wild type controls (Table 2). However, a subtle effect on erythroid differentiation was detectable when colony assays in methylcellulose with bone

| Table 2. Blood Counts of Transgenic Mice and Wild-type Controls |
|---------------------------------------------------------------|
|                  | TG.EMY-1 leukemic | TG.EMY-5 leukemic | TG.EMY-5 healthy | Wild-type controls |
|------------------|------------------|------------------|-----------------|-------------------|
|                  | n = 1            | n = 1            | n = 6           | n = 6             |
| WBC x 10^9/μl    | 3.3              | 41.1             | 5.7 ± 4.2       | 4.9 ± 2           |
| RBC x 10^6/μl    | 0.7              | 3.4              | 8.8 ± 0.6       | 8.6 ± 0.5         |
| HCT%             | 4                | 16               | 46 ± 4          | 47 ± 7            |
| HGB g/100 ml     | 1.4              | 5.2              | 14 ± 0          | 14 ± 1            |
| MCV fl           | 66               | 47               | 53 ± 4.5        | 55 ± 7.8          |
| MCH pg           | 21               | 15               | 16 ± 0.3        | 16 ± 0.6          |
| PLT x 10^9/μl    | 215              | 248              | 1.170 ± 278     | 1.022 ± 144       |

Blood counts were performed on a Technicon H1.
HCT, hematocrit; Hgb, hemoglobin; MCH, mean corpuscular hemoglobin (pg); MCV, mean corpuscular volume (fl); PLT, platelets; RBC, red blood cells; WBC, white blood cells.

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Table 3. **Clonogenicity Assay**

| Cell line | CFU/10^4 cells |
|-----------|----------------|
| EMY-1     | 56/33          |
| EMY-2     | 150/115        |
| EMY-3     | 144/215        |
| EMY-4     | 115/58         |

Numbers of CFU from duplicate plates are given.

Bone marrow cells from 10-wk-old healthy EMY-5 mice and littermate controls were performed (Table 4). On day 7, we observed an about twofold increase in blast-forming unit-erythroid (BFU-E) colonies in the transgenic group. When we analyzed the colonies on day 10, the number of pure erythroid colonies (E) was again increased in the transgenic group. However, no difference in GM colonies, bi-lineage colonies consisting of erythroid and megakaryocytic cells (E/Mg), or multilineage colonies containing erythroid cells (E/multi), was observed. Thus, this effect is confined to relatively late progenitor cells committed to the erythroid lineage, whereas

**Table 4. Frequency of Colony-forming Cells in Bone Marrow from Healthy EMY-5 and Wild-type Mice**

|                  | EMY-5          | Wild-type     |
|------------------|----------------|---------------|
| Cells per femur  | 19.6 ± 2.4     | 19.2 ± 5.6    |
| BFU-E per 10^5   | 10.8 ± 3       | 5.0 ± 2       |
| (day 7)          |                |               |
| Total CFU per 10^6 | 143 ± 21      | 141 ± 28      |
| (day 10)         |                |               |
| GM               | 105 ± 13       | 108 ± 20      |
| E                | 9.3 ± 3.7      | 4.6 ± 0.8     |
| E/Mg             | 25 ± 5.3       | 25 ± 7.6      |
| E/multi          | 2.5 ± 1.4      | 3.3 ± 1       |

Bone marrow cells from 10-wk old EMY-5 and wild-type littermates were assayed in methylcellulose in the presence of kit ligand, IL-1, IL-3, and EPO. BFU-E were counted after 7 d, total CFU and the CFU subtypes after 10 d. Results are expressed as mean ± standard deviation (n = three mice per group). For each mouse, colonies from six plates were counted. GM, granulocyte and/or macrophage colonies; E, erythroid; E/Mg, erythroid and megakaryocytic; E/multi, large (>1 mm) multilineage colonies consisting of erythroid, megakaryocytic, and macrophage or granulocytic cells.

**Figure 5.** Northern analysis of gene expression in EMY cell lines. (A) The same blot was sequentially probed with specific 32P-labeled cDNAs as indicated, except for EPO-R, for which a second blot with the identical RNAs was used. (RP) Was used to normalize for RNA loading. (B) Analysis of the effect of EPO on expression of β-globin in EMY cell lines.
more immature precursors with bi- or multilineage potential seem to be unaffected.

