A Murine Model for B-Cell Lymphomagenesis in Immunocompromised Hosts: c-myc-rearranged B-Cell Lines with a Premalignant Phenotype

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ABSTRACT

Activation of c-myc or bcl-2 protooncogene is a common event in B-cell lymphomagenesis. Alone, each is insufficient to produce lymphoma, prompting the search for the additional steps required to complete the malignant phenotype. Among the existing systems of murine or human B-cell neoplasia, no commonly occurring complementary oncogenic activation has been found. This study introduces a new series of murine B-cell lines with a phenotype suggesting that such additional events might not involve intrinsic growth control, but instead host immune mechanisms which normally suppress tumorigenicity of premalignant B-cells. Four murine B-cell lines were isolated from the long-term culture of normal lymphoid tissue bearing a premalignant phenotype. (a) Their phenotype resembled naturally occurring lymphoid tumors of immunocompromised hosts with regard to c-myc activation, aberrant or absent immunoglobulin expression, preferential rearrangement of the \( \lambda \) light chain locus, and a distinctive pattern of tissue invasion and tumor histology. (b) Their tumorigenicity was dependent on host permissiveness correlated with immunodeficiency status: C.B-17-scid > BALB/c-nu/nu > normal BALB/c > other H-2" strains (NZB x NZW F1, NZB, DBA/2). (c) Host passage selected for malignant variants distinguished by a 10-fold increase in tumorigenicity (as judged by limiting cell dose) and by novel tumorigenicity in nonpermissive syngeneic hosts. These features are analogous to properties of human lymphomas arising in immunocompromised states and, to our knowledge, unique among previously reported murine B-cell lines.

INTRODUCTION

The etiology of B-cell lymphoma has frequently been conceived as a dysregulation of normal, differentiation-specific events (1). A major advance in the understanding of natural B-cell lymphomagenesis has been the recognition of characteristic cytogenetic abnormalities (2), particularly chromosomal translocations involving juxtaposition of specific oncogenes with the immunoglobulin locus (3). The correlation of specific oncogenes (e.g., c-myc and bcl-2) with particular classes of lymphoma (Burkitt's lymphoma and follicular lymphoma, respectively) suggests that these activated oncogenes facilitate neoplastic growth through mechanisms which are specific to the B-cell differentiative stage. The t(14;18) breakpoints in human follicular lymphoma are consistent with an aberrant VDJ rearrangement (4), indicating that the initial transformation event developmentally precedes the manifestation of the malignant phenotype. Therefore, initial transformation is stage specific (1) and requires a prolonged interval for the occurrence of additional transforming events (3).

Similar conclusions may be drawn from studies involving the experimental introduction of various activated oncogenes into defined target cell populations using viral vectors or transgenic approaches (e.g., Refs. 5-11). These oncogenes initially elicit a premalignant state involving polyclonal expansion of characteristic B-cell subpopulations (8-11). As in spontaneous lymphomagenesis, a subsequent latent period is required before a monoclonal, fully malignant population arises.

What is the nature of these additional transforming events in lymphoid neoplasia? An important clue is the remarkable elevation in the incidence of B-cell lymphoma associated with immunodeficiency in the setting of certain genetic disorders (12, 13), viral infections (14-17), pharmacological events (18-21), and advancing age (22). Notably, the onset of B-cell neoplasia can occur abruptly following loss of immune function and rapidly remit when normal immune status is restored (23). It seems unlikely that the increased incidence of lymphoma involves unique transforming events, since the oncogenes identified when studied in normal and immunocompromised settings are the same (13, 14). Instead, accelerated lymphomagenesis may be due to the absence of host immune mechanisms critical to the suppression of premalignant cells, thereby obviating the necessity for additional events.

A second clue involves the CD5 B-cell lineage (24). Oligoclonal expansion of premalignant Ly-1 B-cells is also a common feature in aged animals and in certain genetic backgrounds with accelerated lymphomagenesis (25). An analogous CD5+ B-cell population is observed in humans, which is also distinguished by its remarkable prevalence in chronic lymphocytic leukemia (26). These observations suggest that this B-cell subpopulation comprises a differentiative state particularly prone to development of a successful malignant phenotype.

