Rapid Communication

Peripheral T-Cell Lymphoma in lck<sup>pr</sup>-bcl-2 Transgenic Mice

By Gerald P. Linette, Jay L. Hess, Charles L. Sentman, and Stanley J. Korsmeyer

<sup>t</sup>(14;18) is the most common translocation in human lymphoid malignancy and results in bcl-2 overexpression. bcl-2 blocks apoptosis and constitutes the initial member of a new category of oncogenes, ie, regulators of cell death. Bcl-2 transgenic mice develop follicular hyperplasia and progress to malignant B-cell lymphoma. To assess the oncogenic potential of bcl-2 in the T-cell lineage, a cohort of 68 lck<sup>pr</sup>-bcl-2 transgenic mice and 56 control littermates were monitored for signs of malignancy over a 24-month period. Eighteen (28%) lck<sup>pr</sup>-bcl-2 mice developed diffuse, predominantly large-cell lymphomas at a mean age of 18 months.

B<sub>cl-2</sub> was discovered by cloning the t(14;18)(q32;q21) chromosomal breakpoint characteristic of human follicular B-cell lymphoma. Approximately 85% of follicular and 20% of diffuse B-cell lymphomas display the t(14;18).<sup>4</sup> bcl-2 has the unique role of extending cell survival rather than promoting cell proliferation. Transgenic mice bearing a bcl-2-Ig minigene uniformly develop a polyclonal follicular hyperplasia with a fourfold expansion of mature resting B cells.<sup>5</sup> After a substantial latency period (>9 months), approximately 15% of these transgenic mice develop monoclonal diffuse aggressive lymphomas.<sup>6</sup> The bcl-2-Ig transgenic mouse model proves that extended cell survival is oncogenic within the B-cell lineage. The long latency period, histologic conversion, and progression from poly- to monoclonality to monoclonality implicate secondary genetic changes as part of lymphomagenesis. Approximately half of the high-grade B-cell lymphomas show a c-myc translocation.<sup>6</sup> Eμ-bcl-2 transgenic mice independently derived also show an increased incidence of B-cell lymphoma, confirming the oncogenic potential of bcl-2.<sup>7</sup>

Bcl-2-deficient mice argue that bcl-2’s primary role in the lymphoid system is the maintenance of homeostasis.<sup>8,9</sup> For example, in the thymus, bcl-2 is normally upregulated at the CD4<sup>+</sup>CD8<sup>+</sup> stage during positive selection<sup>10</sup> and mature peripheral T cells retain substantial levels of bcl-2 protein. Bcl-2 homozygous-deficient mice initially show normal lymphoid differentiation; however, with time, both primary and secondary lymphoid organs undergo massive apoptosis leading to a marked lymphopenia.<sup>8,9</sup>

The present study addresses the oncogenic potential of bcl-2 beyond B cells, within the T-cell lineage. Translocations involving chromosome segment 18q21 are uncommon in human T-cell leukemia/lymphomas, and bcl-2 protein levels do not appear to be substantially increased in most T-cell neoplasms. Thus, within the context of T-cell neoplasia, the oncogenic impact of extended cell survival remains uncertain. Consequently, we undertook a longitudinal study of lck<sup>pr</sup>-bcl-2 transgenic mice that overexpress bcl-2 within the thymus as well as in peripheral T cells. We report that lck<sup>pr</sup>-bcl-2 transgenic mice show a marked incidence of peripheral T-cell lymphoma.

Materials and Methods

Mice. lck<sup>pr</sup>-bcl-2 transgenic mice bred on the (B6ScSnJ/F1) background were maintained in a pathogen-free animal facility.<sup>11</sup> Four founder lines (nos. 7, 17, 36, and 37) bearing the lck<sup>pr</sup>-bcl2 transgene were included within the tumor watch cohort. All animals were autopsied within 72 hours of any signs of ill health or at 24 months of age if there were no overt signs of malignancy. Tissues were removed, fixed in 10% buffered formalin, and examined histologically.

Flow cytometry. Single-cell suspensions were prepared from lymphoid organs and stained with fluorescent-conjugated monoclonal antibodies (MoAbs) as described.<sup>10</sup> Cells were analyzed using a FACScan with CellQuest software (Becton Dickinson, Mountain View, CA).

