High Frequency of Myelomonocytic Tumors in Aging Eμ L-*myc* Transgenic Mice

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Summary

Transgenic mice that contain constructs of the L-*myc* gene under the transcriptional control of the immunoglobulin heavy chain enhancer (Eμ) develop thymic hyperplasia and are predisposed to T cell lymphomas. Here we describe a second form of malignancy that occurs in aging Eμ L-*myc* transgenic mice. The mean latency period for the development of this malignancy is longer compared with the Eμ L-*myc* T cell lymphomas but the overall incidence is increased threefold. The histopathological morphology is that of a highly malignant mesenchymal neoplasm that closely resembles human fibrous histiocytoma. The tumor cells were classified as myelomonocytic on the basis of several lineage-specific markers and the lack of rearrangements of the immunoglobulin heavy chain and the T cell receptor β loci. Cultured tumor cells produce macrophage colony-stimulating factor (M-CSF) protein and express the M-CSF receptor, suggesting the involvement of an autocrine loop in this malignancy. Similar to the Eμ L-*myc* T cell lymphomas, these tumors show high-level transgene expression but no detectable levels of endogenous c-*myc* mRNA, directly implicating the deregulated expression of L-*myc* in the generation of this malignancy. Eμ L-*myc* myelomonocytic tumors show consistent trisomy of chromosome 16, implicating this as a secondary event in the development of this tumor. In the light of recent findings that L-*myc* is expressed in human myeloid leukemias and in several human myeloid tumor cell lines, the results described here might implicate L-*myc* in the development of naturally occurring myeloid neoplasias.

The *myc* oncogene family contains three well-defined members: c-, N-, and L-*myc*. All three genes have a similar three-exon structure and encode nuclear phosphoproteins that contain several regions of extensive homology in their amino acid sequences (1). The conservation of the individual *myc* genes through evolution suggests that they exert unique but related functions (2–6). Several studies have shown that *myc* gene products are involved in the regulation of cellular proliferation and differentiation, but their exact function remains to be elucidated (1). The COOH-terminal region of the c-, N-, and L-*myc* proteins contains domains that have been shown to be functional for DNA binding and protein-protein interaction in transcription factors. Recently, a specific DNA binding site has been identified for c-*myc*, suggesting that *myc* proteins act as transcriptional regulators (7).

While c-*myc* is expressed in virtually all dividing cells, the expression of N- and L-*myc* is more restricted and occurs predominantly at earlier stages of development (8–10). Differential expression of *myc* family genes occurs in the development of lymphoid cells. The c-*myc* gene is expressed in all normal and transformed pre-B and B cells, as well as in plasma cells. N-*myc* expression is found in normal and transformed pre-B cells but expression is downregulated when pre-B cells differentiate into B cells and acquire surface receptors (8). Preliminary data suggest a similar expression pattern in T cell development (R. DePinho, unpublished data). L-*myc* expression has not been detected in lymphoid cells with the possible exception of very early precursors (DePinho et al., unpublished data).

All three *myc* genes can cooperate with an activated Ha-*ras* gene to transform primary rat embryo fibroblasts, but the L-*myc* gene has a lower transforming potential compared to c- and N-*myc* (5, 12–16). Activation of the c-*myc* gene has been documented in a wide variety of naturally occurring tumors, but activation of the other two *myc* genes has been found only in a more restricted set of malignancies (17). N-*myc* activation is found in tumors of neuroectodermal origin (2, 18–21), in T cell lymphomas (22, 23), and most recently in Hepatitis B virus–associated hepatocellular carcinoma (24). In naturally occurring tumors, L-*myc* activation has been clearly implicated only in a subset of small cell lung carcinomas (20). However, both N- and L-*myc* are expressed in different subsets of human acute myeloid leukemias and myeloid tumor cell lines (11).

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Constructs containing the c-, N-, or L-*myc* gene under the control of the Ig enhancer element (Eμ) have been introduced into the germline of transgenic mice. The results obtained showed common as well as unique activities (25–32). Eμ N-*myc* and Eμ c-*myc* transgenic mice are similarly predisposed to the development of pre-B and B cell lymphomas (25–30). However, the onset of Eμ N-*myc* tumors was delayed compared with the onset of Eμ c-*myc* tumors (28, 29). Mice that received an Eμ L-*myc* construct virtually identical in its design to the Eμ c- and N-*myc* constructs had features quite different from the Eμ c- and N-*myc* animals (31). Expression of the transgene was predominant in pre-T and T cells, and was accompanied by disturbances of thymic architecture and high level expression of the early T cell marker 1C11 (31). Eμ L-*myc* transgenic mice are predisposed to the development of lymphomas (predominantly thymic T cell tumors), but with a lower incidence and a much longer latency period than Eμ c- or N-*myc* transgenics (31). Here we report that Eμ L-*myc* transgenic animals develop a second, distinct form of malignancy later in life that occurs with a higher frequency than the T cell tumors and apparently originates from myelomonocytic and not from lymphoid cells.

