STRAIN DEPENDENCY OF B AND T LYMPHOMA DEVELOPMENT IN IMMUNOGLOBULIN HEAVY CHAIN ENHANCER (Eμ)-*myc* TRANSGENIC MICE

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The involvement of dysregulated c-*myc* expression has been well established in certain virus- or chemical-induced and naturally developing tumors (1–5). Most of murine plasmacytomas and human Burkitt's lymphomas have been shown to carry a chromosomal translocation involving c-*myc* and Ig genes (2–5). In such tumor cells, only the c-*myc* transcripts from the translocated allele are constitutively expressed, while the normal allele is completely suppressed, suggesting that the translocation affects the expression of the c-*myc* gene of the normal allele as well as that of the translocated one (6, 7). Recently it has been shown that Ig heavy chain enhancer (Eμ)[1]-driven c-*myc* can induce immature B cell tumors in the transgenic mice (8), which is a direct demonstration of the involvement of the translocated c-*myc* gene in B lymphomagenesis. Moreover, tumors developed in such transgenic mice have been demonstrated to be monoclonal or oligoclonal, suggesting that the malignant transformation requires secondary events following an abnormal expression of c-*myc* gene. It is likely that such events might be determined or influenced by factors intrinsic to certain cells, or by environmental and genetic factors. Thus, the transgenic mice carrying activated oncogenes such as c-*myc* can also be a useful system to study such factors influencing tumorigenesis.

To study the genetic or environmental factors that affect the *myc*-induced lymphomagenesis, we introduced the translocated human c-*myc* gene into two inbred strains of mice, C57BL/6 and C3H/HeJ, which have different genetic backgrounds. We have observed the preferential development of T lymphomas in C3H/HeJ transgenic mice, whereas B6 transgenic mice mostly developed B lymphomas. Furthermore, the bone marrow transfer experiments using prelymphomatous transgenic mice suggest that environmental factors might influence the development of T lymphoma in C3H/HeJ mouse.

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[1] *Abbreviations used in this paper:* bi, biotinylated; Cμ, constant region of μ heavy chain; Eμ, Ig heavy chain enhancer; PC, phycocyanin.

Materials and Methods

Production of Transgenic Mice. The Eμ-*myc* used is an 11.5-kb Eco RI fragment from a cosmid DNA clone, cU23, derived from the non-Hodgkin's lymphoma Manca, which consists of a translocated human c-*myc* gene fused head to head with the Cμ gene (Fig. 1) (9). Transgenic mice were produced by microinjecting ~200 copies of the gene into the fertilized eggs of C57BL/6 or C3H/HeJ mice as described (10). The offsprings were screened for the integration of the transgene by Southern blot analysis of DNA from tail tips using the Eco RI/Cla I fragment of human c-*myc* gene as a probe.

Flow Cytometry Analysis. Flow cytometry analysis was performed using a FACS 440 equipped with dual lasers. The mAbs used were FITC-conjugated anti-IgM (rat IgG), FITC-anti-B220 (6B2, rat IgG), FITC-anti-Lyt-2 (53-6, rat IgG), FITC-anti-Thy-1.2 (30H12, rat IgG), FITC-anti-I-A^b (25-9-17, mouse IgG2), biotinylated (bi)-anti-IgD (anti-5a or 5b), bi-anti-B220, bi-anti-L3T4 (GK1.5, rat IgG), bi-anti-Ly-1 (53-7, rat IgG), bi-anti-I-E^k (13/4, mouse IgG2a), and phycocyanin (PC)-labeled anti-CD3 (2C11, hamster IgG). Single cell suspensions were obtained from bone marrow, lymph node, spleen, and thymus in deficient RPMI medium containing 3% FCS and 0.1% NaN_3. Washed cells were incubated with several combinations of various antibodies for 20 min at 4°C. After washing, Texas red avidin was added as a second step to develop biotinylated antibodies. Propidium iodide was included for the last 5 min of a second step to stain dead cells. The stained cells were examined by multiparameter FACS and the list mode data were collected into a VAX11/730 computer and analyzed by a DESK program developed at Stanford University, Stanford, CA.

Southern Blot Analysis. High molecular weight DNA was prepared with 250 μg of proteinase K (Sigma Chemical Co., St. Louis, MO) in 2 ml of 1 × SSC/1 mM EDTA/0.1% $NaDodSO_4$ at 37°C for 3–12 h. After digestion, phenol, phenol/chloroform (1:1, wt/vol), and chloroform extractions were performed and DNA was precipitated with ethanol. 5 μg of the DNA was digested with Xba I for analysis of the Ig heavy chain locus and with Hind III for analysis of the κ chain locus and TCR β chain locus. Digested DNA was separated on 0.5% agarose gel, and transferred to Gene Screen Plus™ Hybridization Transfer Membrane (NEN Research Products, Boston, MA) in a solution of 0.4 N NaOH/0.6 M NaCl. Blots were hybridized with ^{32}P-labeled DNA probes at 65°C in a buffer of 1 M NaCl/0.05 M Tris-HCl, pH 7.5/200 μg/ml salmon sperm DNA. Blots were washed in 2 × SSC at room temperature for 10 min, in 2 × SSC/1% SDS at 65°C for 60 min, in 0.1 × SSC at room temperature for 60 min, and processed by autoradiography. The DNA probes used in this study were a 3.8-kb Xba I fragment of the mouse germline J_H region (11), a 1.7-kb Pst I/Eco RI fragment of Jκ (12), a 1.7-kb Pst I fragment of $Dβ_1$ region (13), and a 2.4-kb Hind III/Eco RI fragment of $Dβ_2$ region (13).

S1 Nuclease Protection Assay. Total RNA was prepared from lymphoma cells and prelymphomatous splenic cells and thymocytes by the guanidium isothiocyanate/$CsCl_2$ method (14). SSDNA probe for S1 analysis was prepared as follows: an Xba I/Pst I fragment of cU23 (9) or an Sst I/Pvu II fragment of PKO-*myc* (6, 15, 16) was cloned into M13mp18 vector and single-stranded ^{32}P-labeled DNA was synthesized by the primer extension method (17). After digestion with Xba I or Sst I, the ssDNA probe was separated by PAGE. For S1 protection assay, 25 μg of total RNA was co-precipitated with the specific DNA probes (100,000 cpm), heated at 95°C for 10 min, and incubated for 14 h at 60°C in 80% formamide/40 mM Pipes/0.4 M NaCl/ 1 mM EDTA. Hybridized materials were treated with 250 U of S1 nuclease (17) for 40 min at 30°C in 280 mM NaCl/50 mM NaoAc (pH 4.6)/4.5 mM $ZnSo_4$/20 μg/ml salmon sperm DNA. After PAGE under denaturing conditions, S1-resistant species were visualized by autoradiography.