Southern blot analysis. Tumor DNA (10 μg) was digested with either HpaI or EcoRI, run on 0.7% agarose gels, and transferred to a nylon membrane. Membranes were hybridized with <sup>32</sup>P-labeled DNA probes and washed and autoradiograms were obtained with Kodak XAR film (Eastman Kodak, Rochester, NY) at −70°C as described.<sup>14</sup> A 0.75-kb EcoRI fragment containing T-cell receptor (TCR) C<sub>γ</sub> sequence and a 6.0-kb BamHI/HindIII fragment containing J<sub>μ</sub> Ig sequences were used as DNA probes.<sup>14</sup>

Western blot analysis. Splenic T cells from mice were purified (>95% CD<sup>3+</sup>) by negative selection using MoAbs and magnetic beads.<sup>10</sup> Western blot analysis of 35 μg cytoplasmic protein per lane was performed using the bcl-2 species-specific (6C8, antihuman bcl-2) MoAbs as described.<sup>11,15</sup> Blots were developed by ECL (Amersham, Arlington Heights, IL).

Results

lck<sup>pr</sup>-bcl-2 transgenic mice express (human) bcl-2 in cortical and medullary thymocytes as well as in peripheral T cells. As previously noted,<sup>12</sup> lck<sup>pr</sup> transgene expression is partially downregulated during T-cell maturation, as indicated by a lower fluorescence intensity of mature peripheral T cells (versus thymocytes) using the 6C8 antibody specific.
for human bcl-2 (Fig 1). Peripheral T cells from lck\textsuperscript{p5}-bcl-2 mice retain substantially higher levels of bcl-2 when compared with T cells from Bcl-2\textsuperscript{+/+} animals, as determined by Western blot (Fig 1, inset). Neither B cells nor other hematopoietic lineages express detectable transgenic bcl-2 protein, consistent with the lineage-restricted expression of the lck promoter.\textsuperscript{15} Thymocytes and peripheral T cells from lck\textsuperscript{p5}-bcl-2 mice are resistant to glucocorticoid-, b-irradiation-, and anti-CD3-induced cell death.\textsuperscript{11} At 12 months of age, lck\textsuperscript{p5}-bcl-2 animals have a threefold to fourfold increase in mature T cells, as monitored by absolute splenic T-cell counts (lck\textsuperscript{p5}-bcl-2 [n = 8], 83 ± 20 × 10\textsuperscript{6} compared with control littermates [n = 4], 25 ± 2.1 × 10\textsuperscript{6}). To assess the oncogenic potential of bcl-2 within the T-cell lineage, a cohort of 68 lck\textsuperscript{p5}-bcl-2 transgenic mice and 56 control (non-transgenic) littermates were monitored for signs of adenopathy and ill health for up to 24 months of age. Of 68 transgenic mice, 18 developed lymphoma at a mean age of 18 months (range, 12 to 23 months; Fig 2). In contrast, only 1 of 56 control littermates developed lymphoma at 19 months of age. In addition, 4 additional tumors were found within the entire tumor watch cohort (2 hepatocellular carcinomas, 1 high-grade sarcoma, and 1 intraductal breast carcinoma; Table 1). No latent tumors were found when healthy animals that survived to 24 months of age were killed.

Histologic analysis shows that most (15 of 18) lymphomas from lck\textsuperscript{p5}-bcl-2 mice were large-cell lymphomas (Fig 3A), with several showing immunoblastic features (Fig 3B). The remaining 3 were classified as diffuse small lymphocytic lymphomas (Fig 3C through E). The single lymphoma discovered in the control group was a diffuse large-cell lymphoma obtained from a mediastinal node. Most cases presented as a rapidly expanding abdominal mass within the mesenteric lymph nodes; however, there were several instances in which a mediastinal or cervical node appeared to be the primary site. The diffuse nature of lymphomas from lck\textsuperscript{p5}-bcl-2 mice is reflected in the frequent finding of splenomegaly, ascites, and extranodal involvement (liver, testis, skeletal muscle, and lung) evident in most animals (Fig 3D and E).