Materials and Methods

Molecular Analyses and Probes. Preparation of genomic DNA from tumor samples and cell lines was performed as previously described (33). DNA blotting procedures were performed as described elsewhere (34, 35). The J_H probe used for Southern analysis is a 2-kb fragment covering J_H 1-4 (36, 37), the $J_H E\mu^-$ probe is an XbaI-EcoRI fragment covering J_H3-4, the Jβ2 probe that was used to detect TCR-β rearrangements is a 1.9-kb HindIII-BamHI fragment containing the Jβ locus (38), and the cκ probe is described in Lewis et al. (39). Preparation of RNA and Northern blotting was as described (33). c-, N-, and L-*myc* probes and the probes specific for Cμ and VhJ558 have already been described (3, 8, 14, 35, 40). The Thy-1 probe is a 1.4-kb ApaI fragment isolated from the 3′ untranslated region of the Thy-1 gene (41), the *fos* probe is a 1.3-kb fragment from the plasmid pfos 1 (42). The c-*myb* probe is a 2.5-kb EcoRI c-DNA fragment (43), the c-*fms* probe is a 3.6-kb fragment from the murine c-DNA (44), the macrophage (M)[1]-CSF specific probe is a 1.8-kb fragment from the murine c-DNA, and the probe specific for granulocyte/macrophage (GM)-CSF is a 1-kb fragment from the murine cDNA.

Tissue Culture Conditions. Single cell suspensions prepared from primary tumor samples obtained at the time of autopsy were grown at different cell densities in RPMI with 20% FCS, 2 mM L-glutamine, with or without 50 mM 2-ME, and antibiotics. Cells were passaged using Trypsin/EDTA. To assay for M-CSF activity in culture supernatants of tumor cells, bone marrow was harvested from femurs of C57Bl6 mice and plated in 1 ml 1% semisolid agar at a density of 10^5 cells per 35-mm culture dish (45). 200 μl of supernatant from each control cell line or from tumor cell cultures or 250 U of purified M-CSF were added to the medium. After an incubation period of 6 d, colonies were enumerated and stained with Giemsa solution for morphological analysis. The assay for phagocytosis was done as described (48). Briefly, the cells were incubated in medium with 3-mm latex beads for 16 h, washed three times while still adhering to the plate, trypsinized, pelleted onto glass slides, and examined microscopically.

Antibody Staining Procedures. Single cell suspensions were made at the time of autopsy from tumor tissue in RPMI supplemented with 5% FCS. Cells were filtered through a cushion of 2 ml of FCS, washed in staining solution (PBS, 1% BSA, 0.1% sodium azide), and incubated on ice for 30 min with either directly FITC-conjugated antibodies or with biotin-labeled antibodies. Cells were washed twice in staining solution after the incubation and if necessary counterstained with FITC- or PE-labeled streptavidin and examined under a fluorescence microscope. Frozen sections were manipulated in a humid chamber at room temperature. They were blocked in PBS/1% BSA for 30 min, washed three times with PBS, incubated with biotinylated antibody in PBS/1% BSA for 30 min, again washed three times in PBS, stained with FITC-coupled streptavidin, washed, mounted, and examined under a fluorescence microscope.

Detection of Myeloperoxidase and Nonspecific Esterase Activity. Reagents to perform tests for myeloperoxidase and naphthyl acetate esterase were purchased as a kit (Sigma Chemical Co.). To obtain cytosmears, cells from tumor cultures or directly explanted from tumor tissue were pelleted onto glass slides, fixed, and treated according to the manufacturer's instructions. Frozen sections of tumor tissue were also used in the naphthyl acetate assay and were treated in the same way as the cytosmears. After the procedure the glass slides were counterstained with hematoxylin/eosin, mounted, and examined microscopically.

Histological Evaluation. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin eosin. Cytosmear preparations were fixed in methanol and stained with Giemsa/White stain.

Chromosome Preparation. Metaphase spreads were prepared from cell suspensions of subcutaneous solid tumors, spleen, mesenteric, and peripheral lymph nodes. The metaphase plates were G-banded according to a slightly modified method of Wang and Fedoroff (58, 59). Between 6 and 10 metaphase plates were karyotyped for each individual tumor. Duplicated chromosomes 16 were identified microscopically in 5–10 plates of high banding quality. Chromosomes were identified according to the criteria specified by the Committee of Standardized Nomenclature (47).

Results

Mesenchymal Tumors in Eμ L-myc Transgenic Mice. The generation and establishment of Eμ L-*myc* transgenic mouse lines with various constructs and the tissue-specific expression pattern of the transgenes has been described (31). Of a population of Eμ L-*myc* transgenic mice observed over a period of 18 mo, ∼10% developed T cell lymphomas with an average latency of 9 mo (31). A distinct second form of malignancy occurs in older transgenics. Of a population of 91 Eμ L-*myc* mice, ∼30% developed these neoplasms with an average latency of 14 mo (Table 1). These tumors were manifest as solid tumors arising either in the skin or in the peritoneal cavity. Histopathological evaluation showed highly invasive masses of malignant mesenchymal cells with a tendency to form giant cells (Fig. 1, *a* and *b*). These malignancies most closely resembled malignant fibrous histiocytoma and arose in seven of nine Eμ L-*myc* transgenic lines with the highest incidence in the line EVL_{cd} 31.14 (Table 1).

[1] *Abbreviation used in this paper:* GM-CSF, granulocyte/macrophage colony-stimulating factor.