Bone Marrow or Fetal Liver Transplantation. 10^7 bone marrow cells treated with anti-Thy-1.2 (IgM mAb, DAIICHI Kagaku) plus complement (LOW-TOX-M Rabbit Complement, Cedar Lane Laboratories, Hornby, Ontario) and 5 × 10^6 of fetal liver cells from prelymphomatous Eμ-*myc* mice were transferred into lethally irradiated normal B6 (878 rad) or normal C3H/HeJ mice (950 rad). At the same time, 10^6 cells from bone marrow or fetal liver of the same Eμ-*myc* mice were inoculated intravenously into unirradiated syngeneic mice. Transgenic mice, whose cells did not induce tumors in unirradiated recipients, were recognized as prelymphomatous ones.

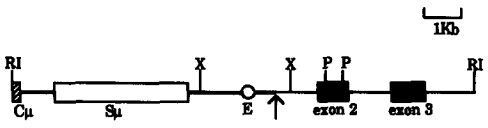

FIGURE 1. Structure of Eμ-*myc* transgene. As the transgene, an 11.5-kb Eco RI fragment containing the Cμ-c-*myc* joining region (*arrow*: recombination site) was prepared from a cosmid DNA clone(cU23) of the Manca cell line that has the chromosomal translocation(t(8:14)(q24;q32)) involving the Cμ-c-*myc* joining region. Exons are shown by solid boxes. Eμ(E) is a transcriptional enhancer element of the human heavy chain locus (9). Restriction sites: RI, EcoRI; X, XbaI; P, PstI.

Results

Developments of B and T Lineage Tumors in B6 and C3H/HeJ Eμ-myc Transgenic Mice. Eμ-*myc* transgenic mice were produced by microinjecting fertilized eggs of both B6 and C3H/HeJ mice with 11.5-kb DNA fragments containing human Eμ-*myc* gene derived from a non-Hodgkin lymphoma cell line, Manca. In nine independent B6 transgenic mice, lymphoma developed by 6 wk of age. The lymphomas involved mainly the thymus, the spleen, and the lymph nodes in these mice. These lymphomas were transplantable into normal syngeneic mice and could be adapted into in vitro culture. The analysis of surface phenotypes and rearrangements of Ig and TCR genes demonstrated that eight of nine B6 transgenic mice developed lymphomas characteristic of B cells. The B lymphomas that developed in the transgenic mice represented various stages of differentiation, as shown in Table I. For instance, a lymphoma in mouse 119 was at pre-B cell stage since the rearrangement of heavy chain gene but not of light chain gene was observed. On the other hand, mouse 316 developed a tumor expressing a phenotype of mature B cells ($IgM^+/IgD^+/B220^+$). As shown in Fig. 2, one or few discrete rearrangements in J_H and J_κ regions were observed in all analyzed B lymphomas except for that certain pre-B cell lymphoma that retained the germline configuration in the J_κ region. These rearrangement patterns of Ig genes indicate that tumors developed in these mice are monoclonal or

TABLE I

Tumors Developed in C57BL/6 Eμ-myc Transgenic Mice

Eμ-*myc* mice	d*	Surface markers of‡ primary tumors	J_H§	J_κ§	TCR-β§	Cell type‖
93(S)¶	40	IgM^+, IgD^-, $B220^+$	R_1,G	R_1,G	G	B
119(S)	35	IgM^-, IgD^-, $B220^+$	R_1,R_2,R_3,G	G	R_1	Pre-B
139(S)	42	IgM^-, IgD^-, $B220^+$	R_1,G	G	G	Pre-B
229(M)	37	IgM^+, $B220^+$	R_1,R_2	R_1	R_1	B
316(S)	42	IgM^+, IgD^+, $B220^+$	ND	ND	ND	B
334(S)	40	IgM^-, $B220^+$	ND	ND	ND	Pre-B?
339(I)	36	IgM^+, $B220^+$	R_1,G	R_1,R_2	G	B
346(S)	39	IgM^+, IgD^-, $B220^+$	ND	ND	ND	B
225(M)	35	$CD4^+$, $CD8^+$, $Thy1.2^+$	ND	ND	ND	T

* Postnatal days when lymphadenopathy or splenomegaly was initially observed.

‡ Surface phenotypes were determined by two-color FACS analysis.

§ Rearrangements of Ig heavy chain (J_H), light chain (J_κ), and TCR β chain genes. G, germline configuration; R_1–R_3 denote distinct rearranged patterns in each tumor.

‖ Cell types of tumors were mainly determined on the basis of FACS data.

¶ Tissues from where the tumors were derived. S, spleen; T, thymus; M, mesenteric lymphnode; I, inguinal lymphnode.

