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Strain Dependency of Cell-type Specificity and Onset of Lymphoma Development in Eμ-*myc* Transgenic Mice

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c-*myc* is a nuclear proto-oncogene that, when activated, induces malignancies in a variety of tissues. Most murine plasmacytomas and human Burkitt's lymphomas have been shown to carry a chromosomal translocation involving c-*myc* and immunoglobulin genes. To study genetic or epigenetic factors that affect *myc*-induced lymphoid cell tumors, we previously introduced the Eμ-*mycΔ* gene lacking its own promoter and first exon into two inbred strains of mice, C57BL/6 and C3H/HeJ. We observed three characteristic features in our transgenic mice. First, T cell lymphoma predominated in the C3H background. Second, both pre-B and B cell lymphoma developed at equal frequency in C57BL/6 transgenic mice. Third, the average age of onset is earlier than that reported by other investigators. To test whether these characteristics are due either to the lack of the promoter region and first exon of the c-*myc* gene in the construct or to the genetic background of the mice, we introduced Eμ-*myc* gene containing the complete c-*myc* gene into fertilized eggs of C57BL/6 and C3H/HeJ mice. The cell-type specificity, differentiation-stage specificity and the average age at onset of lymphoma development were not affected by the transgene construct.

Key words: c-*myc* gene — Lymphoma — Transgenic mouse

It is evident that environmental factors such as chemical carcinogens are involved in tumorigenesis. On the other hand, the incidence and cell type specificity of both spontaneous and induced tumors vary greatly from one to another strain of mouse suggesting that the genetic background can influence the susceptibility to tumor induction. Recent molecular biological studies revealed that at least two mechanisms, the activation of proto-oncogene or inactivation of anti-oncogene, are involved in tumorigenesis, and that multistep events are required for malignant transformation of a cell. However, it is totally unknown how each genetic factor is involved. To make the influence of genetic factor(s) clear, we previously produced transgenic mice by introducing c-*myc* gene lacking the promoter region and first exon activated by immunoglobulin heavy chain enhancer (Eμ-*mycΔ*) into two inbred strain of mice, C57BL/6 (B6) and C3H/HeJ (C3H).[1] In contrast to other reports,[2–4] three characteristic features were observed in these transgenic mice. First, most B6 transgenic mice developed B cell lymphomas, but T cell lymphomas developed in most C3H transgenic mice. That is, the C3H microenvironment in which T cells grow and differentiate was found to increase the incidence of T cell lymphoma in transgenic mice. Second, most of the tumor cells were at the pre-B cell stage in other reports, while tumors arising in our B6 transgenic mice were evenly distributed among pre-B cells and B cells. Third, the average age at onset of our Eμ-*mycΔ* transgenic mice was earlier than that of other groups. For example, Schmidt *et al.*[3] demonstrated that their transgenic mice were normal until 2 months of age and that the time at which half of the mice developed tumors was 114 days. In contrast, our transgenic mice developed tumors as early as 3 weeks of age and half of them developed tumors by 50 days of age. These differences could be either due to the difference of Eμ-*myc* construct, or due to the difference of mouse strain, that is, the genetic background. To distinguish these possibilities, we introduced the Eμ-*myc* gene, that contains the entire c-*myc* gene including promoter region and first exon and Eμ, into fertilized eggs of either C57BL/6 or C3H/HeJ mice. We here report that not only the age at onset but also the cell-type and cell-stage specificity are not affected by the transgene construct used for microinjection, suggesting that the genetic background may be involved in these characteristic features.

MATERIALS AND METHODS

Production of transgenic mouse The Eμ-*myc* gene is a 10.5 kb *Eco*RI-*Hin*dIII fragment which was constructed by inserting a 1.9 kb *Hin*dIII-*Xba*I fragment containing the Eμ into the *Pvu*II site of c-*myc* gene located in the upstream region of c-*myc* promoter (Fig. 1). About 200 copies of the gene were injected into the fertilized eggs of B6 or C3H mice as described.[5] Offspring were screened for the presence of the transgene by Southern blot analysis using the *Pst*I fragment of human c-*myc* gene as a probe. Tissues were lysed with 200 μg of Proteinase K (Sigma) in 1 ml of 50 m*M* Tris (pH 7.6), 100 m*M*