Table 1. *Mesenchymal Tumors in Eμ L-myc Transgenic Mice*

Line		No. of Animals	Tumor	Location	Age
					mo
EL	27.3	26	27.3	Skin	17
			273.20.8	Int/Spl/Skin	10
			273.25	Skin	14
			273.36.13	Skin	13
			273.23.7	Skin	13
			273.39	Spl/LN/Kidney	16
	29.7	8	–		
	35.6	18	356.21.15	Int/Skull	14
EVL	18.1	7	181.26	Skin/LN	6
			181.21.15	Skin	13
			181.FG.7	Skin/Head	11
	20.14	2	215.5.9	Skin/Int	15
EVL_{cd}	31.14	15	314.10.10	Skin	12
			314.10.1	Skin/Head	16
			314.10.15	Skin/Head	14
			314.10.9	Skin	14
			314.10.12	Skin	12
			314.10.13	Skin/Head	14
			314.23	Skin	12
			314.10	Skin	12
			314.5	Skin	17
			314.8	Skin	13
			314.14	Skin	16
			31.14	Skin	18
			314.33	Skin	17
			314.21.7	Skin	13
			314.21.4	Skin/Head	15
	33.42	6	342.10	LN/Spl/Skin	11
			342.13.5	Skin/Muscle	13
			342.12.19	Skin	11
			342.13.8.15	Skin/Head	14
	42.12	9	42.12	Skin	23
Total		91	31		

Representation of all mesenchymal tumors that arose in a population of 91 Eμ L-*myc* transgenic mice. No tumors were observed during a time period of 18 mo in a population of 50 nontransgenic littermates. Average time of tumor onset was 14 mo. Int, intestinal cavity; Spl, spleen; LN, lymph node.

Cells explanted from these tumors were usually not adaptable to tissue culture conditions and could be kept only for two or three passages, with the exception of the tumor 314.8, where >20 passages were achieved (Table 2). In three of five cases tested, tumors were transplantable into syngeneic animals (Table 2). The transplantability was tested using cells directly obtained at autopsy of the animals. For the tumor 314.8, cells were also successfully transplanted after tissue culture.

Table 2. *Cell Explants of Myelomonocytic Tumors*

Tumor		Growth*	Transplant‡	MPO§	Phagocytosis‖
EL	273.23.7	+/–	–	+	+
	273.39	+	+	+ +	+
EVL	214.5.9	+/–	–	+	+
	181.26	+/–	ND	+/–	ND
EVLcd	314.8	+ + +	+	+ +	+
	314.14	+/–	+	+	+

* Growth of cells obtained from tumor tissue according to their approximate doubling time. + + +, cells were split 1:3 after 3–4 d; +, cells were split 1:3 after 8–10 d; +/–, cells were split after 2–3 wk.
‡ 1–1.5 ml of cultured tumor cells were injected at a density of 10^6–10^7 cells/ml intraperitoneally and subcutaneously into syngeneic animals.
§ +/–, low; +, medium; + +, high intensity of staining with a substrate specific for myeloperoxidase (Sigma Chemical Co., St. Louis).
‖ All tumor cells showed approximately the same degree of phagocytic activity. In the case of 214.5.9, only 50% of the cells showed phagocytic activity. The negative controls for the phagocytosis assay were 38B9 pre-B cells and NIH 3T3 fibroblasts.

Mesenchymal Tumors in Eμ L-myc Transgenic Mice Consist of Myelomonocytic and Not of Lymphoid Cells. To identify the exact cell type of these mesenchymal tumors, several lymphoid lineage markers were first tested. Southern analysis of DNA from mesenchymal tumors revealed no rearrangements of the Igμ heavy, the Igκ light chain locus, or the TCR β locus (Table 3). We did not detect transcripts specific for the T cell surface marker Thy-1 (Fig. 2), the TCR α or γ genes, or the lymphoid cell–specific enzyme TdT (not shown). However, transcripts hybridizing to an Ig constant region (Cμ)-specific probe were found in the control T cell tumor 181.6 and in all mesenchymal tumors, except tumor 273.25 and 342.10. These transcripts correspond in size (2.2 and 2.6 kb) to germline Cμ transcripts (40) (Fig. 2). A major Cμ-specific transcript of 2.4 kb, corresponding to a mature Cμ mRNA, was detected in the control B cell tumor 356.3. and in tumors 273.25 and 342.10 (Fig. 2). Rehybridization of the same blot with an Ig c-κ probe showed the absence of Ig κ light chain messenger RNA in all tumors except 273.25 and 342.10 (Fig. 2). Material from tumor 273.25 was no longer available, but a histopathological reevaluation of tumor 342.10 showed the presence of both lymphoid and mesenchymal malignancies. It is likely that the mature Ig μ and Ig κ transcripts seen in both cases stem from lymphoid tumor cells contaminating the mesenchymal tumor (not shown). 342.10 tumor cells in culture failed, however, to produce a nonadherent lymphoid cell population, arguing that in this case mature Cμ transcripts and, therefore, Ig rearrangements could be carried by the mesenchymal cells. However, the absence of mature Ig μ heavy and Ig κ light chain messenger RNA and the lack of Ig and TCR gene rearrangements in the majority of the

