Myeloid, B and T lymphoid and mixed lineage thymic lymphomas in the irradiated mouse

Emma Boulton, Helen Cleary and Mark Plumb

MRC Radiation and Genome Stability Unit, Chilton Didcot, Oxfordshire OX11 ORD, UK
1To whom correspondence should be addressed at: Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK
Email: map12@le.ac.uk

Thymic lymphoma is a very common spontaneous and/or induced malignancy in both inbred mice and in transgenic mouse models of human cancer. Although a thymic lymphoma is defined as thymus-dependent T-cell malignancy, diagnostic criteria vary between studies and considerable heterogeneity has been reported. To define and classify the thymic lymphomas that arose in our study of X-irradiated (CBA/H × C57BL/6)F1, F1 backcross and F1 intercross mice, 66 thymic lymphomas were immunogenotyped for immunoglobulin heavy chain (Igh) and T-cell receptor β (TCRβ) gene rearrangements, and/or analysed for expression of lineage-specific markers and allelic loss on chromosome 4. The data indicate that 33% of the thymic lymphomas are very similar to mouse radiation-induced acute myeloid (AML) and mixed lineage (IgHβ, TCRββ) pre-B lympho-myeloid (L-MLs) leukaemias, 33% are mixed lineage (IgHβ, TCRββ) B/T lymphoid and <33% can be described as single lineage (IgHβ, TCRββ) T-cell malignancies. As the myeloid and L-ML leukaemias are not thymus-dependent this suggests that a malignant myeloid or pre-B lympho-myeloid cell can colonize the spleen to give an AML or L-ML leukaemia, or can colonize the thymus where TCRβ gene rearrangement(s) may be induced to give the mixed lineage thymic lymphomas. Thus, assuming the single lineage T-cell thymic lymphomas fulfil the criteria of a thymus-dependent T-cell malignancy, thymic lymphomas are comprised of at least three distinct malignancies.

Introduction

Gene targeting and the generation of transgenic mice which are either deficient for a specific gene, or which over-express a gene, have been extensively used to test the function of oncogenes or tumour suppressor genes implicated in human cancers. Both the inactivation of genes involved in the maintenance of genomic stability or the enforced expression of (proto-)oncogenes result in an increased susceptibility to spontaneous or induced cancer in transgenic mice. Although the (proto-)oncogenes and tumour suppressor genes tend to be associated with specific malignancies in humans, the most common malignancy that arises in transgenic mice is thymic lymphoma. Many inbred mouse strains are also highly susceptible to radiation- and/or chemical carcinogen-induced thymic lymphoma (2–11), suggesting that thymic lymphoma is the predominant malignancy that arises in mice as a consequence of genetic instability and/or carcinogen exposure.

As a mouse model of an induced haemopoietic malignancy, thymic lymphomas have been extensively analysed to identify tumour suppressor and susceptibility loci. Loss of heterozygosity (LOH) studies of induced mouse thymic lymphomas have identified five tumour suppressor loci on chromosome 4, in addition to loci on chromosomes 2, 5, 6, 11, 12, 16 and 19 (11–21), and the inactivation of the p15INK4b (chromosome 4) and Ikaros (chromosome 11) tumour suppressor genes has been reported (13,20,22–24). Together with susceptibility/resistance loci on chromosomes 2, 4, 5 and 7 identified in genetic studies (25,26), at least 13 loci on nine chromosomes have been implicated in mouse spontaneous and/or induced thymic lymphomagenesis.

The diagnosis of thymic lymphomas in the mouse does not generally follow the strict criteria used to diagnose human haemopoietic malignancies, with diagnosis relying on an enlarged thymus (with laboured breathing) and/or limited serology and immunogenotype analyses, but considerable heterogeneity has been observed (2,3,5–10,12,13,27–30). Although in vivo transplantation experiments following whole body X-irradiation have shown that exposure induces thymus-dependent pre-leukaemic cells which require the thymus microenvironment for progression to full malignancy (32–35), non-thymic mixed phenotype B-myeloid lymphomas arise in thymectomized mice (36,37), evidence that while the thymus may not be essential for malignant transformation, it may determine the type of malignancy.