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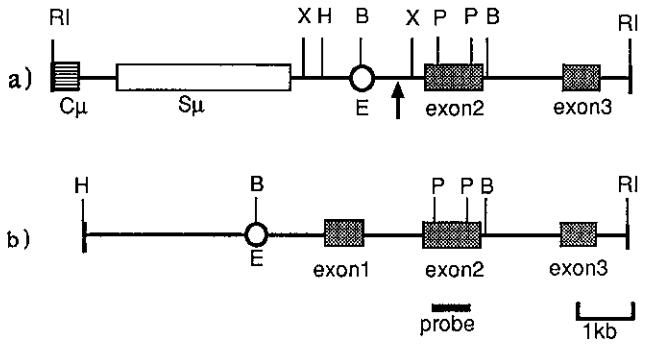

Fig. 1. The structures of transgenes. a) Eμ-*myc*Δ gene. Eμ-*myc*Δ transgene is an 11.5 kb *Eco*RI fragment containing the Cμ-c-*myc* joining region (arrow; recombination site) and is derived from the human lymphoma, Manca cell line. b) Eμ-*myc* gene. Eμ-*myc* transgene is a 10.5 kb *Hin*dIII-*Eco*RI fragment. A 1.9 kb *Hin*dIII-*Xba*I fragment containing the immunoglobulin heavy chain enhancer (Eμ) is inserted into the *Pvu*II site located upstream of c-*myc* gene. Restriction sites: RI, *Eco*RI; X, *Xba*I; P, *Pst*I; H, *Hin*dIII; B, *Bgl*II.

EDTA, 1% SDS, at 37°C for overnight. They were treated with phenol/chloroform (1:1 v/v), precipitated with isopropyl alcohol and dissolved in TE (10 m*M* Tris-HCl, pH 7.5/1 m*M* EDTA).

Northern blot analysis The total RNA was prepared from the tissues by the guanidium isothiocyanate/$CsCl_2$ method.[6] RNAs were subjected to electrophoresis in a 1% agarose gel containing 6.6% formaldehyde, transferred to nylon membranes (Biodyne) and hybridized with ^{32}P-labeled *Pst*I fragment of c-*myc* gene (Fig. 1).

Flow cytometry analysis Flow cytometry analysis was performed using a FACScan (Becton Dickinson). The monoclonal antibodies used were FITC-anti-IgM (Zymed), FITC-anti-Thy1.2, PE-anti-L3T4, FITC-anti-Lyt2 (Becton Dickinson), anti-B220 (6B2 rat g2a,k), and FITC-anti-RAT k. Single cell suspensions were obtained from lymph nodes in PBS containing 2% FCS and 0.1% NaN_3. These cell suspensions were incubated with antibodies for 20 min at 4°C. The stained cells were examined and analyzed by FACScan equipped with the Consort 30 program.

RESULTS

Onset and cell-type specificity As reported previously, B6 transgenic mice carrying Eμ-*myc*Δ mostly developed B cell lymphomas by 6 weeks of age and most C3H transgenic mice developed T cell lymphomas by 5 weeks of age. Nine independent B6 transgenic mouse lines carrying Eμ-*myc* gene were newly obtained and they started to develop lymphomas at 5 weeks of age and usually died by about 11 weeks of age. Cell surface marker analysis was performed on 4 lines. Two cases were B220(+), IgM(+) B cell lymphomas, and another two cases were B220(+), IgM(−) pre-B cell lymphomas. On the other hand, among four independent C3H transgenic mouse lines carrying Eμ-*myc*, two of them developed T cell lymphomas by 8 weeks of age and one developed B cell lymphoma at around 11 weeks of age. Another mouse, F_0-24, suddenly died before developing lymphoma, but his transgenic offspring mostly developed T cell lymphomas. Ages at onset of these offspring were 3 to 18 weeks of age. As most of them developed tumors at 12 weeks of age, they could be maintained and used for the following analyses. We mated

Table I. Tumors Developed in Eμ-*myc* Transgenic Mice

Strain	No. of mice	Surface marker			Cell type
		B220	IgM	Thy1.2	
B6	2	(+)	(−)	(−)	preB
	2	(+)	(+)	(−)	B
C3H	1	(+)	(+)	(−)	B
	2	(−)	(−)	(+)	T
CBF1	7	(+)	(+)	(−)	B
	5	(−)	(−)	(+)	T
CBF2	3	(+)	(−)	(−)	preB
	2	(+)	(+)	(−)	B
	1	(−)	(−)	(+)	T

Surface phenotypes were determined by FACS analysis.