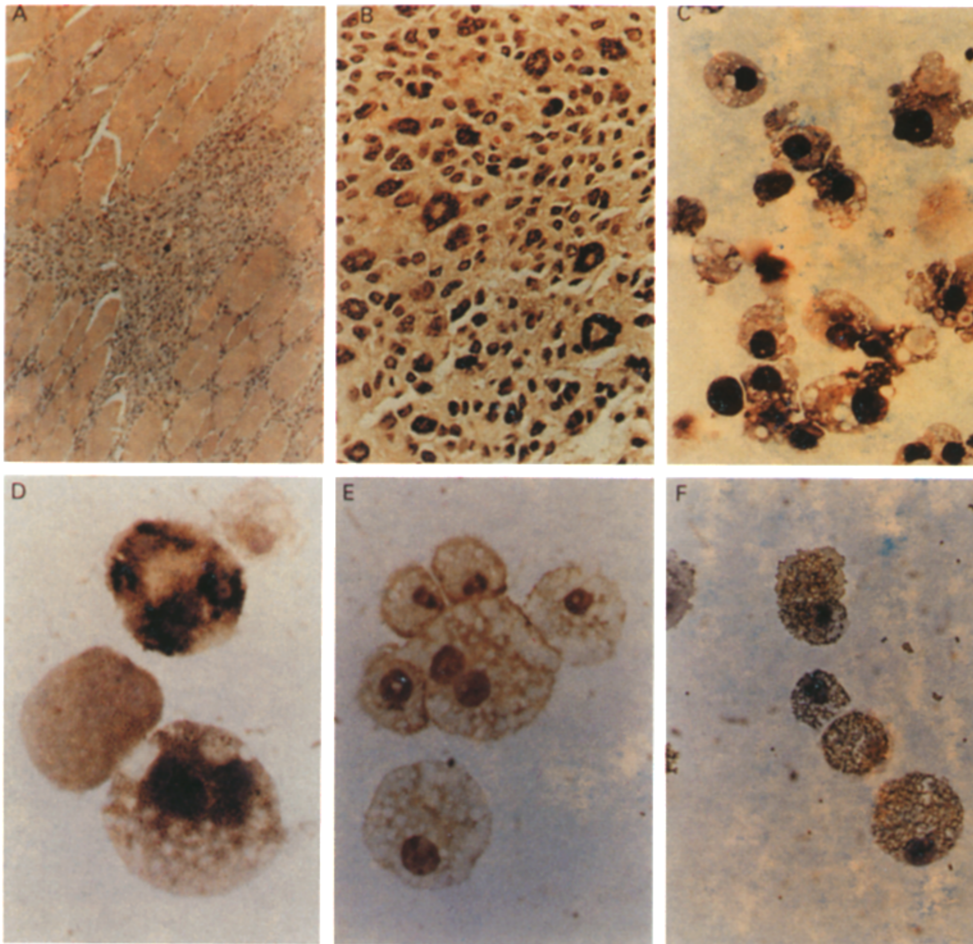

Figure 1. (*A*) Skeletal muscle invaded by mesenchymal tumor cells stained with hematoxylin/eosin. (*B*) Formation of tumor giant cells in a paraffin section of tumor tissue stained with hematoxylin/eosin. (*C*) Cytosmear of tumor cells colored with Giemsa/White stain. (*D*) Cells after reaction with substrates specific for naphthyl acetate esterase or (*E*) myeloperoxidase. (*F*) Cells after incubation with 3.5-mm latex beads. The cells shown in *C*, *D*, *E*, and *F* were from tumor 314.8. Positive controls for the myeloperoxidase- and naphthyl-specific esterase assay were neutrophils from bloodsmears, cytosmears from the myeloid cell lines RAW, P338, and M1S2. Negative controls consisted of cytosmears from the Abelson line 38B9, NIH3T3 fibroblasts, and a frozen section of a thymic lymphoma derived from an Eμ L-*myc* transgenic animal.

cases clearly classifies the Eμ L-*myc* mesenchymal tumors as nonlymphoid.

Giemsa-stained cytosmears from explanted tumor cells showed a morphology consistent with monocytes and macrophages (Fig. 1 *C*). A number of assays specific for myelomonocytic cells were performed: all tumors showed phagocytic activity, the presence of myeloperoxidase, naphthyl-specific esterase, and expression of the surface marker Mac-1 (Fig. 1, and Tables 2 and 3). The cells derived from mesenchymal neoplasms that arise in aging Eμ L-*myc* transgenic animals were therefore classified as myelomonocytic.

Oncogenes and Growth Factors. The nuclear proto-oncogenes c-*myb* and c-*fos* are expressed at different stages during the development of lymphoid and myeloid cells; c-*myb* transcripts are easily detected in pre-B cells and myeloid precursor cells, but expression is downregulated in mature lymphoid cells and during terminal myeloid differentiation. The c-*fos* gene is expressed at high levels in mature myeloid cells but is not readily detected in lymphoid cells (46, 48–50). To investigate the expression pattern of c-*myb* and c-*fos* in Eμ L-*myc* lymphoid and myelomonocytic tumors, RNA from both tumor types was analyzed by Northern blotting. Levels of c-*myb*-specific RNA are high in the Eμ L-*myc* lymphoid tumors compared with 38B9 pre-B cells with the exception of the tumor 356.3, a mature B cell tumor and tumor 342.10, which might represent a mixture of myelomonocytic and lymphoid tumor cells. The level of c-*fos* message is low to undetectable (Fig. 3 *B*). Compared with the same set of controls, the reverse situation is found for the myelomonocytic tumors, i.e., very low levels of c-*myb* and high levels of c-*fos* expression (Fig. 3 *A*). A similar inverse correlation of c-*myb* and c-*fos* expression between lymphoid and myeloid tumor cells has been reported (46), and most probably reflects the lineage commitment and differentiation stage of the tumor cells. This again points to the myeloid origin of the mesenchymal Eμ L-*myc* tumors.