Our laboratory has successfully established a series of long-term Ly-1 (CD5) B-cell lines from the in vitro culture of normal murine lymphoid tissue (27, 28). We hypothesized that, since these cell lines were selected for immortal growth in vitro, they would bear common transformation events. Since they had not yet encountered in vivo selection, they would not manifest additional characteristics necessary to evade host defenses. Therefore, the cell lines should be either nonmalignant or malignant only in hosts with relevant immune deficits. The present study demonstrates that most of these cell lines possess an activated c-myc locus and are fully malignant in immunocompromised but not immune competent hosts. These cell lines thus phenotypically and operationally are premalignant, and they offer a new system to dissect the physiologically relevant host immune mechanisms which may normally suppress malignant progression.

MATERIALS AND METHODS

Cell Lines. AJ9 is a cloned CD5+ B-cell line derived from T-depleted male A/J splenic lymphocytes; it has been previously described and characterized (27, 28). BD1L-1 and BD1L-2 are cloned cell lines established by long-term cultures of splenic lymphocytes from 6- to 8-wk

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female BALB/c mice as described (Footnote 5; Ref. 28). DAC-1 and DAC-2 are cloned cell lines derived from T-cell depleted BWF₁ lymphocytes as described (Footnote 6; Ref. 28). Cells were passaged in vitro in growth medium consisting of RPMI 1640, 10% fetal calf serum (HyClone, Logan, UT), 2 mM L-glutamine, 5 x 10⁻³ M 2-mercaptoethanol (Sigma, St. Louis, MO), and antibiotics (penicillin, 50 units/ml; streptomycin, 50 μg/ml).

Animals. Female BALB/cJ, male NZB, male NZW, female DBA/2, and male and female BWF₁ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Female CBA/Ca mice were obtained from Bantin and Kingman (San Rafael, CA). They were maintained at the UCLA Center for Health Sciences Vivarium. C.B-17-scid and BALB/c-nu/nu (nu) were maintained in a sterile vivarium unit at UCLA/Warren Hall. All aspects of animal care and experimentation were approved by the UCLA Chancellor’s Animal Research Committee and were in accordance with current guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Tumorigenicity. Cells obtained from log-phase cultures were suspended in sterile phosphate-buffered saline and injected into recipient groups (5 to 10 mice/group; recipient age, 7 to 14 wk) by the specified route (i.v., i.p.) and cell number. In some cases, recipients were irradiated (450 to 750 cGy) 2 to 4 h before receiving the injection. For progression studies, tumor tissue was gently teased between frosted slides, washed once in RPMI 1640, and either injected directly into animals or cultured overnight in growth medium.

RESULTS

The B-Cell Lines Phenotypically Are Similar to Lymphoid Tumors in Immunocompromised Hosts. We recently reported a detailed characterization of the molecular and antigenic phenotype of the murine B-cell lines used in this study. We were impressed by the phenotypic similarity of these cell lines to the B-cell neoplasms observed in certain immunocompromised hosts, particularly in the setting of cyclosporine therapy (18–21) and AIDS (14). A summary of this comparison is shown in Table 1.

In humans, lymphomas in the immunocompromised setting typically involve c-myc translocation to an immunoglobulin locus (13, 14, 32). Markedly elevated levels of c-myc transcripts have been detected in conjunction with a tandemly amplified c-myc locus in an initial series of CD5+ cell lines developed in our laboratory (e.g., AJ9; Ref. 33). Southern blot analysis of the c-myc locus in the more recently obtained cell lines generally revealed rearrangements of a single c-myc allele (Fig. 1A). The c-myc restriction fragments differed in size from immunoglobulin-rearranged bands, but c-myc rearrangements to the immunoglobulin locus, particularly variant translocations, can occur at distant sites not detectable by conventional Southern analysis (3). We have no direct evidence that the c-myc rearrangements in these cell lines were of this type. Northern analysis demonstrated that the c-myc rearranged alleles were usually associated with high expression levels of an aberrant, elongated c-myc transcript (Fig. 1B). No instances of bcl-2 rearrangement were detected in any of these cell lines (data not shown).

The B-Cell Lines Are Hyperdiploid. Cytological examination of all the cell lines revealed prominent nuclear and overall cell dimensions, suggesting the presence of hyperdiploidy. By karyotypic analysis, all cell lines were hyperdiploid: DAC-1, 62 ± 5.0; DAC-2, 71 ± 2.1; BDL-2, 64 ± 4.6; and AJ9, 52 ± 0.5 chromosomes. A detailed cytogenetic analysis of AJ9 (Fig. 2) demonstrated the presence of numerous marker chromosomes, chromosomal deletions, and duplications; notably, double minute chromosomes were absent. In situ chromosomal hybridization revealed that the amplified c-myc locus in this cell line was associated with a predominant marker chromosome localization.