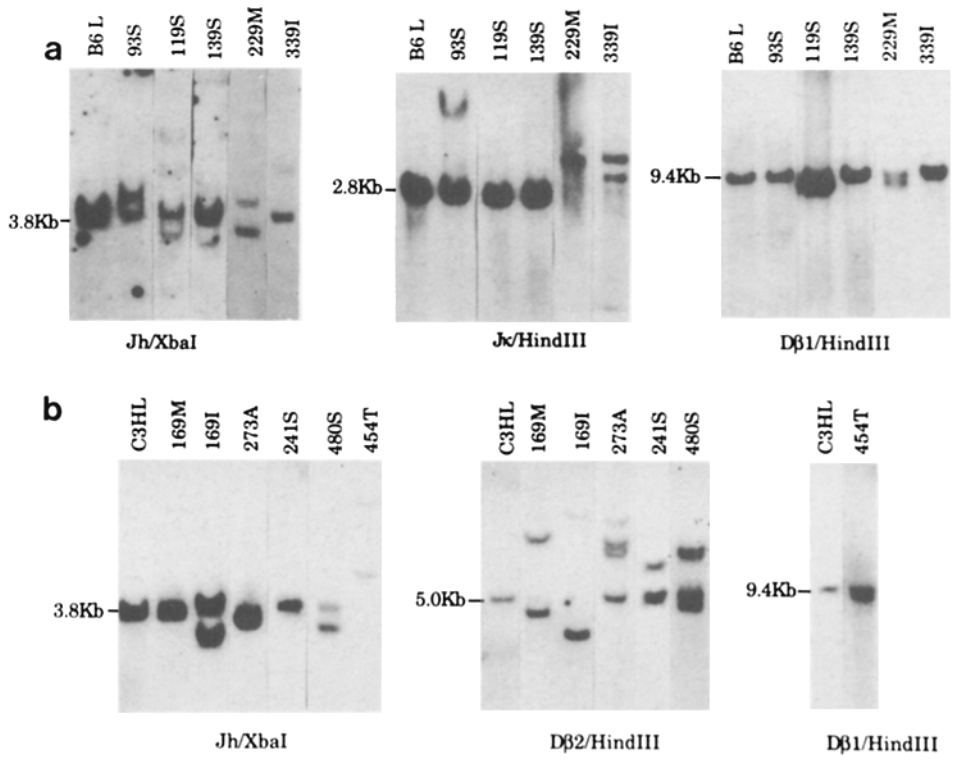

FIGURE 2. Monoclonal or oligoclonal tumors arose in B6 and C3H/HeJ Eμ-*myc* transgenic mice. Southern blot analysis was performed with DNA from B6 liver, tumors of B6 transgenic mice (93, 119, 139, 229, 339), C3H/HeJ liver, and tumors of C3H/HeJ transgenic mice (169, 273, 241, 480, 454). The blots were hybridized with J_H, J_κ, Dβ1, Dβ2 probes (see Materials and Methods). Rearrangements at the Ig heavy chain, light chain, and TCR β chain loci of tumors in B6 transgenic mice (*a*) and C3H/HeJ transgenic mice (*b*) are shown. Tissues from where DNA were prepared. L, liver; S, spleen; T, thymus; M, mesenteric lymphnode; I, inguinal lymphnode; A, axillary lymphnode.

oligoclonal. One pre-B cell lymphoma developed in mouse 119 and a B cell lymphoma in mouse 229 had also the rearrangement of TCR β chain gene. One mouse (225), developed a $CD4^+$, $CD8^+$, Thy-1.2^+ lymphoma. These results indicate that Eμ-*myc* B6 transgenic mice mostly develop B cell tumors although these mice also have a potential to develop T cell tumors at low frequencies.

Seven C3H/HeJ transgenic mice carrying Eμ-*myc* gene were produced. Around 5 wk of age, all developed lymphomas that involved mainly the mesenteric lymph nodes and the spleen but rarely the thymus. In contrast to B6 transgenic mice, six of seven C3H/HeJ transgenic mice developed lymphomas that express T cell phenotypes. B cell lymphoma arose only in one C3H/HeJ transgenic mouse (454). The surface phenotypes of the tumors developed in these mice are summarized in Table II and representative FACS analyses are shown in Fig. 3. The differentiation stages of T lymphomas were variable in each C3H/HeJ transgenic mice. For instance, lymphomas that developed in mouse 480 expressed both CD4 and CD8. Mice 241 and 450 developed $CD4^+/CD8^-$ and $CD4^-/CD8^+$ tumors, respectively. Some lym-

TABLE II
Tumors Developed in C3H/HeJ Eμ-myc Transgeneic Mice

Eμ-*myc* mice	d*	Surface markers of‡ primary tumors	J_H§	J_κ§	TCR-β§	Cell type‖
169(M)¶	30	$CD4^+$, $CD8^+$, $CD3^+$, Thy-1.2$^+$ $CD4^-$, $CD8^+$, $CD3^+$, Thy-1.2$^+$	G	G	R_1,R_2	T
169(I)	30	$CD4^+$, $CD8^+$, $CD3^+$, Thy-1.2$^+$ $CD4^-$, $CD8^+$, $CD3^+$, Thy-1.2$^+$	R_1,G	G	R_1	T
272(M)	35	$CD4^+$, $CD8^+$, $CD3^+$, Thy-1.2$^+$	ND	ND	ND	T
273(A)	33	$CD4^+$, $CD8^-$, $CD3^+$, Thy-1.2$^+$ $CD4^-$, $CD8^+$, $CD3^+$, Thy-1.2$^+$ $CD4^-$, $CD8^-$, $CD3^+$, Thy-1.2$^+$	R_1,G	ND	R_1,R_2,G	T
241(S)	35	$CD4^+$, $CD8^-$, $CD3^+$, Thy-1.2$^+$	G	ND	R_1,G	T
450(S)	30	$CD4^-$, $CD8^+$, $CD3^+$, Thy-1.2$^+$	R_1,G	G	R_1,G	T
480(S)	34	$CD4^+$, $CD8^+$, $CD3^+$, Thy-1.2$^+$	R_1,G	ND	R_1,R_2,G	T
454(T)	35	IgM^+, $B220^+$	R_1	R_1,G	R_1,G	B

* Postnatal days when lymphadenopathy or splenomegaly was initally observed.
‡ Surface phenotypes were determined by two-color FACS analysis.
§ Rearrangements of Ig heavy chain (J_H), light chain (J_κ) and TCR-β genes. G, germline configuration; R_1 and R_2 denote the distinct rearrangement patterns in each tumor.
‖ Cell types of tumors were mainly determined on the basis of FACS analysis.
¶ Tissues from where tumor cells were prepared. M, mesenteric lymphnode; I, inguinal lymphnode; A, axillary lymphnode; S, spleen; T, thymus.

phomas were found to consist of two or three subpopulations expressing distinct phenotypes: $CD4^+/CD8^+$ and $CD4^-/CD8^+$ in mouse 169 and $CD4^+/CD8^-$, $CD4^-/CD8^+$ and $CD4^-/CD8^-$ in mouse 273. As shown in Fig. 2, in Southern blot analysis of T cell tumors developed in C3H/HeJ transgenic mice, the discrete bands representing the rearranged TCR β chain gene were observed in each tumor, indicating that these T cell tumors are monoclonal or oligoclonal. Two tumors derived from mesenteric lymph nodes and inguinal lymph nodes of mouse 169 showed distinct patterns of TCR-β gene rearrangement although they displayed similar surface phenotypes ($CD4^+/CD8^+$ and $CD4^-/CD8^+$), demonstrating that two tumors could arise independently in a single transgenic mouse. However, tumor cells expressing distinct phenotypes in each lymph node of this mouse appear to be of the same clonal origin. For example, tumor cells from a mesenteric lymph node showed only two rearranged bands and no germline band of TCR-β gene. This result suggests that cells might be differentiating from $CD4^+/CD8^+$ to $CD4^-/CD8^+$ in these tumors. Although three subpopulations of tumor cells were found in mouse 273, we could not determine from their rearranged pattern of TCR β chain gene whether each subpopulation was derived from a single clonal origin. The rearrangement of J_H gene was also observed in these tumors, except in lymphomas developed in mesenteric lymph nodes of mouse 169 and in spleen of mouse 241, which conserved the germline configuration of J_H alleles. These results indicate that, in contrast to B6 transgenic mice, C3H/HeJ transgenic mice preferentially develop T cell tumors.