To investigate the possibility that myeloid/granulocyte–specific growth factors or receptors are expressed in Eμ L-*myc* myelomonocytic tumors and may have contributed to the establishment of these neoplasias, RNA from fresh tumor tissue was analyzed for the expression of M-CSF, G-CSF, GM-CSF, IL-3, and the M-CSF receptor c-*fms*. Expression of c-*fms* was detected in all myelomonocytic tumors (Fig. 3 *A*). Transcripts specific for IL-3 and G-CSF were not readily detected in the myelomonocytic tumors, but transcripts for GM-CSF were found in tumor 214.5.9 (Fig. 3 *A*). Expression of M-CSF was found in most of the myelomonocytic tumors (Fig. 3 *A*). The sizes of the M-CSF transcripts are 1.6 and 4 kb cor-

Table 3. *Myelomonocytic Tumors: Lymphoid and Myeloid Markers*

Tumor		NSE*	Mac-1‡	Jhr§	κr§	J_{β}2r§
EL	273.25	ND	ND	–	–	–
	273.23.7	+++	+	–	–	–
	273.39	+++	+	–	–	–
EVL	215.5.9	++	+	–	–	–
	181.26	++	+	–	–	–
EVLcd	314.10.13	++	+	–	–	–
	314.23	+/–	+	–	–	–
	314.5	+	+	–	–	–
	314.8	++	+	–	–	–
	314.14	++	+	–	–	–
	31.14	ND	+	–	–	–
	314.33	ND	+	–	–	–
	314.21.7	+++	+	–	ND	–
	342.10	+++	+	–	ND	–
	342.12.19	+++	+	–	ND	–

* The assay for naphthyl acetate esterase was performed either with cells directly explanted from a tumor and pelleted onto glass slides or by using sections of frozen tumor tissue according to the manufacturer's protocol (Sigma Chemical Co.).
‡ Staining of tumor cells with Mac-1 was carried out on cytosmears or on cells in suspension. The percentage of Mac-1-positive cells was 80% for the tumor 273.39, 75% for 273.23.7, 76% for 314.10.13, 90% for 214.5.9, >95% for 314.5, >95% for 314.8, 90% for 31.14, 84% for 314.14, 43% for 314.21.7, and 85% for 314.33. In all other cases the staining was done on frozen sections of tumor tissue, and was not quantified.
§ Jhr, κr, J_{β}2r: rearrangements of the J region of the heavy chain locus, the κ light chain locus, and the T cell receptor β locus, respectively.

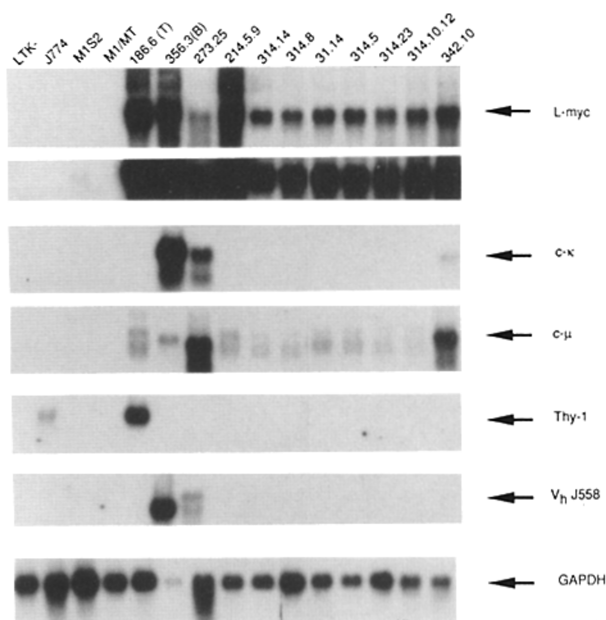

Figure 2. Northern analysis of 10 μg total RNA derived from Eμ L-*myc* myelomonocytic tumors and controls. Duplicate blots were sequentially hybridized with the indicated probes. To control for uniform loading, the blots were probed with a GAPDH-specific DNA fragment. From left to right were LTK$^-$ fibroblasts, the myeloid cell lines J774, M1S2, and M1/MT, a T cell and a B cell lymphoma derived from Eμ L-*myc* transgenics (181.6 [T] and 356.2 [B] respectively), and nine different myelomonocytic tumors that arose in Eμ L-*myc* transgenic mice. The second panel from the top represents a longer exposure of the first panel.

responding to alternative splicing (51). Both messages were found in tumor 342.10 but only the 4-kb message was detected in all other tumors (Fig. 3 *A*).

The presence and activity of M-CSF protein was also tested in supernatants of four different myelomonocytic tumor cell cultures by assaying the outgrowth of macrophages from normal bone marrow. Freshly aspirated bone marrow cells were treated with supernatant from a tumor cell culture or the appropriate controls. The supernatants from three of four tumor cell cultures produced colonies that exclusively displayed macrophage morphology (Table 4), indicating that they produce functional M-CSF. The supernatant from tumor cell culture 215.5.9 produced 50% macrophage colonies and 50% granulocyte colonies (Table 4), showing that tumor 214.5.9 secretes both functional GM-CSF and M-CSF. The lymphoid control cell line 38B9 did not yield colonies, but all the myeloid control cell lines did provoke the outgrowth of either macrophage, granulocyte, or mixed colonies, demonstrating their production of either M-CSF or GM-CSF (Table 4).

Karyotypic Abnormalities in Eμ L-myc Tumors. Four myelomonocytic tumors (nos. 1–4, Table 5), two lymphomas (nos. 5 and 6), and one plasmacytoma (no. 7) that developed in the Eμ L-*myc* transgenic lines 31.14, 27.3, 29.7, 42.12, and 18.1 were karyotyped (Table 5). A trisomy of chromosome 16 was observed in all four myelomonocytic tumors as a single and consistent chromosomal aberration (Table 5, Fig. 4). Trisomy 15 was a nonrandom chromosomal change in the lymphomas nos. 5 and 6. Trisomy 16 was also observed in lymphoma no. 5. The plasmacytoma (no. 7) showed trisomy of chromosome 9 and a translocation of chromosomes 14 and X (Table 5). Since all were primary tumors, the likelihood of secondary chromosomal change was minimal. This is also reflected by the absence or very low frequency of chromosomal rearrangements (marker chromosomes) in all seven tumors and also by their strict modal chromosome nos. 41 and 42, respectively (Table 5).