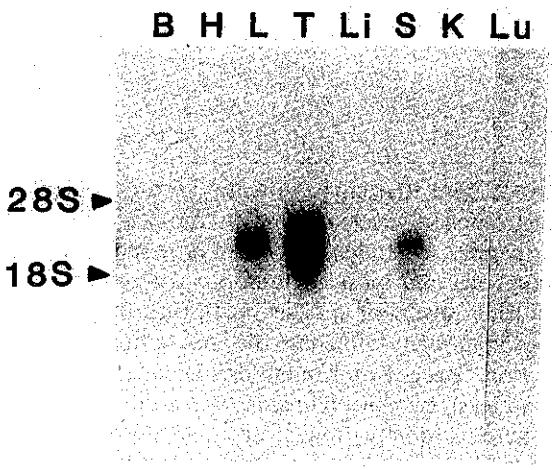

Fig. 2. RNA blot analysis in Eμ-*myc* transgenic mice. Eμ-*myc* transgenes were expressed in thymus, spleen, lymph node. B, brain; H, heart; L, lymph node; T, thymus; Li, liver; S, spleen; K; kidney; Lu; lung.

this line with B6 mice to obtain C3H×B6 hybrid F_1 (CBF_1) transgenic mice. All transgenic CBF_1 offspring developed lymphomas. Among 12 cases analyzed, seven cases were B220(+), IgM(+) B cell lymphomas and five cases were Thy1.2(+) T cell lymphomas. We then mated CBF_1 transgenic mice with B6 mice to obtain backcross progeny (CBF_2). Five of 6 CBF_2 transgenic mice developed B lymphomas. Two cases were B220(+), IgM(+) B cell lymphomas and three cases were B220(+), IgM(−) pre-B cell lymphomas. One case was T cell lymphoma. These results (Table I) suggest that the cell type specificity of lymphoma was determined by the genetic background of the mouse strain, and that B cell lymphoma predominates in the B6 background.

Northern blot analysis demonstrated that Eμ-*myc* genes were expressed in lymphoid tissues including spleen, thymus, and lymph node (Fig. 2). This result is consistent with the expression pattern of Eμ-*myc*Δ transgenic mouse.

Cell-stage of B lymphoma in B6 transgenic mice In both Eμ-*myc* and Eμ-*myc*Δ transgenic mice, two classes of lymphoma, pre-B and B, occurred at similar frequencies in B6 transgenic mice. This is in marked contrast to other reports in which pre-B cell lymphomas predominantly develop.[2-4] Interestingly, this situation is similar to that observed in double transgenic mice carrying both Eμ-*myc* gene and a transgene encoding the membrane-bound from of the immunoglobulin heavy chain (mIgμ).[7]

Tumor incidence We examined tumor occurrence as a function of age in Eμ-*myc* transgenic mice (Fig. 3). They developed lymphomas as early as 3 weeks of age and half of them developed tumors by 50 days of age. This incidence of lymphomas in Eμ-*myc* transgenic mice is virtually identical to that of Eu-*myc*Δ transgenic mice. The data of Schmidt *et al.*[3] are also shown in Fig. 3 for comparison of the kinetics of tumor appearance.

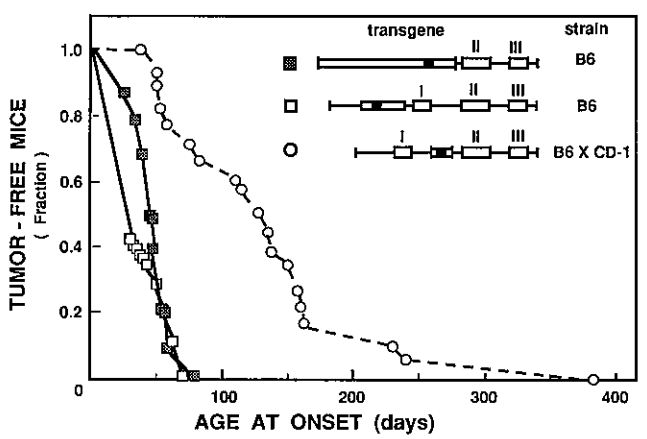

Fig. 3. Tumor occurrence as a function of age in Eμ-*myc* transgenic mice. Tumor onset in days is plotted against the percentage of animals that remain tumor-free. The data of Schmidt *et al.*[3] (o) are also shown.