A number of other mouse strain-specific spontaneous or induced haemopoietic malignancies have been described in inbred mice, and include pre-B lymphomas (38), lymphocytic leukaemia (39), plasmacytoma (40), acute myeloid leukaemia (AML) and a mixed lineage early pre-B lympho-myeloid leukaemia (L-ML) (41,42). Although it is generally accepted that there is a strong genetic component, which determines the type and/or incidence of the spontaneous or induced haemopoietic malignancy in the mouse, like thymic lymphomas, the malignancies tend to be poorly defined. The absence of consistent and stringent diagnostic criteria in the classification of mouse leukaemias and lymphomas raises the possibility of misdiagnosis and makes the comparison of data from different studies and/or in different mouse strains difficult.

There is indirect evidence that mouse thymic lymphomas may represent more than one distinct malignancy: (i) the immunogenotype and phenotype analyses of thymic lymphomas have revealed considerable heterogeneity (2,3,5–10,12,13,27–31); (ii) LOH studies have revealed widespread allelic loss which is in part mouse strain-specific (11–21); (iii) the incidence of p15INK4b tumour suppressor gene inactivation by allelic loss and promoter methylation in radiation-induced thymic lymphomas varies from 20 to 80% depending on the genetic background and carcinogen treatment (22–24); and

Abbreviations: AML, acute myeloid leukaemia; L-ML, pre-B lympho-myeloid leukaemias; LysM, lysozyme M; MPO, myeloperoxidase.
(iv) mixed lineage L-ML/lymphomas arise in thymectomized mice (36,37).

Our recent molecular studies of mouse radiation-induced AML, which had been diagnosed by leukaemic blood cell morphology revealed two distinct malignancies: AML and a mixed lineage early B L-ML (41). We have therefore used the same approach to classify thymic lymphomas which arose in 3 Gy X-irradiated (CBA/H×C57BL/6)F1 backcross and intercross mice. Thymic lymphoma DNA was screened for immunoglobulin heavy chain (IgH) and T-cell receptor β (TCRβ) gene rearrangements, and revealed all four possible immunogenotype combinations. Thymic lymphoma RNA was also screened for expression of lineage specific/restricted markers and a significant proportion found to express both lymphoid (VpreB1) and myeloid [myeloperoxidase (MPO), lysozyme M (LysM)] markers. Together with LOH studies of chromosome 4, our data suggest that many of the thymic lymphomas are mixed lineage B and/or T lympho-mylloidal malignancies, and in many respects are similar to AML and L-ML leukaemias that arose in the same mice (41). Thus, whilst the thymus does not appear to be essential for malignant progression, the thymus microenvironment may induce TCRβ gene rearrangements in myeloid and B L-ML cells which colonize the thymus to yield the observed mixed lineage thymic lymphomas.

Materials and methods

Mouse irradiations

CBA/H and C57BL/6 mice were from the Harwell colony. Mice, 8–12-week-old (CBA/H×C57BL/6)F1 (n = 89), F1×CBA/H (n = 1087), F1×C57BL/6 (n = 314) and F1×F1 (n = 142), were exposed to a single acute dose of 3.0 Gy X-rays at 0.5 Gy/min (250 kV constant potential, HVL 1.2 mm Cu). The animal studies were carried out under guidance issued by the MRC in ‘Responsibility in the use of animals for medical research’ (July 1993) and Home Office Project Licence No. PPL 30/689 and 30/1272.

Leukaemias and lymphomas were diagnosed by micropscopic examination of blood, bone marrow, spleen and thymus. The average weight of an adult mouse thymus is ~0.01 g, and a thymic lymphoma defined in the first instance as a malignancy resulting in an enlarged thymus weighing >0.1 g (42). Thymic lymphomas were snap frozen for subsequent molecular analyses.