Downregulation of c-myc Expression in Myelomonocytic Tumors. A downmodulation of the expression of endogenous *myc* genes has invariably been observed in tumors that contain activated *myc* genes or transcriptionally deregulated *myc* transgenes (17, 21, 25, 28, 29, 31). For c-*myc*, this effect is achieved by the deregulation of its expression alone; in the case of N- and L-*myc*, a high level of expression may also be necessary for the downmodulation of endogenous *myc* expression (25, 28, 29, 31, 52). All Eμ L-*myc* myelomonocytic tumors described here produce levels of L-*myc* mRNA that are comparable to the levels expressed in Eμ L-*myc* lymphoid

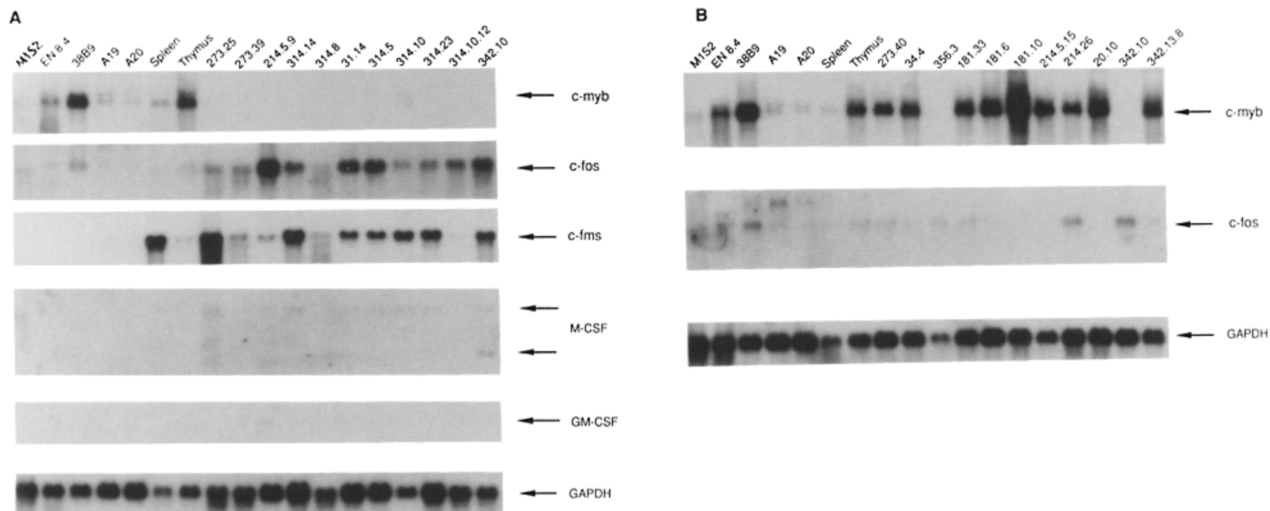

Figure 3. (*A*) Expression of c-*myb*, c-*fos*, c-*fms*, M-CSF, and GM-CSF in Eμ L-*myc* myelomonocytic tumors and controls. EN 8.4 is a B cell tumor derived from a Eμ N-myc transgenic animal; A19 and A20 represent RNA samples from LPS-stimulated splenocytes. Duplicate blots were prepared with 10 μg of total RNA from the indicated source per lane. RNA loading was controlled by hybridization with GAPDH. (*B*) Duplicate blots showing expression of c-*myb* and c-*fos* in Eμ L-*myc* lymphoid tumors and controls. The last hybridization was performed with the GAPDH probe to ensure uniform loading.

Table 4. *Production of M-CSF in Myelomonocytic Tumors*

Supernatant	CFU/10^5 cells/ 0.2 ml SN‡	Colony morphology* Mac	Gran	Mac/Gran mix
			%	
Controls:§				
Medium	0			
38B9	0			
J774	109 ± 2	82	7	11
M1S2	1 ± 1	100		
P388	8 ± 3	100		
WD/pLJ	48 ± 2	65	6	29
M-CSF (250U)	47 ± 4	100		
Tumor cells:				
314.8	15 ± 8	100		
181.26	8 ± 2	100		
214.5.9	7 ± 1	50	50	
273.23.7	6 ± 1	100		

* The numbers indicate the percentage of colonies that consist of macrophages or granulocytes or the percentage of colonies that show a mixture of both cell types.

‡ Given are the numbers of CFU developed after the treatment of normal murine bone marrow cells plated at a density of 10^5 per 35-mm dish in semisolid agar with 200 μl of supernatant of the indicated cell lines.

§ 38B9 is an Abelson virus–transformed pre-B cell line, J774, M1S2, P388, and WD/pLJ are myeloid control cell lines.

tumors (Figs. 2 and 5). As there is no endogenous L-*myc* expression in normal murine lymphoid or myeloid cells, including macrophages and their precursors (8, 31, and T. Möröy and P. E. Fisher, unpublished results), a third exon murine L-*myc* probe was used to detect expression from the Eμ L-*myc* transgene. c-*myc* mRNA is undetectable in Eμ L-*myc* myelomonocytic tumors (Fig. 5). This downregulation of endogenous c-*myc* expression has also been observed in Eμ L-*myc* lymphomas (31) and implicates the deregulated expression of L-*myc* directly in the tumorigenic process.