Lymphomas were further characterized by flow cytometry and antigen receptor rearrangements for lineage classification. The T-cell origin of all lymphomas studied was apparent by intense staining with anti-CD3 antibody as well as an absence of reactivity with the B-cell-specific antibody to B220 (data not shown). Further analysis indicated that these neoplasms were predominantly of the CD4\textsuperscript{+}CD8\textsuperscript{+} subset; in fact, only 1 tumor (7-16) was phenotyped as CD4\textsuperscript{+}CD8\textsuperscript{+} (Table 1). In addition, the lymphomas were judged to be clonal, as determined by TCR \(\beta\) rearrangement on Southern blot analysis (Fig 4). Ig heavy chain genes remained in the germline configuration, consistent with their proposed T-cell origin (data not shown). No c-myc gene rearrangements were noted among the T-cell lymphomas tested (Table 1).

**DISCUSSION**

Molecular cloning of the t(14;18)(q32;q21) chromosomal breakpoint showed a novel proto-oncogene, bcl-2.\textsuperscript{16} bcl-2 is the founding member of a new family of proteins dedicated to regulating programmed cell death.\textsuperscript{16} bcl-2-Ig transgenic mice uniformly display follicular hyperplasia due to an accumulation of resting peripheral IgM\textsuperscript{+}IgD\textsuperscript{+} B cells. After a substantial latency period, bcl-2-Ig mice develop diffuse large-cell, often immunoblastic lymphoma recapitulating the natural history of the human disease.\textsuperscript{17} Thus, violation of homeostasis through the repression of cell death can be a primary oncogenic event in B cells.\textsuperscript{17} The long latency period and histologic conversion to large-cell lymphoma evident in...
Table 1. Summary of Tumors

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Age (mo)</th>
<th>Tumor Site</th>
<th>Phenotype*</th>
<th>TCR</th>
<th>Jm</th>
<th>myc</th>
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<tr>
<td>lckP'-bcl-2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>17-1-5-1-20</td>
<td>14</td>
<td>SLL</td>
<td>MedLN</td>
<td>CD4</td>
<td>C</td>
<td>G</td>
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<tr>
<td>36-1-3-5-2</td>
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<td>SPL</td>
<td>CD4</td>
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<td>SLL</td>
<td>MedLN</td>
<td>CD4</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>36-23-35</td>
<td>16</td>
<td>Mixed LCL</td>
<td>CLN</td>
<td>CD4</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>36-1-6</td>
<td>18</td>
<td>Mixed LCL</td>
<td>MedLN</td>
<td>CD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-1-5-1-2-6</td>
<td>14</td>
<td>Mixed LCL</td>
<td>MLN</td>
<td>CD4</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>17-1-5</td>
<td>17</td>
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<td>MLN</td>
<td>CD4</td>
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<tr>
<td>7-16</td>
<td>19</td>
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<tr>
<td>37-7</td>
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<tr>
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<tr>
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<tr>
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<td>MedLN</td>
<td>CD4</td>
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<tr>
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<td>MLN</td>
<td>CD4</td>
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</tr>
<tr>
<td>17-1-15-5</td>
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<td>MLN</td>
<td>CD4</td>
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<td>G</td>
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<tr>
<td>36-1-3-5-2-1-12</td>
<td>10</td>
<td>Hepatocellular carcinoma</td>
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<td></td>
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<tr>
<td>17-1-16</td>
<td>16</td>
<td>High-grade sarcoma</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Nontransgenic controls
| 17-13-30 | 20 | LCL | MedLN | CD4 | | |
| 36-1-3-5-5-19 | 19 | Hepatocellular carcinoma | | | |
| 17-1-5-5-1-5 | 14 | Intraductal breast carcinoma | | | |

The founder line is designated by the first number in the series of each mouse.

Abbreviations: LCL-I, large-cell lymphoma, immunoblastic; LCL, large-cell lymphoma; SLL, small lymphocytic lymphoma; MLN, mesenteric lymph node; MedLN, mediastinal lymph node; CLN, cervical lymph node; spl, spleen; C, clonal rearrangement present; G, germline configuration.

*Lymphomas were classified by flow cytometry as belonging to either the CD4 T-cell subset (CD3+CD4'CD8-) or CD8 T-cell subset (CD3+CD4-CD8'). In addition, lymphomas from lckP'-bcl-2 mice stained positive with 6C8 MoAb (antihuman bcl-2) in Western blotting assays and flow cytometry.

the transgenic mouse model (as well as in human lymphoma) implicate secondary somatic changes required for tumorigenesis. Indeed, deregulated c-myc expression is frequently found in malignant B-cell lymphomas from these transgenic mice. The present study, we sought to determine the oncogenic potential of bcl-2 in a different lineage. We find that mice bearing the lckP'-bcl-2 transgene develop T-cell hyperplasia followed by diffuse malignant T-cell lymphoma, indicating that repression of cell death can be a primary mechanism of tumorigenesis in this lineage as well.

