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.
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 transgensics (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 Jμ probe used for Southern analysis is a 2-kb fragment covering Jμ-1-4 (36, 37). The Jκ/Eμ probe is an XbaI-EcoRI fragment covering Jκ3-4, 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 Apal fragment isolated from the 3' untranslated region of the Thy-1 gene (41), the Jμ probe is a 1.3-kb EcoRI fragment isolated from the 3' untranslated region of the Thy-1 gene (41), 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 Apal fragment isolated from the 3' untranslated region of the Thy-1 gene (41), the Jμ probe is a 1.3-kb EcoRI fragment containing the Jμ locus (41), the Jκ/Eμ probe is a 2.5-kb EcoRI c-cDNA fragment (43), the c-myc probe is a 3.6-kb fragment from the murine c-cDNA (44), the macrophage (M)1-CSF specific probe is a 1.8-kb fragment from the murine c-cDNA, and the probe specific for granulocyte/macrophage (GM)-CSF is a 1-kb fragment from the murine c-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 μM 2-DE, 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 C57B16 mice and plated in 1 ml 1% semisolid agar at a density of 105 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 cytospins, 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 cytospins. 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/Wright 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 transgensics. 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 manifested 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 EVLcd 31.14 (Table 1).
Table 1. Mesenchymal Tumors in Eμ L-myc Transgenic Mice

| Line | No. of Animals | Tumor Location | Age (mo) |
|------|----------------|----------------|---------|
| EL   | 27.3 26        | 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      | 14      |
| EVL  | 18.1 7         | Skin/LN        | 6       |
|      | 181.21.15      | Skin           | 13      |
|      | 181.FG.7       | Skin/Head      | 11      |
| 20.14| 2              | 215.5.9        | 15      |
| EVLcd | 31.14         | 314.10.10      | 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         | 11      |
|      | 342.13.5       | Skin/Muscle    | 13      |
|      | 342.12.19      | Skin           | 11      |
|      | 342.13.8.15    | Skin/Head      | 14      |
| 42.12| 9              | 42.12          | 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 106-107 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 IgH heavy, the Igk 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 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 κ heavy and Ig κ light chain messenger RNA and the lack of Ig and TCR gene rearrangements in the majority of the...
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-l* | Jhr* | k* | Jαβ* |
|--------|------|--------|------|----|------|
| 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  | +     | +    | -  | -    |
|        | 314.1  | ND    | +    | -  | -    |
|        | 314.33  | 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.).

1 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 314.14, 84% for 314.43% 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, Kr, Jαβ: rearrangements of the J region of the heavy chain locus, the k light chain locus, and the T cell receptor β locus, respectively.

![Figure 2](image-url)
Table 4. Production of M-CSF in Myelomonocytic Tumors

| Supernatant | CFU/10^5 cells/0.2 ml SN* | Mac/Gran/mix | Colony morphology* |
|-------------|--------------------------|-------------|-------------------|
| Controls:   |                          |             |                   |
| Medium      | 0                        |             |                   |
| 38B9        | 0                        |             |                   |
| J774        | 109 ± 2                  | 82          | 7                 |
| M152        | 1 ± 1                    | 100         |                   |
| P388        | 8 ± 3                    | 100         |                   |
| WD/pLJ      | 48 ± 2                   | 65          | 6                 |
| 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, M152, P388, and WD/pLJ are myeloid control cell lines.

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

The myelomonocytic tumors 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 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. Mőrő, unpublished results) but was detected in lymphoid cells, including pre-B cells (31, and T. Mőrő, 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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Received for publication 19 June 1991 and in revised form 30 September 1991.
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