Lymphocyte Activation and Eμ-myc mRNA Expression in Eμ-myc Transgenic mice at the Prelymphomatous Stage. The above results suggest the possibility that the strain depen-

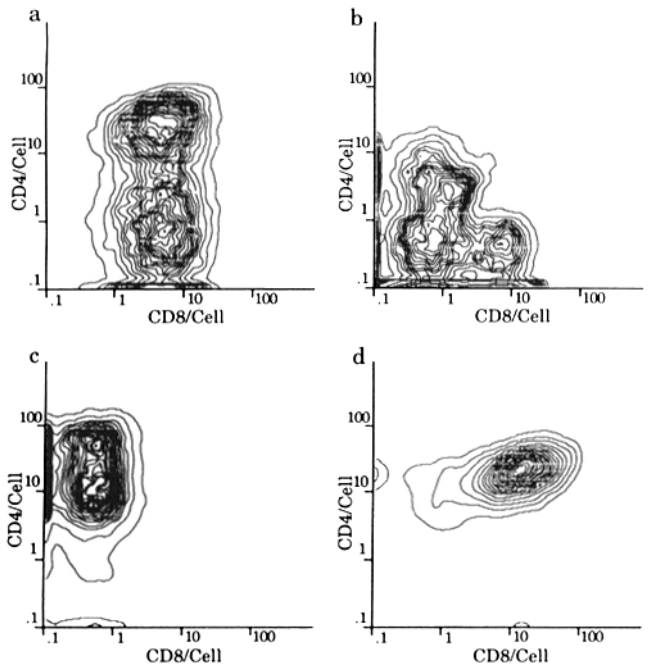

FIGURE 3. Surface phenotypes of the tumors developed in C3H/HeJ Eμ-*myc* transgenic mice. Tumors developed in mouse 169 (*a*), mouse 273 (*b*), mouse 241 (*c*), and mouse 480 (*d*) were stained with biotinylated anti-CD4, FITC anti-CD8, TR-AV, and PI. Vertical axis represents CD4(TR) and horizontal axis means CD8(FITC). (*a*) $CD4^+/CD8^+$ and $CD4^-/CD8^+$ T lymphomas developed in mesenteric lymphnode of mouse 169. (*b*) $CD4^+/CD8^-$, $CD4^-/CD8^+$, and $CD4^-/CD8^-$ T lymphomas in axillary lymphnode of mouse 273. (*c*) $CD4^+/CD8^-$ T lymphoma in spleen of mouse 241. (*d*) $CD4^+/CD8^+$ T lymphoma in spleen of mouse 480.

dency of B and T lymphoma development might be due to preferential activation of B- or T-lineage cells in B6 or C3H/HeJ transgenic mice, respectively. To test this possibility, we analyzed the size and surface phenotypes of bone marrow cells, spleen cells, and thymocytes from the prelymphomatous transgenic mice that had not developed tumors. As shown in Fig. 4, $B220^+$ cells from bone marrow and spleen of both B6 and C3H/HeJ transgenic mice were found to be larger in size and strongly expressed class II antigens compared with those of normal mice, indicating that B-lineage cells were similarly activated in prelymphomatous transgenic mice of both strains. However, cell size and numbers of each T cell subsets in thymus derived from both B6 and C3H/HeJ transgenic mice were normal, as shown in Fig. 5. These results suggest that high frequency of T lymphoma development in C3H/HeJ transgenic mice may not be simply due to the preferential activation of T lymphocytes.

The second possible explanation for a high incidence of T lymphoma in C3H/HeJ mice is that the Eμ-*myc* gene might be expressed in T-lineage cells of C3H/HeJ mice but not of B6 mice. To determine whether there is any difference of Eμ-*myc* mRNA expression among B and T lymphomas as well as prelymphomatous T and B cells from B6 and C3H/HeJ transgenic mice, RNAs were prepared from these cells and the expression of the transgene was analyzed. As shown in Fig. 6, four protected bands were observed in B and T lymphomas derived from B6 and C3H/HeJ transgenic mice, respectively, when the fragment of human genomic c-*myc* DNA was used