The B-Cell Lines Are More Rapidly and Completely Malignant in Immunocompromised Hosts. Five representative cell lines (AJ9, BDL-1, BDL-2, DAC-1, and DAC-2) were tested for their tumorigenicity in hosts ranging from normal to deficient in immune function: untreated syngeneic; irradiated syngeneic; BALB/c-nu/nu; and C.B-17-scid. Cells were inoculated either i.v. or i.p. at doses ranging from 1 x 10⁶ to 2 x 10⁷ cells
Generally, the i.p. versus the i.v. route resulted in much more rapid outgrowth (e.g., for C.B.-17-scid hosts, DAC-1, 24 versus 70 days; DAC-2, 20 versus 62 days) and in some cases a dramatic increase in tumor take (DAC-1, 100% versus 35%). This may reflect the fact that the peritoneum is particularly favorable for localization and expansion of normal and transformed Ly-1 B-cells (24-28). Increasing the dose of i.v.-injected DAC-1 cells in scid mice to 2 x 10^7 cells did not change the frequency of tumor take (data not shown). Nude mice were significantly less susceptible than were C.B.-17-scid to in vivo growth of cell lines, measured by increased tumor latency (BDL-2, 18 versus 40; DAC-2, 20 versus 27 days) and a significant decrease in tumor take (DAC-1, 100% versus 44%; DAC-2, 100% versus 33%).

In contrast, BWF1 mice were resistant to the tumorigenicity of all cell lines regardless of dose (1 x 10^6 to 2 x 10^7), route (i.p., i.v.), irradiation (450 or 750 cGy), sex, or age of recipient (7, 10, 20 wk old). The last two parameters were tested in case the hereditary autoimmune disease that increases with age and is accelerated in female animals influenced tumorigenicity in these hosts.

The BALB/c-derived cell lines, BDL-1 and BDL-2, were tumorigenic in syngeneic hosts, but with a prolonged latency compared with irradiated BALB/c +/+ , nu/nu, or C.B.-17-scid hosts (Table 2). Irradiation of syngeneic mice greatly increased the rate of BDL-2, but not BDL-1 outgrowth. These results may have reflected either the increased malignant potential of the BDL versus DAC cell lines or the decreased resistance of BALB/c versus BWF1. This was a critical issue, since both the nu/nu and scid hosts used in these experiments derived from a BALB/c genetic background. Therefore, the BWF1-derived cell lines, DAC-1 and DAC-2, were tested for tumorigenicity in BALB/c mice (Table 2). While they were nonmalignant when inoculated i.v., they were significantly tumorigenic when inoculated i.p., although much less so than in nude or scid hosts. This indicated a relative deficiency in host resistance of BALB/c versus BWF1.

This phenotype was distinct to BALB/c, since other H-2d strains generally did not generally allow the outgrowth of these cell lines (Table 4). We conclude that tumorigenicity of the B-cell lines correlated with host immune status. However, other strain-specific mechanisms, possibly independent of specific immune function, may also be critical to the host resistance (34).

The Pattern of Tissue Invasion and Morphology Is Similar to the Lymphoma of Immunocompromised Hosts. The pattern of tissue involvement and cytology of B-cell line tumors was influenced by cell type, recipient, mode of inoculation, and dose. Representative histology is shown in Fig. 3.

All four cell lines had cytological features most consistent with immunoblastic sarcoma by the Pattengale-Taylor classification (12), the most common lymphoma in immunocompromised hosts (Table 1). Following i.v. inoculation, intrasplenic growth was most prominent for all the cell lines and appeared to initially localize to the marginal zone of the white pulp. BDL-1 and BDL-2 but not DAC-1 and DAC-2 consistently involved peripheral lymph nodes. The liver was involved with nodular lymphoproliferation arising in portal areas in all cases except BDL-1 (i.v., normal syngeneic). Involvement of blood and bone marrow was only detected following i.v. injection of BDL-1 and BDL-2. Other extralymphoid sites of lymphomatous growth (lung, kidney, brain) were in all cases uninvolved.

Intraperitoneal tumor and ascites occurred in all cases of i.p. inoculation. DAC-1 and DAC-2 typically produced single large
immunocytomas attached by a delicate stalk to the peritoneal wall. In contrast, BDL-1 and BDL-2 grew as innumerable small peritoneal nodules. Extraperitoneal involvement was uncommon except for BDL-2 which commonly invaded the spleen.