DISCUSSION

Several studies on transgenic mice carrying activated *myc* gene have been reported,[2-4] and we noticed three different results in comparison with other groups. One is the difference of cell-type specificity of lymphoma development. Most C3H transgenic mice developed T cell lymphomas regardless of transgene construct. Previously we showed that the C3H microenvironment is involved in the susceptibility of T cell lymphoma by means of a bone marrow transplantation experiment.[1] Interestingly, the thymus epithelium, but not lymphocytes in thymus, is important for the development of thymoma in BUF/Mna rat (O. Taguchi, personal communication). This thymoma is a dominantly inherited disease which is determined by the single gene, Tsr-1. However, in our case the frequency of T and B cell lymphoma was almost the same in CBF_1 transgenic mice, but B cell lymphomas predominantly developed in CBF_2 generation. Thus, T cell lymphoma is not a dominant trait determined by a single gene, but is under polygenic control. In any case, our results showed that the transgene construct did not affect the cell-type specificity, and that the genetic background may influence the cell-type specificity of lymphoma.

The second characteristic feature is the stage-specificity of B cell tumors. Most groups reported that Eμ-*myc* transgenic mice developed a narrow spectrum of pre-B cell lymphomas. That is, the pre-B cells are most susceptible to transformation to Eμ-*myc*. This was confirmed by Nussenzweig *et al.*, who showed that the membrane-bound form of the immunoglobulin heavy chain (mIgμ) transgene suppresses the development of the early onset pre-B cell lymphomas in Eμ-*myc* transgenic mice.[7] In our experiment, both pre-B and B cell lymphomas developed at equal frequency in B6 transgenic mice regardless of transgene construct. These results suggest that genetic factors also influence the differentiation stage specificity of B lineage lymphoma.

The third feature is the tumor occurrence as a function of age. In contrast to other reports, our transgenic mice develop lymphomas as early as 3 weeks of age and all of them die by 20 weeks of age. Concerning this question, there are two possibilities. Initially we used Eμ-*myc*Δ construct which lacks the promoter region and the first exon of c-*myc* gene. This region is often lost in the translocated c-*myc* gene found in some human lymphomas and murine plasmacytomas[8] and v-*myc* also lacks the first exon of the c-*myc* gene.[9-11] In addition, several studies have shown that the first exon of c-*myc* gene and

its upstream region play an important role in the negative control of c-*myc* gene transcription.[12–15] Other investigators have reported that c-*myc* mRNA lacking the first exon is more stable than the complete c-*myc* mRNA.[16–18] These results suggested that the lack of first exon and promoter could be the reason for early onset of lymphoma in our transgenic mice. We hence produced transgenic mice by introducing a Eμ-*myc* gene containing the complete c-*myc* gene. We compared the occurrence of lymphomas as a function of time in these transgenic mice with that of previously produced Eμ-*myc*Δ transgenic mice. As shown above, the kinetics of tumor occurrence in both B6 and C3H Eμ-*myc* transgenic mice were exactly the same as those in Eμ-*myc*Δ mice. Although the molecular mechanisms for early onset of lymphoma development in B6 or C3H transgenic mice are not known yet, other events necessary for tumor development may occur more easily in these inbred strains of mice. Actually the abnormality of chromosome 6 was observed in all cases of T lymphoma that developed in C3H transgenic mice. Recently, two groups identified a novel oncogene, bmi-1 whose activation is dramatically accelerated in pre-B cell lymphomagenesis in Eμ-*myc* transgenic mice by the use of provirus tagging.[19, 20] Whether the activation of bmi-1 is also observed in our transgenic mice, and if so whether this activation occurs more easily in an inbred strain of mice are important questions to address. Further investigation is needed.