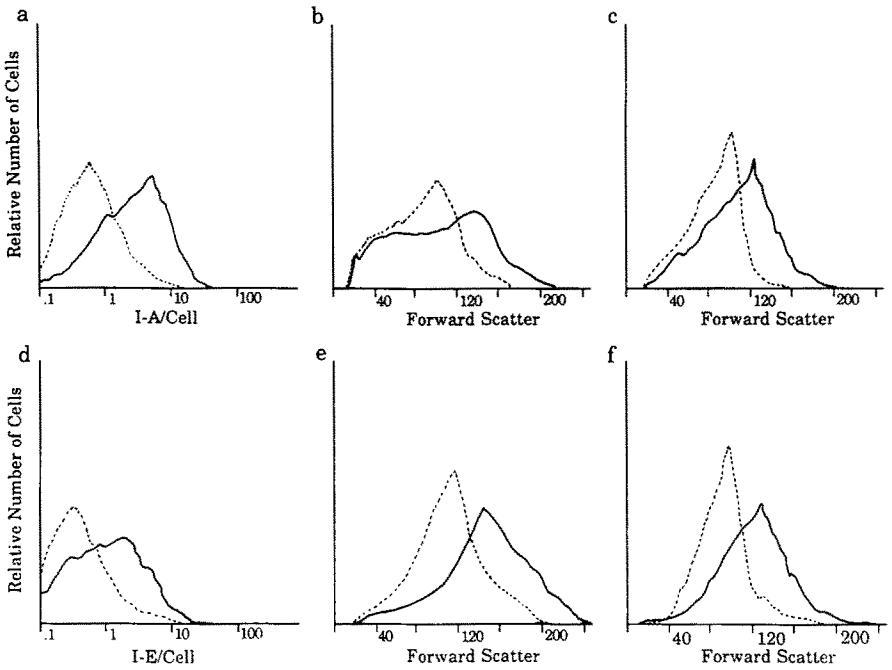

FIGURE 4. Characterization of bone marrow cells and splenocytes from prelymphomatous Eμ-*myc* B6 (*a–c*) and C3H/HeJ (*d–f*) mice. Solid lines denote Eμ-*myc* cells, and broken lines denote normal cells. (*a*) Class II antigen expression on B220$^+$ bone marrow cells between B6 transgenic (*solid line*) and normal littermates (*broken line*) are compared by fluorescence intensities. (*b*) Sizes of B220$^+$ bone marrow cells from B6 mice. (*c*) Sizes of B220$^+$ splenocytes from B6 mice. (*d*) Class II antigens expression on B220$^+$ bone marrow cells from C3H/HeJ mice. (*e*) Sizes of B220$^+$ bone marrow cells from C3H/HeJ mice. (*f*) Sizes of B220$^+$ splenocytes from C3H/HeJ mice. Three independent prelymphomatous B6 or C3H/HeJ transgenic mice were examined. The representative FACS profiles of one B6 and one C3H/HeJ transgenic mouse were presented in the figure, since all analyzed mice showed similar patterns.

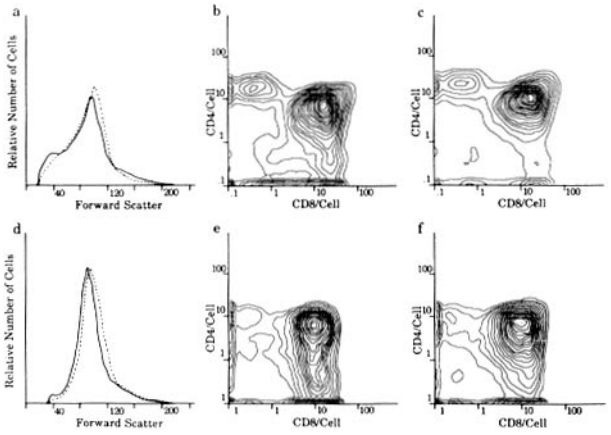

FIGURE 5. Characterization of thymocytes from prelymphomatous Eμ-*myc* B6 and C3H/HeJ mice. (*a*) Sizes of thymocytes from B6 transgenic (*solid line*) and normal littermates (*broken line*) are compared by forward light scatter. (*b*) Staining pattern for CD4 vs. CD8 of thymocytes from normal B6 mice. (*c*) Staining pattern for CD4 versus CD8 of thymocytes from Eμ-*myc* B6 mice. (*d*) Sizes of thymocytes from C3H/HeJ transgenic (*solid line*) and normal littermates (*broken line*). (*e*) Staining pattern for CD4 versus CD8 of normal C3H/HeJ thymocytes. (*f*) Staining pattern for CD4 versus CD8 of Eμ-*myc* C3H/HeJ thymocytes. Three independent pre-lymphomatous B6 or C3H/HeJ transgenic mice were examined. The representative FACS profiles of one B6 and one C3H/HeJ transgenic mouse were presented in the figure since all analyzed mice showed the similar patterns.