Immunogenotype

DNA was prepared from thymic lymphomas and ~15 μg of restriction enzyme-digested DNA was resolved by 1% (w/v) agarose gel electrophoresis and transferred to Genescreen (NEN Life Science Products, Boston, MA) nylon membranes. HindIII DNA digests were probed with the pTcrb-J2 probe to detect TCRβ gene rearrangements, and EcoRI and BamHI DNA double digests probed with p5’ (JH) to screen for immunoglobulin heavy chain (IgH) gene rearrangements (41,42).

Lymphoma phenotype

Where possible total cellular RNA was also prepared from ~50% of the thymic lymphoma, and from control adult spleen and thymus, for northern blot analyses. RNA (~10–20 μg) was resolved by 1.0% (w/v) denaturing gel electrophoresis, transferred to Genescreen membranes, and probed with MPO, LysM, CD19, PU1, VpreB1 and RAG1 cDNA probes (41,42). Normal bone marrow and thymus were used as positive controls for VpreB1 and RAG1, respectively. Equivalent RNA loading was confirmed by ethidium bromide staining and/or hybridizations with glutathione peroxidase (GPx) and transforming factor β (TGFB) cDNA probes. Probes were labelled with [α-32P]dATP (3000 Ci/mmol; Amersham Biosciences, Amersham, UK) using the Random Prime labelling kit (Invitrogen, Paisley, UK).

Loss of heterozygosity

The polymorphic chromosome 4 microsatellite markers used in LOH analyses of tail and lymphoma DNA from the same mouse had already been described (41,43). Genetic map positions are from the Mouse Genome Database, and microsatellite primer sequences were from the Jackson Laboratory, except for MP-15-3* on chromosome 4 (44).

Results

Thymic lymphoma immunogenotype

In a lifetime study involving 1632 X-irradiated (CBA/H×C57BL/6)F1 and F1 backcross and F1 intercross mice, 66 thymic lymphomas were diagnosed as defined by an enlarged (>10-fold) thymus (42). Thymic lymphomas have been described as T-cell malignancies (2,3,5–10,12,13,27–30), so in a retrospective study we sought to confirm this by immunogenotyping thymic lymphoma DNA for IgH and TCRβ gene rearrangements by Southern blot analyses. Unexpectedly, and as illustrated in Figure 1 and summarized in Table I, germline (IgHc and TCRβc) and rearranged (IgHr and TCRβr) alleles were readily detectable in the thymic lymphomas, and all four immunogenotype combinations represented.

The detection of mono- and bi-allelic gene rearrangements by Southern blot analysis, together with the relative intensities of the germline and rearranged bands on the Southern blots (Figure 1 and data not shown), is clear evidence of the clonal expansion of a malignant cell carrying a rearranged gene(s) in the thymus, and we can infer (Figure 1) that contamination of the lymphoma by normal untransformed cells in most cases is low. Further evidence that the malignancies are clonal in origin and contain <20% contaminating normal cells can be inferred from LOH studies (see below).

Although mono- and bi-allelic IgH and TCRβ gene rearrangements were detected, two rearranged alleles in addition to the germline configuration allele (Figure 1A, lanes 6 and 7; Figure 1B, lane 7 and data not shown), and three or more rearranged alleles (Figure 1A, lane 10 and data not shown) were also detected. Given that the mean latency of the
distinct malignancies can be classified according to their immunogenotype: B cell (IgHR, TCRβ), T cell (IgHG, TCRβ) and mixed lineage B/T (IgHR, TCRβ). IgHR, TCRβ lymphomas cannot be classified.

**Thymic lymphoma phenotype**

Mouse haemopoietic malignancies commonly have a leaky differentiation block, so although they are clonal in origin, the proportion of differentiating cells expressing one or more lineage-specific markers can vary considerably. In mouse AML, for example, metamyelocytes can account for 2–40% of the white blood cell count and MPO gene expression in the leukaemic spleen can vary by orders of magnitude (41). Similarly, mixed lineage malignancies are also commonly observed in mice (37,41,46,47). Many mouse haemopoietic malignancies are therefore not composed of a single homogenous cell type, but are classified by the presence of specific immature haemopoietic progenitor cells in haemopoietic tissue(s) which are either not normally present in that tissue, or which are present in much higher numbers than normal.