**Search for Secondary Events Involved in Transformation of EMY Cell Lines.** To assess the possibility that the secondary events frequently associated with Friend virus or spleen focus forming virus transformation might also be involved in leukemogenesis in our transgenic mice, we analyzed the expression of Fli-1 (27) and Spi-1 (25, 26) in EMY cell lines (Fig. 5 A). Spi-1 was not expressed in EMY erythroleukemic cell lines and therefore does not play a role. In contrast, Fli-1 mRNA was expressed in three of the five cell lines (EMY-1, EMY-2, and EMY-4). Thus, activation of Fli-1 could be one of the mechanisms in the leukemogenesis in these TG.EMY mice.

Friend helper virus can activate dormant endogenous mink cell focus viruses (MCFV) to express the oncogenic truncated envelope protein gp55 (41). To rule out the possibility that the deregulated expression of the c-MYC transgene could activate endogenous MCFV, we analyzed expression of gp55 protein in metabolically labeled EMY cell lines by immunoprecipitation with antienvelope antibodies (Fig. 6). In MEL cells this antiserum (31) recognizes both the wild-type envelope protein (gp70) and the mutated gp55, as well as an uncharacterized intermediate of ~60 kd (42). In EMY cell lines we found no envelope protein. The bands visible in EMY-2 display a faster mobility than gp55 and on longer exposure appeared in all lanes, including the preimmune sera panel. Therefore, the c-MYC transgene does not activate dormant MCFV and does not induce expression of gp55. Thus, despite the fact that EMY cells resemble Friend virus–derived MEL cells in some respects, they have been generated by a distinct mechanism.

**Discussion**

We have generated transgenic mice that are prone to developing erythroleukemias because of the deregulated expression of the c-MYC proto-oncogene. We have derived erythroleukemia cell lines from these animals that morphologically resemble proerythroblasts (Fig. 3 C) and constitutively express the erythroid markers EPO-R and β-globin (Fig. 5 A). The occurrence of erythroleukemia infers that the regulatory elements necessary for expression in the erythroid compartment are present in our construct. Interestingly, committed hematopoietic precursors deficient for the GATA-1 gene display a block in erythropoiesis at the proerythroblast stage, as determined by an in vitro differentiation assay of GATA-1–embryonic stem cells, indicating that GATA-1 is required for differentiation to later stages of erythroid development (43).

The reproducible observation of leukemias with exclusively erythroid characteristics was unexpected, since the endogenous GATA-1 gene is also expressed in other hematopoietic lineages, such as mast cells, megakaryocytes, and eosinophils (5-8). The regulatory sequences from the GATA-1 gene used in this transgenic construct might include the elements necessary for erythroid expression only. Consistent with this notion we found that the transgene was not expressed in mast cells derived from the TG.EMY-5 line (Fig. 4). This suggests that the region of the mouse GATA-1 gene comprising 2.7 kb of the 5' upstream region and 1.5 kb of the first intron lacks element(s) required for expression in mast cells in vivo. The EMY cell lines did not display megakaryocytic markers, such as acetylcholinesterase (35, 36) or 4A5 (44). We were unable to determine expression of the transgene in megakaryo-
expression. The levels of leukemia in the majority of our transgenic mice, however, in our transgenic cell lines (Fig. 6). The early onset of erythroid retroviruses activated expression of Friend spleen focus virus envelope protein gp55, a mechanism that has been excluded from Friend erythroleukemia in this transgenic strain, it should provide a useful system in which to identify the secondary genetic events that can collaborate with c-MYC to accelerate tumor formation in the erythroid lineage. The use of insertional mutagenesis with retrovirus or appropriate genetic crosses with other tumor-predisposing transgenic lines should be particularly fruitful in this regard.
We wish to thank Cathie Daugherty for assistance with the tissue culture, Susi Wehrli and Valerie Quesniaux for the bone marrow colony assays, and Juanita Campos-Torres for cyttofluorometry. We thank David Seldin, Michael Shen, and Alan D'Andrea for comments on the manuscript, Robert D. Cardiff for reviewing the histopathology, and André Tichelli for reviewing the bone marrow morphology.

R. C. Skoda was supported in part by the Schweizerische Stiftung für Medizinisch-Biologische Stipendien.

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Received for publication 20 April 1994 and in revised form 13 December 1994.

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