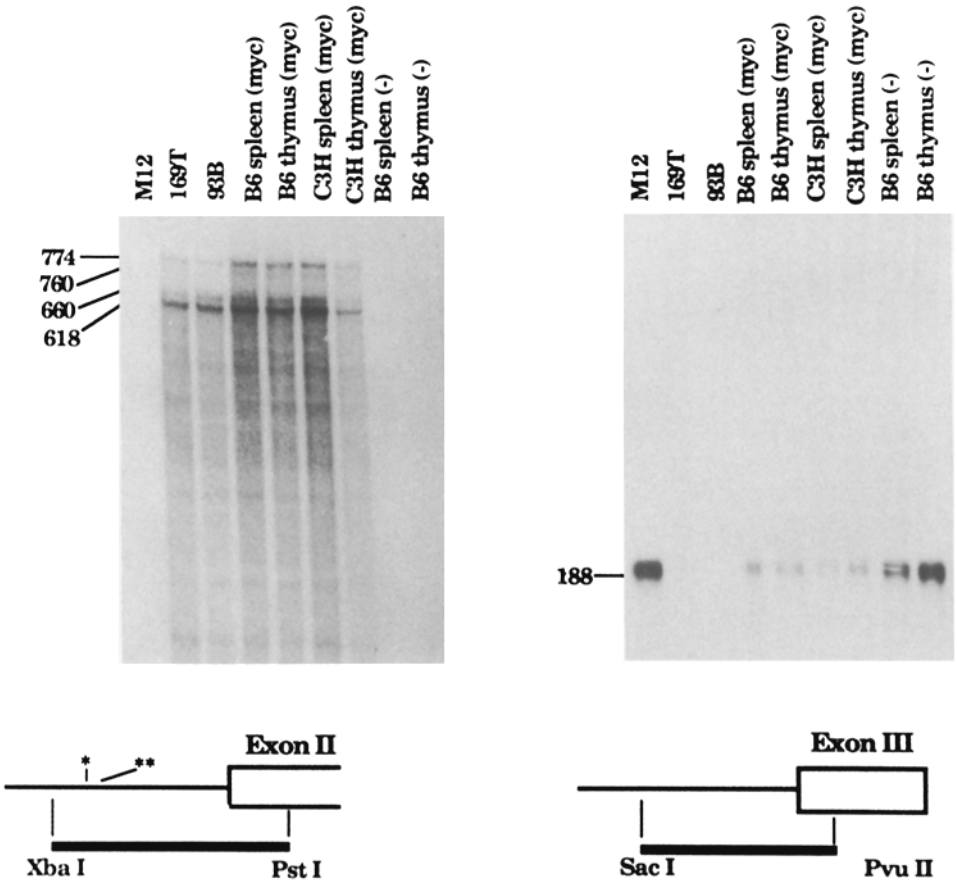

FIGURE 6. S1 nuclease protection assay of transgenic and endogenous c-*myc* RNA transcription. (*Left*) The human specific c-*myc* probe was hybridized with total RNAs prepared from M12 (mouse B cell line), 169T (C3H/HeJ Eμ-*myc* T lymphoma), 93B (B6 Eμ-*myc* B lymphoma), B6 prelymphomatous Eμ-*myc* spleen, B6 prelymphomatous Eμ-*myc* thymus, C3H/HeJ prelymphomatous Eμ-*myc* spleen, C3H/HeJ prelymphomatous Eμ-*myc* thymus, B6 normal spleen, and B6 normal thymus. The reported cryptic promoters in intron 1 of human c-*myc* were indicated (* and **) and represented by protected bands of ~774 and ~760 nucleotides. Bands of ~660 and ~618 nucleotides also represent the probes specifically protected by human c-*myc* gene transcripts. (*Right*) The mouse-specific c-*myc* probe was used for S1 nuclease analysis. Mouse endogenous c-*myc* transcripts were detected at 188 nucleotides. In addition to mice shown in this figure, one B6 and two C3H/HeJ transgenic mice were examined and also found to express almost equal amounts of human c-*myc* mRNA in the spleen and the thymus. FACS analysis showed that $B220^+$ B cells in the thymus from a B6 transgenic, a control B6, a C3H/HeJ transgenic, and a control C3H/HeJ mouse were 1.7, 1.1, 1.3, and 1.0%, respectively, indicating that Eμ-*myc* mRNA in the thymus of transgenic mice is not due to contaminating B cells.

as a probe. The two larger bands of ~774 and ~760 nucleotides represent the mRNA transcribed from cryptic promoters in intron 1 of the human c-*myc* gene as previously reported (9). Smaller but intense bands of ~660 and ~618 nucleotides were consistently observed in lymphomas from transgenic mice but not in control cells, indicating that these bands also represented the transcripts of human c-*myc* gene.

These four bands were also observed in splenocytes and thymocytes from prelymphomatous B6 and C3H/HeJ transgenic mice but not in those of control mice. Expression levels of Eμ-*myc* mRNA were found to be almost comparable among splenocytes and thymocytes in both strains. Endogenous murine c-*myc* expression was completely inhibited in B and T lymphomas of both strains. The expression of murine c-*myc* mRNA was lower in prelymphomatous B and T cells of transgenic mice than in those of normal mice. These results demonstrated that the Eμ-*myc* gene is equally expressed in prelymphomatous B and T cells from B6 and C3H/HeJ transgenic mice, and that the expression of endogenous c-*myc* gene was suppressed in cells expressing Eμ-*myc* mRNA.

Transfer of Bone Marrow or Fetal Liver Cells from Prelymphomatous Transgenic Mice into Normal Mice. No differences of Eμ-*myc* expression and lymphocyte activation were observed between B6 and C3H/HeJ transgenic mice. These results suggested the possibility that environmental factor(s) may enhance the development or the growth of B and T lymphomas in B6 and C3H/HeJ transgenic mice, respectively. However, it was impossible to carry out the crossing experiment of the transgenic mice to study this possibility, since such mice died from tumors before giving rise to progeny. Therefore, bone marrow or fetal liver transfer experiments were done by using primary transgenic mice. Bone marrow or fetal liver cells of prelymphomatous B6 or C3H/HeJ transgenic mice were transferred into lethally irradiated normal B6 and C3H/HeJ mice, and the recipient mice were monitored for the development of tumors for 1 yr. 10 of 11 B6 and 7 of 9 C3H/HeJ recipients developed lymphomas between 65 and 95 d after transplantation. As shown in Table III, 6 of 9 C3H/HeJ mice transferred with bone marrow cells from either B6 or C3H/HeJ transgenic mice developed T lymphomas within 3 mo. Only one C3H/HeJ, which received bone marrow

TABLE III

Bone Marrow or Fetal Liver Transfer from Eμ-myc Transgenic Mice to B6 and C3H/HeJ Mice

	Tumors developed in B6 recipients				Tumors developed in C3H/HeJ recipients			
Donors*	n‡	B§	T§	(−)§	n‡	B§	T§	(−)§
B6 transgenic	8	4	4	0	6	1	3	2
C3H transgenic	3	1	1	1	3	0	3	0
Total	11	5	5	1	9	1	6	2

Bone marrow cells (5×10^6–1×10^7) or fetal liver cells (5×10^6) from Eμ-*myc* transgenic mice were transplanted into irradiated normal B6 or C3H/HeJ mice. The cell type of tumors developed in recipients were determined by multiparameter FACS analysis.

* Donor cells were prepared from bone marrow or fetal liver of B6 transgenic mice (91, 3-22, 3-24-2, 3-24-5, 3-25) and C3H/HeJ transgenic mice (4-30). Bone marrow or fetal liver cells from a single donor were transfered into one or several recipients; bone marrow of 91 to one B6, fetal liver of 3–22 to one B6, fetal liver of 3-24-2 to two B6 and two C3H/HeJ, fetal liver of 3-24-5 to two B6 and two C3H/HeJ, bone marrow of 3-25 to two B6 and two C3H/HeJ, and bone marrow of 4-30 to three B6 and three C3H/HeJ recipients.

‡ Number of recipient mice.

§ Number of recipient mice that developed B (B) or T lymphomas (T) or no lymphoma (−).

cells from B6 transgenic mice, developed B lymphoma. This indicates that hematopoietic stem cells from B6 as well as C3H/HeJ transgenic mice can give rise preferentially to T lymphomas in C3H/HeJ recipients. When bone marrow or fetal liver cells from B6 transgenic mice were transferred into irradiated B6 recipients, the development of T lymphomas increased up to 50%. This result indicates that the frequency of T lymphoma development increases in the environment of irradiated B6 mice, since primary B6 transgenic mice mostly developed B lymphomas as described earlier. To determine whether tumors developed in recipients were derived from independent clonal origins, we analyzed the integration of the transgene and the rearrangements of Ig heavy chain and TCR β chain genes in B and T lymphomas derived from different recipients that had been transferred with bone marrow cells from the same B6 transgenic mouse (3-25). Bone marrow cells from this mouse (3-25) were transferred into two B6 and two C3H/HeJ recipients. B lymphomas developed in one B6 and one C3H/HeJ recipient and T lymphomas developed in the others. As shown in Fig. 7, all lymphomas that developed in different recipients showed the Eμ-*myc* integration and the distinct rearrangement pattern of Ig heavy chain and TCR β chain genes, indicating that these tumors are derived from the same donor bone marrow, but different clonal origins. All these results suggest that T lymphomas