As IgH and TCRβ gene rearrangements are absolutely specific to B- and T-cell lineages, respectively, at least three distinct malignancies can be classified according to their immunogenotype: B cell (IgHR, TCRβ), T cell (IgHG, TCRβ) and mixed lineage B/T (IgHR, TCRβ). IgHR, TCRβ lymphomas cannot be classified.

Thymic lymphoma phenotype

Mouse haemopoietic malignancies commonly have a leaky differentiation block, so although they are clonal in origin, the proportion of differentiating cells expressing one or more lineage-specific markers can vary considerably. In mouse AML, for example, metamyelocytes can account for 2–40% of the white blood cell count and MPO gene expression in the leukaemic spleen can vary by orders of magnitude (41). Similarly, mixed lineage malignancies are also commonly observed in mice (37,41,46,47). Many mouse haemopoietic malignancies are therefore not composed of a single homogenous cell type, but are classified by the presence of specific immature haemopoietic progenitor cells in haemopoietic tissue(s) which are either not normally present in that tissue, or which are present in much higher numbers than normal.

As the source of malignant thymic lymphoma cells in the mouse is the thymus, and as the thymus is the site of T-cell differentiation in vivo, the use of T-cell-specific cell surface antigens to characterize the lymphomas is potentially complicated by the presence of normal thymic cells, and may explain the considerable heterogeneity observed in thymic lymphoma analyses using CD4, CD8, CD3, TL, IL-2R, MEL-14 and H2-K antigens (3,6,27,31). Although 61% of the thymic lymphomas in this study exhibited an unequivocal T-cell phenotype (TCRβ gene rearrangements, Table I), the origin and classification of the IgHR (40%, Table I) and IgHG, TCRβ (29%; Table I) thymic lymphomas was unclear. To address this issue, thymic lymphomas were analysed for the expression of lineage-specific/restricted genes not normally expressed, or expressed at very low levels, in a normal adult thymus.

To further characterize the thymic lymphomas, total cellular RNA was prepared from 19 thymic lymphomas, which were large enough to permit both DNA and RNA preparations. RNA was analysed for the expression of lineage-specific/restricted markers by northern blot (Figure 2).

Only a significant difference in the abundance of an mRNA in a thymic lymphoma compared with a normal thymus can be attributed to the lymphoma cells, so enrichment (+) of an

<table>
<thead>
<tr>
<th>Thymic lymphoma immunogenotype</th>
<th>F1</th>
<th>F1×CBA/H</th>
<th>F1×C57BL/6</th>
<th>F1×F1</th>
<th>TL</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 89</td>
<td>n = 1087</td>
<td>n = 314</td>
<td>n = 142</td>
<td>(%) 66</td>
<td>(%) 1632</td>
<td></td>
</tr>
<tr>
<td>IgHR, TCRβ</td>
<td>1 (1.1)%</td>
<td>9 (0.8)%</td>
<td>8 (2.5)%</td>
<td>1 (0.7)%</td>
<td>19 (29%)</td>
<td>1.2</td>
</tr>
<tr>
<td>IgHG, TCRβ</td>
<td>2 (2.2)%</td>
<td>7 (0.6)%</td>
<td>7 (2.2)%</td>
<td>5 (3.5)%</td>
<td>21 (32%)</td>
<td>1.3</td>
</tr>
<tr>
<td>IgHR, TCRβG</td>
<td>3 (0.3)%</td>
<td>3 (0.9)%</td>
<td>1 (0.7)%</td>
<td>7 (11%)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>IgHG, TCRβG</td>
<td>2 (2.2)%</td>
<td>11 (1%)</td>
<td>3 (0.9)%</td>
<td>3 (2%)</td>
<td>19 (29%)</td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>5 (5.6)%</td>
<td>30 (2.8)%</td>
<td>21 (6.7)%</td>
<td>10 (11%)</td>
<td>66 (100%)</td>
<td>4</td>
</tr>
</tbody>
</table>

aGenetic background, where n is the number of mice irradiated.
b(%): per cent thymic lymphoma incidence in each genetic background.
cGenetic background identified with each immunogenotype.
dIncidence: thymic lymphoma lifetime incidence as a per cent of the total number of mice irradiated (1632) irrespective of genetic background.