Discussion

Myelomonocytic Tumors in Eμ L-myc Transgenic Mice. Eμ L-*myc* transgenic mice are predisposed to T cell lymphomas, but later develop a second form of neoplasia diagnosed as malignant fibrous histiocytoma. These malignancies occur with a high incidence (31%) in Eμ L-*myc* transgenic mice after a mean latency period of 14 mo. The tumor cells were classified as myelomonocytic and nonlymphoid on the basis of a number of lineage-specific markers. The expression patterns of c-*myb* and c-*fos* in these tumors correlate inversely with the pattern found in Eμ L-*myc* lymphoid tumors and are characteristic of more mature myelomonocytic cells (46).

Histiocytic or monocytic sarcomas have already been described to occur in mice and were then classified according to morphological features. The tumor infiltrate in these sarcomas contained giant cells that were also observed to occur in the Eμ L-*myc* tumors. More recently, histiocytic lymphomas were found in transgenic mice that contain an activated N-*ras* gene under the transcriptional control of the Ig heavy chain enhancer and the SV40 promotor (53). The tumor cells dis-

Table 5. *Chromosomal Constellation in Eμ L-myc Tumors*

Tumor no.	Construct	Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	Tr (X;14)	M1	M2	M3	Modal Chr.no.	Location*
1	EVL	18.1	2	1	2	2	2	2	2	2	1.9	2	1.8	1.7	2	2	2	3	2	2	2	2	–		0.3	0.2	0.6	41	s.c.
2	EVL_{cd}	31.14	2.1	2	2	1.9	2	2	2	2	2	2	2	2	2.4	2.1	2	3	2	2	2	1	1					42	s.c.
3	EVL	18.1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	–					41	s.c.
4	EVL_{cd}	42.12	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	–					41	s.c.
5	EL	27.3	2	2	2	2	2	2	2	2	2	2	2	2	1	2	3	3	2	2	2	1	1					42	pln
6	EL	27.3	2.1	2.1	2	2	2.1	2	2.2	2.5	2.6	2	2.1	2	2.9	2	3	2.2	2.7	1.9	2	0.8	–	1				42	spl/mln
7	EL	29.7	2	2	2	2	2	2	2	2	2.9	2	2.2	2	2	2	2	2.1	2	0.8	0.8	1	1					41	spl/mln

Summary of the results of a chromosomal analysis of seven Eμ L-myc myelomonocytic and lymphoid tumors.
* Tissue taken for chromosome preparation: s.c., subcutaneous tumor; pln, peripheral lymph node; spl, spleen; mln, mesenteric lymph node.

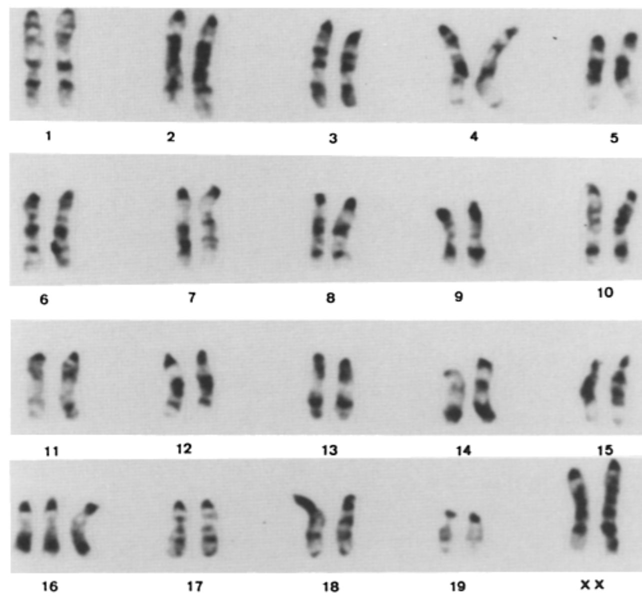

Figure 4. Chromosomal configuration of Eμ L-*myc* myelomonocytic tumor cells (tumor no. 3).

play the Mac-1 antigen on their surface, but in contrast to the Eμ L-*myc* malignancies, do not show phagocytic activity or form giant cells (53). These tumors were classified as a reticulum cell sarcoma type A (53). Similar to the Eμ L-*myc* transgenic mice, EμSV N-*ras* animals also develop thymic T cell lymphomas that occur with a lower incidence than the myeloid tumors (53).

Other cases of monocyte/macrophage tumors were observed in BALB/c mice at very high frequency when infected with a c-*myc*-containing retrovirus and treated with pristane (54). In some cases retroviral insertion upstream of the M-CSF gene activated the production of M-CSF. As the tumor cells bear the M-CSF receptor (c-*fms*), an autocrine loop was installed and permitted indefinite growth (54, 55). We have demonstrated the expression of the M-CSF receptor (c-*fms*) and the presence of functional M-CSF in Eμ L-*myc* myelomonocytic tumors. It is therefore not unlikely that a similar autocrine mechanism is responsible for the full transformation and growth of these tumor cells. However, further studies of

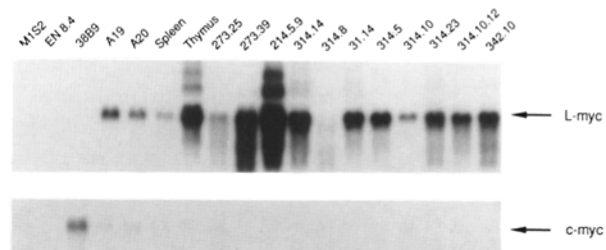

Figure 5. Comparison of the levels of expression of L-*myc* and c-*myc* in Eμ L-*myc* myelomonocytic tumors and control cells by Northern analysis of 10 μg of total RNA from the indicated sources (as in Fig. 3 *A*). Duplicate blots were prepared and controlled for uniform loading by rehybridization with a GAPDH-specific probe (not shown).

growth interference with anti-M-CSF antibodies are necessary to show the existence of an autocrine loop in Eμ L-*myc* myelomonocytic tumors.