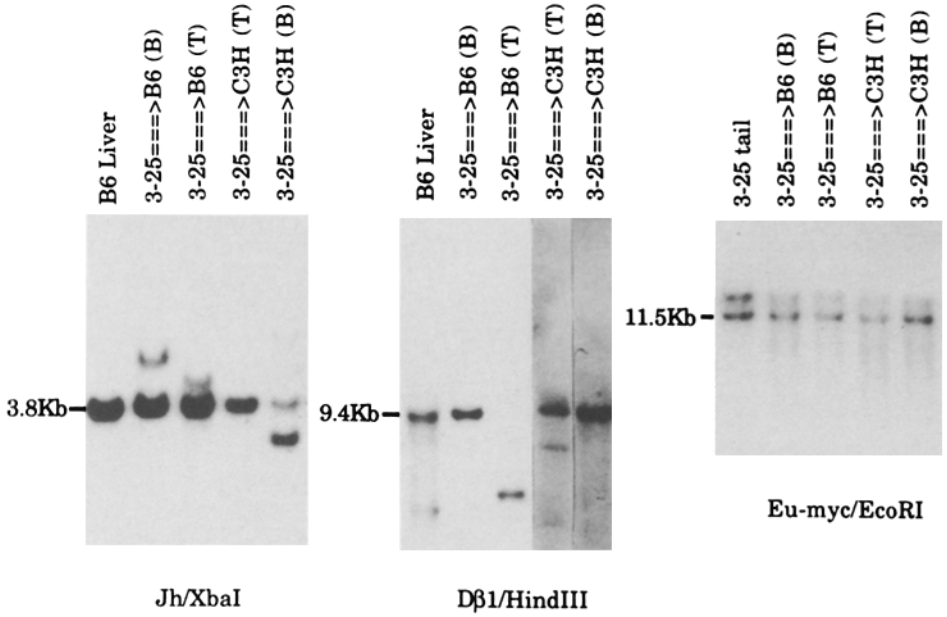

FIGURE 7. Tumors of distinct clonal origins were developed in respective recipients that were transferred with bone marrow cells from a single Eμ-*myc* transgenic mouse. 10^7 bone marrow cells from the B6 transgenic mouse (3-25) were transferred into two B6 and two C3H/HeJ recipient mice. DNA were prepared from tumors developed in each recipient mouse and analyzed for the rearrangements of Ig heavy chain and TCR β chain genes. Integration of the Eμ-*myc* transgene in these tumors demonstrated that they were of donor origin. 3-25→B6 (*B*): B lymphoma developed in one B6 recipient. 3-25→B6 (*T*): T lymphoma developed in another B6 recipient. 3-25→C3H (*T*): T lymphoma developed in one C3H recipient. 3-25→C3H (*B*): B lymphoma developed in another C3H recipient. 3-25 tail: the DNA extracted from the tail of 3-25 Eμ-*myc* B6 transgenic mouse. B6 liver: the DNA extracted from the liver of B6 mouse.

preferentially develop or grow in the environment of C3H/HeJ mice, and that irradiated mice might provide the similar environment that supports the development of T cell tumors.

Discussion

In this study, we have produced transgenic mice carrying the Eμ-*myc* gene by microinjecting the transgene into B6 or C3H/HeJ fertilized eggs and have shown that both B and T cell lymphomas can arise in these mice. Most lymphomas that developed in B6 transgenic mice were of B cell type as expected from the construction of the transgene in that human c-*myc* gene was under the control of Ig enhancer element. In contrast to B6 transgenic mice, C3H/HeJ transgenic mice preferentially developed T cell tumors. Several studies on productions of transgenic mice carrying Eμ-*myc* have been reported (8, 18, 19). Such transgenic mice have been shown to develop mostly pre-B cell tumors. These findings are in striking contrast to our results. However, they have injected the transgene into (B6 × SJL)F2 or (B6 × CD1)F_1, both of which retain partially B6 background. Moreover, the SJL strain has been shown to develop frequently spontaneous reticulum cell sarcomas that are of pre-B cell origins (20). Thus, our results raised the possibilities that the strain dependency of B or T lymphoma development might exist. Therefore, it is interesting to know what determines the development of B or T lymphoma in our B6 or C3H/HeJ transgenic mice, respectively.

The phenotypic analysis of lymphocytes from B6 and C3H/HeJ transgenic mice at a prelymphomatous stage showed increases in cell size and MHC class II expression of B cells from such mice of both strains, which is in good agreement with the finding by Langdon et al. (21). However, the expression of Eμ-*myc* transgene did neither perturb the development of T cells in thymus or increase the size of T cells in the transgenic mice of both strains. The S1 nuclease analysis of splenocytes and thymocytes demonstrated that almost equal amounts of human c-*myc* mRNA were expressed in spleens and thymuses of both strains of transgenic mice, indicating that there is no difference in the transgene expression in B and T cells of B6 and C3H/HeJ transgenic mice. These results suggest that a high incidence of B or T lymphomas in B6 or C3H/HeJ transgenic mice could not be simply explained by the differential activation of or the differential Eμ-*myc* expression in B or T lineage cells.