![Fig. 2. Thymic lymphoma mRNA expression profiles. Representative northern blots containing total cellular RNA (~20 µg) prepared from control spleen (lane 1) or thymus (lane 2) or thymic lymphomas (lanes 3–20). Blots were probed with MPO, LysM, CD19, PU1, VpreB1, Rag1, TGFβ and/or GPX cDNA probes (41,42). Thymic lymphoma immunogenotypes are: IgHR, TCRβ; lanes 3, 5–7, 9, 12, 15–19. IgHG, TCRβ; lanes 4, 8, 13. IgHR, TCRβG; lanes 10, 11, 14, IgHG, TCRβG; lane 20.](image)
mRNA species in an individual lymphoma was initially defined as >2-fold, and depletions (<) as <0.5-fold, compared with its level in control thymus, or as positive (+) when that mRNA is not detectable in thymus (VpreB1). The differences in mRNA levels considered informative are therefore larger than those that might be attributed to gel loading (GPX and TGFβ controls, Figure 2).

As illustrated in Figure 2, and with reference to control adult thymus (lane 2), a significant number of the lymphomas exhibit enrichment of MPO (lanes 7, 9, 16, 18 and 20), LysM (lanes 5, 9, 11, 13, 17, 19 and 20), CD19 (lanes 5, 6, 8–10, 12–14, 18 and 19), VpreB1 (lanes 4, 13 and 16) and PU1 (lanes 3–20) mRNAs. No one thymic lymphoma exhibited significant enrichment of Rag1 mRNA compared with normal thymus, so Rag1 is not informative as a lymphoid marker.

The detection of VpreB1 mRNA by northern blot in three thymic lymphomas is particularly informative as VpreB1 gene expression occurs very early (Pro-B → Pre-B) in B-cell maturation and is undetectable in normal mouse thymus and spleen, but is detected in bone marrow, which is the site of early B-cell maturation in vivo (41), and data not shown. As it is highly unlikely that normal early B progenitor cells would leave the bone marrow and colonize the thymus in sufficient numbers to give detectable levels of VpreB1 mRNA, the abnormal gene expression observed in the lymphoma, together with the clonal IgH gene rearrangements detected by Southern blot (Figure 1 and Table I), is strong evidence that at least some of the lymphomas are early B-cell malignancies that originated in the bone marrow.

The enrichment of CD19 mRNA compared with normal thymus (Figure 2) is observed irrespective of whether IgH gene rearrangements are detected by Southern blot analysis of thymus (Figure 2) is observed irrespective of whether those that might be attributed to gel loading (GPX and TGFβ controls, Figure 2).

The immunogenotype (Table I) and mRNA expression profiles (Figure 2) of the thymic lymphomas suggest that some may be related to the AML (IgHF, TCRβG, MPO+, CD19+, VpreB1+), or L-ML (IgHF, TCRβG, MPO+, LysM+, VpreB1+/−), or CD19+, VpreB1−/− leukemia whose mRNA expression profiles were defined with reference to normal spleen (41). Compared with normal spleen, five of 19 (26%) thymic lymphomas are enriched for LysM mRNA (Figure 2, lanes 9, 11, 13, 17 and 19), whereas no significant enrichment of CD19 mRNA was observed. This is remarkably similar to the AMLs and L-MLs which were either negative or not significantly enriched for CD19 mRNA, whereas 6% of the AMLs and 22% of the L-MLs were enriched for LysM mRNA (41).

Excluding CD19, PU1 and Rag1 as not informative, and comparing LysM mRNA levels with control spleen, the immunogenotype and MPO, LysM and VpreB1 mRNA expression profiles (Figure 2) permit the further classification of the lymphomas:

(i) IgHF, TCRβG, LysM+/MPO+, VpreB1+: myeloid (lanes 6, 7, 9, 12, 17 and 19).