In many respects, lymphomas from lckP'-bcl-2 mice are similar to the B-cell lymphomas previously described from bcl-2-Ig mice. In general, these malignancies are best classified as diffuse, large-cell non-Hodgkin's lymphomas. In the current study, 15 of the 18 neoplasms from lckP'-bcl-2 mice were either large-cell or mixed small lymphocytic and large-cell lymphomas. The large-cell lymphomas showed a range of histologic appearances with some showing a vaguely monocytoid (Fig 3A) or histiocytic appearance with pale cytoplasm similar to that seen in human peripheral T-cell lymphomas. Two other lymphomas showed a high mitotic rate with features best classified as immunoblastic type (Fig 3B). Phenotypically, both B- and T-cell lymphomas appear to be derived from mature peripheral lymphocytes that accumulate in the secondary lymphoid organs within either transgenic line. Interestingly, the mesenteric nodes are the most common primary site of involvement. A second feature is the substantial latency period required for histologic conversion and transformation. However, it appears that T-cell lymphomas require a somewhat longer latency period because the mean age at diagnosis was 18 months (range, 12 to 23 months); in contrast, the mean age of bcl-2-Ig mice harboring malignant lymphoma was 13.5 months (range, 7 to 21 months). Perhaps this finding reflects an inherent difference of B cells when confronted with somatic changes that alter cellular proliferation. For example, c-myc and cyclin D1 expression is frequent gain of function mutations that result in a proliferative advantage within B-cell lymphomas. In our study, we were unable to find any evidence of c-myc or cyclin D1 overexpression in T-cell lymphomas from lckP'-bcl-2 mice (data not shown). Thus, it appears that secondary genetic alterations that accompany lymphomagenesis are not necessarily common to B and T cells.

It is clear that disruption of apoptotic pathways either through gain of function (ie, bcl-2) or loss of function mutations (ie, p53) can have a profound impact on cancer susceptibility. Recent evidence suggests that p53 is a required component of an apoptotic pathway used in response to DNA damage.25-27 p53-deficient mice are predisposed to malig-
Fig 3. Histopathology of Ick<sup>Δ</sup>-bcl-2 lymphomas. Formalin-fixed paraffin sections were stained with hematoxylin and eosin and examined microscopically. (A) 17-13-10: Large-cell lymphoma from a 22-month-old transgenic mouse with a mediastinal mass approximately 1 cm in diameter associated with invasion of the mainstem bronchus and skeletal muscle of the chest wall. Original magnification × 670. (B) 17-1-15-5: Large-cell immunoblastic lymphoma from a 18-month-old transgenic mouse with abdominal distention, ascites, splenomegaly, and extensive adenopathy involving the mesenteric and mediastinal nodes. Numerous mitotic figures are present in a representative section of the mesenteric node. Examination of tissue sections from the spleen, liver, and testis also showed lymphoma, predominantly large-cell type. Original magnification × 670. (C) 17-1-5-39: Small lymphocytic lymphoma from a 20-month-old transgenic mouse found to be tachypneic. On autopsy, splenomegaly and extensive mediastinal adenopathy were noted. Original magnification × 670. (D) 17-1-5-39: Invasion of the liver parenchyma by small lymphocytic lymphoma taken from the case described above. Note the extensive infiltration and complete effacement of hepatocytes by lymphoma. Original magnification × 134. (E) 17-1-5-39: Invasion of lung by small lymphocytic lymphoma from the case described above. Original magnification × 134.
I~k~"bcl-2 of through 12Kb- nancy. most notably T-cell In this study, en-
eral T-cell lymphoma. Thus, increasing the resistance to
oncogenic to long-lived lymphocytes due to their capacity
death programs are repressed, extended cell survival clearly
of function study with bcl-2 argues that, when apoptotic
susceptible to genetic alterations that may normally lead
to re-enter the cell cycle with antigen stimulation. This gain
helpful discussions. We acknowledge Lynn White, Pam Coda. and

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