In contrast to our transgenic mice, the Eμ-*myc* transgenic mice produced by others (8, 18, 21, 22) have been shown to express the transgene specifically in B lineage cells. The reason for this discrepancy is not clear at the moment. However, it has been reported that the transgenic mice introduced with productively rearranged μ chain gene express the transgene in certain population of T as well as B cells (23). Furthermore, several pieces of evidence have also been reported that Ig enhancer element can be active in T as well as B cells (24–27). Thus it is reasonable to assume that the expression of human c-*myc* gene is also under the influence of the adjacent Ig enhancer even in T cells. We used the human Eμ-*myc* gene cloned from a non-Hodgkin lymphoma, Manca, in which human Ig enhancer has been translocated into intron 1 of the c-*myc* gene (9). Other studies used the Eμ-*myc* construct, in which the mouse or the human c-*myc* gene is under the control of the murine Ig enhancer (8, 18, 19). One of the three was the naturally translocated Eμ-*myc* and the others were the artificially constructed ones. Therefore, it is possible that the extent of Eμ-*myc*

gene expression in T cells may reflect particular constructs. It should be noted that the Eμ-*myc* construct used in this study lacks exon 1. The exon 1 is known to be lost in the translocated c-*myc* gene of some of human Burkitt's lymphoma cells and murine plasmacytoma cells (5). v-*myc* also lacks the equivalent sequence (28–30). Some investigators have proposed that the exon 1 sequence plays an important role in the negative control of c-*myc* gene transcription (31–33). Others have suggested that the untranslated sequence of c-*myc* mRNA derived from exon 1 may be involved in the translational control (34, 35). Furthermore, it has been also reported that c-*myc* mRNA lacking exon 1 sequence is more stable than the complete c-*myc* mRNA (36–38). It is thus possible that our transgene might be more active than the *myc* construct used by others, which contained exon 1 (8, 18) or heterologous SV40 promoter (19). Alternatively, the Eμ-*myc* transcripts of the transgenic mice produced here might be more stable.

There are two possible explanations for a high incidence of T lymphoma in the C3H/HeJ transgenic mice; (*a*) the frequency of transformation of B and T cells might be intrinsically determined, or (*b*) the development or growth of T lymphoma in C3H/HeJ mice might be accelerated by certain environmental factors such as growth factors or endogenous viruses. One piece of evidence supporting the former possibility is that the C3H/HeJ strain has a functional defect in B cells and is a low responder to LPS (39, 40). Therefore, this defect might be involved in less frequent B lymphoma development in transgenic mice of this strain. However, we could not observe any difference in Eμ-*myc*–induced distortion of B lymphocyte activation in prelymphomatous transgenic mice of both strains, as discussed above, suggesting that the defect of C3H/HeJ did not affect Eμ-*myc*–induced B cell activation.

The microenvironment of each strain is also an important factor that influences the growth or development of lymphomas. When the bone marrow or fetal liver cells from the prelymphomatous B6 or C3H/HeJ transgenic mice were transferred to lethally irradiated C3H/HeJ mice, the developed lymphomas were mostly of T cell type. Half of the tumors developed in irradiated B6 mice, which received hemopoietic stem cells from B6 and C3H/HeJ transgenic mice, were also of T cell type. Although the number of experiments was small because of the limited availability of transgenic mice, these results indicate the presence of the necessary conditions in C3H/HeJ to support the growth or the development of T lymphomas, respectively. Furthermore, irradiation might modify the environment of B6 mice, resulting in increased development of T cell lymphomas.

One of the environmental factors affecting lymphoma development in the transgenic mice might be an endogenous retrovirus. Recently, Klinken et al. (41) demonstrated that v-*raf* could convert the phenotype of B lymphomas developed in Eμ-*myc* transgenic mice to that of macrophages. This suggests that retrovirus infection could have an influence on the lineage determination of tumors. However, the high incidence of T lymphomas in C3H/HeJ transgenic mice cannot be explained simply by the phenotype conversion of tumors from B to T lineage since some of T cell lymphomas developed in C3H/HeJ transgenic mice conserved the germline configuration of Ig heavy chain genes.

Kaplan et al. (42–45) have shown the high incidence of T cell lymphomas of graft origins in the irradiated mice that receive unirradiated thymus graft and have isolated radiation leukemia virus (RadLV) as the agent that induces tumors of graft origin by indirect mechanisms in irradiated recipients. The recent study by Lie-

berman et al. (46) has confirmed the indirect mechanism of radiation-induced lymphomagenesis but has demonstrated that such lymphomagenesis is mediated by the transmissible agent that is not identical to RadLV. Recently it has also been reported that irradiation of hematopoietic stroma cells induces the production of certain growth factor(s) that support the development of myelogenous leukemia (47). Supposing such an agent or a growth factor exists even at considerably low levels in normal C3H/HeJ, it might positively affect the development or growth of T lymphoma in the prelymphomatous transgenic mice of C3H/HeJ. Irradiation of B6 mice may induce such an agent or growth factor and provide the environment to support the T lymphoma development. However, the identification of viral agents or factors, which influence the development of T and B cell tumors, will require further studies.

In contrast to Eμ-*myc* transgenic mice produced by others (8, 18, 19, 48), our C3H/HeJ transgenic mice developed mostly T lymphomas, which represent various differentiation stages of T cells. Although a number of transgenic mice carrying various activated oncogenes have been reported, the incidence of T lymphoma is considerably low (see reference 49 for review). Recently it has been reported that murine leukemia virus (MuLV) infection accelerated the development of T lymphoma in Eμ-*pim*-1 transgenic mice, although uninfected transgenic mice developed T lymphomas at a low frequency (5–10%) and after a relatively long latent period (3–7 mo) (50). Similarly to T lymphomas in our C3H/HeJ transgenic mice, tumors developed in Eμ-*pim*-1 represented different T cell subpopulations, $CD4^+/CD8^+$ tumors and $CD4^+/CD8^-$ tumors. Therefore, those transgenic mice and ours and tumors developed in such mice will be very useful for the study of T cell differentiation pathways, as well as for the elucidation of the mechanism of T lymphomagenesis.

Summary

The transgenic mice were produced by injecting eggs of B6 and C3H/HeJ mice with the human Eμ-*myc* gene. Preferential development of B lymphomas was observed in the B6 transgenic mice, whereas the C3H/HeJ transgenic mice developed mostly T lymphomas. The phenotypic activation of B lineage cells but not of T lineage cells was detected in the prelymphomatous transgenic mice of both strains. The transgene was similarly expressed in B and T cells of the transgenic mice of both strains. These results suggest that a high incidence of T lymphomas in the C3H/HeJ transgenic mice may not be due to the preferential activation of or the preferential Eμ-*myc* expression in T lymphocytes. When the bone marrow or fetal liver cells from the prelymphomatous transgenic mice of both strains were transferred into irradiated normal C3H/HeJ mice, most of the recipients developed T lymphomas. Moreover, even when irradiated B6 mice received the hematopoietic stem cells from the prelymphomatous B6 transgenic mice, the incidence of T lymphoma increased up to 50%. These findings suggest that B6 and C3H/HeJ mice might provide the environment that supports the development or growth of B and T lymphomas, respectively, and that such an environment could be modified by irradiation of the mice.

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