(ii) IgHF, TCRβG, MPO+, LysM−, VpreB1+: proB lymphoid (lane 16).

(iii) IgHF, TCRβG, MPO+, LysM+, VpreB1+: preB lymphoid (lane 13).

(iv) IgHF, TCRβG, MPO+, LysM+, VpreB1−/+: early B lymphoid (lanes 4 and 8).

(v) IgHF, TCRβG, MPO+, LysM−, VpreB1+: T lymphoid (lanes 10 and 14).

(vi) IgHF, TCRβG, MPO+, LysM+, VpreB1+: T lymphoid (lane 11).


Thus, the immunogenotype and/or mRNA expression analyses using three lineage-specific markers permits the sub-classification of ~80% of the thymic lymphomas and reveals an unexpected complexity.

**Loss of heterozygosity**

Allelic loss on chromosome 4 is frequently detected in radiation-induced thymic lymphomas, and five thymic lymphoma suppressor regions (TLSR1–5) mapped (17–19,21). As allelic loss at TLSR5 has been preferentially detected in mouse radiation-induced AML and L-ML leukemias (41), and some of the thymic lymphomas appeared to be related to the leukemias, the thymic lymphomas in this study were screened for LOH on chromosome 4. As most of the lymphomas arose in F1 backcross or intercross mice (Table I), there is a 50% probability of homozygosity at any given microsatellite marker. Similarly, only those lymphoma DNA samples that contained <20% contaminating normal cells will reveal LOH (41,42).

Twenty-five out of 66 thymic lymphomas exhibited LOH on chromosome 4, further evidence that the malignancies are clonal in origin and that there are low levels of contaminating normal cells in the lymphomas. Twenty thymic lymphomas were informative for both proximal and distal breakpoints (Figure 3).

Ninety-two per cent (23 out of 25) exhibit LOH at D4Mit286, and a 3.4 cM minimally deleted region inferred between D4Mit102 and D4Mit214 irrespective of immunogenotype. This 3.4 cM interval maps to the same TLSR5 tumour suppressor gene locus mapped in both radiation-induced AML (~50% LOH incidence) and L-ML (>95% LOH incidence) leukemias (41). Furthermore, the maternally transmitted CBA/H allele at D4Mit286 was preferentially lost in 20 out of 23 of the thymic lymphomas as it was in 25 out of 28 of radiation-induced AMLs and L-MLs (41), further evidence that many of the thymic lymphomas are the same or very similar to the radiation-induced leukemias.

TLSR1 has been associated with the inactivation of the p15INK4B tumour suppressor gene by allelic loss and promoter hypermethylation (22), and the MP15-3’ polymorphic microsatellite marker mapped to the INK4a locus (44). Only three thymic lymphomas in our study exhibit LOH at MP15-3’, two of which also exhibit LOH at D4Mit286/TLSR5 (Figure 3). Two additional thymic lymphomas are not informative at MP15-3’, but exhibit LOH at D4Mit286/TLSR5 so LOH at MP15-3’ cannot be excluded. Thus, although five out of 25 lymphomas potentially exhibit allelic loss at the TLSR1 locus, only one out of 25 exhibits LOH at TLSR1 (MP15-3’) but not...
6) F1 was a simple recessive or dominant C57BL/6 genetic trait. Susceptibility to radiation-induced T-cell thymic lymphomas in the mouse thymus, include myeloid, B and T lymphoid and malignancies that can colonize and undergo clonal expansion by haemopoietic, myelomonocytic leukaemia or mixed lineage L-ML leukaemias in our study arose in the bone marrow but fortuitously colonized the thymus where variable T-cell differentiation was induced. Furthermore, as the thymus microenvironment is required for TCRβ gene rearrangements, this raises the possibility that on occasion TCRβ gene rearrangements can be induced in AML or L-ML cells that colonize the thymus and give rise to the observed B/T and T-myeloid mixed lineage thymic lymphomas. This is consistent with the relative under-representation of IgHβ, TCRβG lymphomas in the thymus (0.4%; Table I) compared with the lifetime incidence of IgHβ, TCRβG lymphomas in the thymus (1.2%; Table I) and IgHβ, TCRβG L-ML leukaemias in the spleen (~8%) (41).