Incidence and Latency Period of Eμ L-myc Malignancies. One of the most striking features of Eμ L-*myc* myelomonocytic tumors is their increased latency period. The mean age at which transgenic mice develop this malignancy is 14 mo compared with 9 mo for the development of lymphoma (31). One explanation for this phenomenon could be that immunosurveillance is impaired in older mice and that this effect is even exacerbated by the expression of an L-*myc* transgene in lymphoid cells. Age-associated thymic atrophy in humans is believed to be responsible for senescence of the immune system and subsequent development of autoantibodies and tumors (56). However, one would also expect the number of T cell lymphomas to rise.

As the tumorigenic process is a multistep phenomenon, it is also conceivable that additional steps leading to myelomonocytic tumors occur rarely in younger animals but are more frequent in older mice. This would explain a longer latency as well as a higher tumor incidence in later life.

It has been shown that in some circumstances the number of myeloid cells, especially the macrophage population, rises and the lymphoid population decreases in aging animals. Such a scenario could provide more myeloid than lymphoid target cells for transformation by the L-*myc* transgene and could also increase the occurrence of random secondary events that are necessary in the tumor formation process thereby leading to a higher number of myelomonocytic tumors in older animals.

Another major difference between Eμ L-*myc* lymphoid and myelomonocytic tumors is the correlation of their incidence with transgene copy number. Eμ L-*myc*, like Eμ N-*myc*, lymphoid neoplasms arise with the highest incidence in high-copy number lines (>10 copies) (28, 31), but Eμ L-*myc* myelomonocytic tumors do not show such a correlation. The highest incidence is observed in the line 31.14, a low-copy number line. Animals from this line show all the prelymphomatous features typical for Eμ L-*myc* transgenics, but have so far failed to develop lymphoma (31).

Cellular Origin of Eμ L-myc Myelomonocytic Tumors. The original cell type that has undergone transformation-related changes and given rise to the myelomonocytic tumors in Eμ L-*myc* transgenic mice is not easily identified. Several lineage-specific markers classify the tumor cells as myelomonocytic. However, the activity of the Ig enhancer and the presence of germline Cμ transcripts could be consistent with early lymphoid cells, e.g., a pre-B cell precursor, or a bipotential myeloid/lymphoid precursor cell that differentiated during or after the transformation into a myelomonocytic cell. It has been reported that the transfection of tumorigenic pre-B cells with v-*raf* or the infection of normal pre-B cells from long-term bone marrow cultures with *fms* can force pre-B cells to undergo lineage switch and to differentiate into macrophages (48, 57), suggesting that the commitment of pre-B cells to the lymphoid lineage is not absolute and lineage switch can be induced by external stimuli. Considering that expression of the Eμ L-*myc* transgene was not detected in normal macrophages from bone marrow and peritoneum (T. Möröy, unpublished results) but was detected in lymphoid cells, including pre-B cells (31, and T. Möröy, unpublished results), it is conceivable that the myelomonocytic tumors observed in Eμ L-*myc* transgenics originate from a transformed early lymphoid precursor that has undergone a lineage switch during later stages of differentiation.

Chromosomal Aberrations in Eμ L-myc Tumors. Trisomy 16 occurs systematically and in a nonrandom fashion in Eμ L-*myc* myelomonocytic tumors, suggesting that this chromosomal aberration represents a second step in the generation of myelomonocytic malignancies. It is conceivable that chromosome 16 carries one or several genes that are involved either in the early transformation events or in later stages of tumor progression. Previous genetic studies have revealed trisomy of chromosome 15 in murine T and B cell lymphomas and erythroleukemias irrespective of the inducing agent (58–60). In these cases it has been suggested that the chromosomal imbalance was a late event in tumorigenesis and is associated with the progression of the tumor rather than with its initiation (61). It is very likely that the same situation applies to the Eμ L-*myc* myelomonocytic tumors, but further studies are needed to identify the elements of chromosome 16 responsible for such an enhancement of tumor progression.

Downmodulation of Endogenous c-myc Expression. The downmodulation of the endogenous *myc* gene expression in tumors in which one member of the *myc* family genes is activated is well documented (21, 25, 27–29). Expression of the L-*myc* gene is efficient in crossregulating c- and N-*myc* expression in lymphoid tumors (31). The absence of c-*myc* expression in Eμ L-*myc* myelomonocytic tumors is consistent with these findings and implicates the transcriptionally deregulated L-*myc* gene directly in the tumor formation process. The exact mechanism by which crossregulation of *myc* gene expression is achieved remains speculative. It is clear, however, that high level expression of one *myc* family member alone is insufficient. For example, coordinate high level expression of c- and N-*myc* does occur in Abelson-transformed pre-B cell lines derived from Eμ N-*myc* transgenic mice, but no endogenous c-*myc* expression is detected in spontaneously arising Eμ N-*myc* pre-B cell tumors (52). Thus, crossregulation of *myc* genes in a transformed cell can only be achieved if one member of the *myc* family is causally implicated in the transformation process.

L-*myc* is expressed in the mouse monocyte precursor line M1S2 (Fig. 1), but also in human myeloid leukemias and in a subset of human myeloid tumor cell lines (11). In addition, L-*myc* is clearly capable of predisposing transgenic animals to the development of myelomonocytic tumors. It is therefore conceivable that L-*myc* plays a pivotal role in the development of at least a subset of myeloid tumors.

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