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REFERENCES

1) Yukawa, K., Kikutani, H., Inomoto, T., Uehira, M., Bin, S. H., Akagi, K., Yamamura, K. and Kishimoto, T. Strain dependency of B and T lymphoma development in immunoglobulin heavy chain enhancer (Eμ)-*myc* transgenic mice. *J. Exp. Med.*, **170**, 711–726 (1989).
2) Adams, J. M., Harris, M. A., Pinkert, C. A., Corcoran, L. M., Alexander, W. S., Cory, S., Palmitter, R. D. and Brinster, R. L. The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature*, **318**, 533–538 (1985).
3) Schmidt, E. V., Pattengale, P. K., Weir, L. and Leder, P. Transgenic mice bearing the human c-*myc* gene activated by an immunoglobulin enhancer: a pre-B-cell lymphoma model. *Proc. Natl. Acad. Sci. USA*, **85**, 6047–6051 (1988).
4) Suda, Y., Aizawa, S., Hirai, S., Inoue, T., Furuta, Y., Suzuki, M., Hirohashi, S. and Ikawa, Y. Driven by the same Ig enhancer and SV40 T promoter ras induced lung adenomatous tumors, *myc* induced pre-B cell lymphomas and SV40 large T gene a variety of tumors in transgenic mice. *EMBO J.*, **6**, 4055–4065 (1987).
5) Yamamura, K., Kikutani, H., Folsom, V., Clavton, L. K., Kimoto, M., Akira, S., Kashiwamura, S., Tonegawa, S. and Kishimoto, T. Functional expression of a microinjected E_α^d gene in C57BL/6 transgenic mice. *Nature*, **316**, 67–69 (1985).
6) Maniatis, T., Fritsch, E. F. and Sambrook, J. "Molecular Cloning," pp. 187–206 (1982). Cold Spring Harbor Laboratory, New York.
7) Nussenzweig, M. C., Schmidt, E. V., Shaw, A. C., Sinn, E., Campos-Torres, J., Mathey-Prevot, B., Pattengale, P. K. and Leder, P. A human immunoglobulin gene reduces the incidence of lymphomas in c-*myc*-bearing transgenic mice. *Nature*, **336**, 446–450 (1988).
8) Klein, G., and Klein, E. *myc*/Ig juxtaposition by chromosomal translocations: some new insights, puzzles and paradoxes. *Immunol. Today*, **6**, 208–215 (1985).
9) Alitalo, K., Bishop, J. M., Smith, D. H., Chen, E. Y., Colby, W. W. and Levinson, A. D. Nucleotide sequence of the v-*myc* oncogene of avian retrovirus MC29. *Proc. Natl. Acad. Sci. USA*, **80**, 100–104 (1983).
10) Watson, D. K., Reddy, E. P. Duesberg, P. H. and Papas, T. S. Nucleotide sequence analysis of the chicken c-*myc* gene reveals homologous and unique coding regions by comparison with the transforming gene of avian myelocytomatosis virus MC29, *gag-myc*. *Proc. Natl. Acad. Sci. USA*, **80**, 2146–2150 (1983).
11) Westaway, D., Payne, G. and Varmus, H. E. Proviral deletions and oncogene base substitutions in insertionally mutagenized c-*myc* alleles may contribute to the progression of avian bursal tumors. *Proc. Natl. Acad. Sci. USA*, **81**, 843–847 (1984).
12) Leder, P., Battey, J., Lenoir, G., Moulding, C., Murphy, W., Potter, H., Stewart, T. and Taub, R. Translocations among antibody genes in human cancer. *Science*, **222**, 765–771 (1983).
13) Dunnick, W., Shell, B. E. and Dery, C. DNA sequence near the site of reciprocal recombination between a c-*myc* oncogene and an immunoglobulin switch region. *Proc. Natl. Acad. Sci. USA*, **80**, 7269–7272 (1983).
14) Rabbitts, T. H., Foster, A., Hamlyn, P. H. and Baer, R. Effect of somatic mutation within translocated c-*myc* gene in Burkitt's lymphoma. *Nature*, **309**, 592–597 (1984).
15) Lipp, M., Schilling, R., Wiest, S., Laux, G. and

Bronkamm, G. W. Target sequences for cis-acting regulation within the dual promoter of the human c-*myc* gene. *Mol. Cell. Biol.*, **7**, 1393–1400 (1987).
16) Eick, D., Piechaczyk, M., Henglein, B., Blanchard, J. M., Traub, B., Kofler, E., Wiest, S., Lenoir, G. M. and Bornkamm, G. W. Aberrant c-*myc* RNAs of Burkitt's lymphoma cells have longer half-lives. *EMBO J.*, **4**, 3717–3725 (1985).
17) Piechaczyk, M., Yang, J. Q., Blanchard, J. M., Jeanteur, P. and Marcu, K. B. Posttranscriptional mechanisms are responsible for accumulation of truncated c-*myc* RNAs in murine plasma cell tumors. *Cell*, **42**, 589–597 (1985).
18) Rabbitts, P. H., Forster, A. Stinson, M. A. and Rabbitts, T. H. Truncation of exon 1 from the c-*myc* gene results in prolonged c-*myc* RNA stability. *EMBO J.*, **4**, 3727–3733 (1985).
19) Lohuizen, M. V., Sjef Verbeek, S., Scheijen, B., Wientjens, E., Gulden, H. and Berns, A. Identification of cooperating oncogenes in Eμ-*myc* transgenic mice by provirus tagging. *Cell*, **65**, 737–752 (1991).
20) Haupt, Y., Alexander, W. S., Barri, G., Klinken, S. P. and Adams, J. M. Novel zinc finger gene implicated as *myc* collaborator by retrovirally accelerated lymphomagenesis in Eμ-*myc* transgenic mice. *Cell*, **65**, 753–763 (1991).