The thymic lymphomas analysed in this study arose in X-irradiated (CBA/H×C57BL/6)F1, F1 backcross and F1 intercross mice. As CBA/H mice are susceptible to radiation-induced AML and L-ML (41), and C57BL/6 are susceptible to radiation-induced thymic lymphomas (4), the genetic background of the hybrid mice analysed must contribute to the complexity of the lymphomas observed. Differences in the thymic lymphoma incidence and/or immunogenotype would be expected in the (CBA/H×C57BL/6)F1, (CBA/H×C57BL/6)F1×CBA/H and (CBA/H×C57BL/6)F1×C57BL/6 mice if susceptibility to radiation-induced T-cell thymic lymphomas was a simple recessive or dominant C57BL/6 genetic trait. However, as shown in Table I, a comparison of the lifetime incidence of thymic lymphomas as a whole or subclassified according to their immunogenotype, reveals no obvious or genetically interpretable difference in the five genetic backgrounds studied.

Early dose–response studies of thymic lymphoma induction by ionizing radiation in inbred C57BL/6 mice clearly demonstrated that exposure to fractionated doses of radiation is considerably more efficient in inducing thymic lymphomas than single acute exposure to the same total dose (4), and the generally accepted irradiation regime involves four weekly doses of 1.6–2.5 Gy starting at 4–5 weeks of age (1,2,4,9,12,13,16,17,20,27). In contrast, the thymic lymphomas in our study arose in mice exposed to a single acute dose of 3 Gy X-rays at 8–12 weeks of age, so a more mature haemopoietic system at the time of exposure to a single dose of X-rays may account for the complexity of the thymic lymphomas observed. However, it should be noted that: (i) the last 1.6–2.5 Gy exposure in the thymic lymphoma fractionated irradiation protocol occurs at 7–8 weeks of age; (ii) 40 out of 66 (61%; Table I) of the thymic lymphomas analysed in this report can be defined as T-cell malignancies as they have TCRβ gene rearrangement(s); (iii) myeloid, plasmacytomas and mixed lineage pre-B L-ML and lymphomas arise in thymectomized mice (36,37) and are very similar to the mixed lineage L-ML and thymic lymphomas in our study; and (iv) spontaneous thymic lymphomas in transgenic mouse models can arise at an age of >18 months (1).

A number of T-cell-specific cell-surface antigens, including CD4, CD8, CD3, TL, IL-2R, MEL-14 and H-2K, have been employed to classify mouse thymic lymphomas, and heterogeneity attributed to either a continuous spectrum of mature and immature phenotypes (3,6,27,31), or to the cortical or medullary origin of the lymphoma cell within the thymus (9). As B or myeloid cell markers are not commonly used, the classification of a T-cell thymic lymphoma based on T-cell-specific markers alone is unsatisfactory, particularly as plasmacytomas, myelomonocytic leukaemia or mixed lineage IgHβ, Cds5+, Nk1.1+, Mac1+, LysM+ B lympho-myeloid non-thymic lymphomas arise in thymectomized mice (36,37). Significantly, many studies of mouse haemopoietic malignancies that do use multi-lineage-specific markers have revealed an unexpected complexity (29,36,37,41,46–49), which is comparable with that described here, supportive evidence that at least some of the thymic lymphomas represent malignancies that arose in the bone marrow but fortuitously colonized the thymus where variable T-cell differentiation was induced.

LOH studies of induced mouse thymic lymphomas have mapped 12 tumour suppressor loci on eight chromosomes, including five loci (TLSR1-5) on chromosome 4 (11–21). TLSR1-5 map to positions 42, 71, 64, 39 and 16 cM on chromosome 4, respectively (41), and the incidence of LOH detected at each locus in (C57BL/6×BALB/c)F1 and/or (C57BL/6×RF/1)F1 radiation-induced thymic lymphomas varies between 20.4 (TLSR5) and 32.6–40% (TLSR2 and TLSR1) (17,18,21,22). In contrast, allelic loss in mouse radiation-induced AML and L-ML leukaemias is considerably more specific when the malignancies are classified according to the criteria used in this study. Allelic loss on chromosome 2 is detected in >95% of AMLs and <15% L-MLs, whereas allelic loss at TLSR5 is detected in >95% of L-MLs and ~50% of AMLs (41). Our observation that the preferential loss of the maternally transmitted CBA/H allele at TLSR5 is observed in
92% of thymic lymphomas (Figure 3), and that at most 25% of thymic lymphomas exhibit allelic loss at TLSR1, is consistent with the proposal that at least 70% of the thymic lymphomas analysed in this study are related to AML and L-ML leukemias.

Allelic loss at TLSR1 is associated with promoter methylation of the p15INK4b tumour suppressor gene promoter, but the incidence of p15INK4b gene promoter methylation in radiation-induced thymic lymphomas varies from 20 to 88% depending on genetic background and/or radiation quality (22,24). Prior to the immunogenotype analyses of the thymic lymphomas described in this report, p15INK4b gene promoter methylation was detected in 21% of the thymic lymphomas we analysed (24). A re-evaluation of our data indicates that p15INK4b gene promoter methylation in the thymic lymphomas is immunogenotype-dependent as it is detected in five of 11 (45%) IgHβ, TCRββ lymphomas and one of 11 (9%) IgHγ, TCRββ lymphomas, but was not detected in IgHα, TCRββ and in IgHγ, TCRββ lymphomas (data not shown), further supportive evidence for distinct malignancies.

The Pax5 gene maps to the chromosome 4 TLSR5 minimally deleted region mapped in the LOH studies (Figure 3). Appropriately stimulated Pax5 deficient pre-B and pro-B cells can develop into erythroid, myeloid and lymphoid cell lineages, suggesting that Pax5 suppresses alternative lineage choices in vivo, or that Pax5 deficient pre-BI cells dedifferentiate as far back as the pluripotent stem cell (50,51). The identification of mixed lineage B-myeloid, B/T-lymphoid and T-lymphoid thymic lymphomas and their apparent relationship to AML and mixed lineage L-ML leukemias, together with the presence of subclonal IgH and TCRβ gene rearrangements, suggests that the differentiation block in these murine haemopoietic malignancies is extremely weak and that limited (de-) differentiation can be induced following malignant transformation. Furthermore, the two Pax5 alleles are independently regulated during B-cell development, and one allele is predominantly expressed in early progenitors and mature B cells (52), indicating that the loss of the active allele in the appropriate cell is sufficient to inactivate the gene. As the maternally transmitted CBA/H allele is preferentially lost in murine thymic lymphomas (this report), AMLs and L-MLs (41), Pax5 is an excellent candidate for the TLSR5 tumour suppressor gene.

The classification of mouse haemopoietic malignancies is inconsistent, and is complicated further by differences in genetic background and carcinogen treatments. The data presented here suggest that at least some of the heterogeneity observed in studies of radiation-induced thymic lymphomas can be attributed to the fact that distinct malignancies can undergo clonal expansion in either the thymus or the spleen, and yet in the case of the thymic lymphomas, they fulfill the generally accepted criteria used to define a thymic T-cell malignancy—clonal expansion in the thymus and TCRβ gene rearrangements. The data also suggest that allelic loss at specific tumour suppressor gene loci and/or specific tumour suppressor gene inactivation is considerably more specific to a particular haemopoietic malignancy than implied by the 12 thymic lymphoma tumour suppressor gene loci reported in the literature (11–21).

Acknowledgements

Supported by the Medical Research Council and by the Leukaemia Research Fund. H.C. was supported by an MRC Research Studentship.

References


Received February 5, 2002; revised March 26, 2002; accepted April 2, 2002

Myeloid, B and T lymphoid and mixed lineage thymic lymphomas

1085