The B-Cell Lines Are Uniformly Malignant. Limiting numbers of BDL-2 cells were inoculated i.v. into untreated syngeneic recipients (Fig. 4). Below $1 \times 10^6$ cells, there was a dose-dependent reduction in the rate of onset of malignant disease, and no tumorigenicity was detected below an inoculum of 1000 cells. Limiting numbers of DAC-1 and DAC-2 cells were also tested for tumorigenicity in BALB/c +/+ and nu/nu recipients. Results were similar for all combinations of cells and recipients, so only the data for DAC-1 in BALB/c +/+ are shown (Fig. 4). DAC-1 was almost uniformly malignant at $1 \times 10^7$ cells but nontumorigenic at $1 \times 10^5$ cells. Tumor formation was undetectable in BWF$_1$ recipients at all doses ($<2 \times 10^7$ cells), again reflecting the marked permissiveness of BALB/c compared with BWF$_1$ mice.

The inefficient tumorigenicity of DAC cells suggested that malignant growth may be due to rare tumorigenic cells (i.e., $<1/1000$) preexisting in the starting population. To evaluate this possibility, tumorigenicity was tested in a series of BDL-2 and DAC-2 subclones. If tumor formation were due to rare preexisting tumorigenic cells, then only $\sim 1$ of $10^3$ or 1 of $10^4$ clones of BDL-2 and DAC-2 was expected to be tumorigenic, respectively. However, all subclones of BDL-2 and DAC-2 were equally tumorigenic when tested in BALB/c (6 BDL-2 subclones, 3 DAC-2 subclones; data not shown).

Malignant Progression of Host-passaged c-myc B-Cell Lines. The B-cell lines were initially derived on the basis of sponta-
The indicated recipients were given injections of $1 \times 10^6$ (or in the case of BWF1, 5 to $10 \times 10^6$) cells, then monitored for up to 120 days, and sacrificed when moribund.

**Table 2** B-cell line tumorigenicity

<table>
<thead>
<tr>
<th>Cell line and strain</th>
<th>Route</th>
<th>BWF1</th>
<th>BALB/c</th>
<th>BALB/c-nu/nu</th>
<th>C.B-17-Scid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>Irradiated</td>
<td>Untreated</td>
<td>Irradiated</td>
</tr>
<tr>
<td>BDL-1 (BALB/c)</td>
<td>i.p.</td>
<td>NM* (0/5)</td>
<td>ND</td>
<td>79 ± 14* (3/5)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>ND</td>
<td>ND</td>
<td>75 ± 25 (10/14)</td>
<td>78 ± 21 (11/14)</td>
</tr>
<tr>
<td>BDL-2 (BALB/c)</td>
<td>i.p.</td>
<td>NM (0/5)</td>
<td>ND</td>
<td>68 ± 19 (5/11)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>ND</td>
<td>ND</td>
<td>32 ± 9.2 (19/20)</td>
<td>19 ± 1.8 (19/19)</td>
</tr>
<tr>
<td>DAC-1 (BWF1)</td>
<td>i.p.</td>
<td>NM (0/20)</td>
<td>NM (0/5)</td>
<td>32 ± 7.4 (9/32)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>NM (0/30)</td>
<td>NM (0/15)</td>
<td>45 (1/10)</td>
<td>ND</td>
</tr>
<tr>
<td>DAC-2 (BWF1)</td>
<td>i.p.</td>
<td>NM (0/20)</td>
<td>NM (0/10)</td>
<td>34 ± 11 (5/19)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>NM (0/30)</td>
<td>NM (0/5)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* NM, not malignant; ND, not determined.
* Number of tumors/number of animals.
* Median ± SD of day of survival.

**Table 3** Pattern of tissue infiltration following i.v. inoculation

<table>
<thead>
<tr>
<th>Host</th>
<th>BALB/c untreated</th>
<th>BALB/c irradiated</th>
<th>C.B-17-Scid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>Control</td>
<td>0.25 ± 0.06*</td>
<td>1.25 ± 0.15</td>
<td>0.045 ± 0.03</td>
</tr>
<tr>
<td>BDL-1</td>
<td>0.41 ± 0.23</td>
<td>1.55 ± 0.41</td>
<td>0.43 ± 0.35</td>
</tr>
<tr>
<td>BDL-2</td>
<td>1.40 ± 0.55</td>
<td>3.40 ± 0.12</td>
<td>0.75 ± 0.21</td>
</tr>
<tr>
<td>DAC-1</td>
<td>0.19 ± 0.10</td>
<td>0.75 ± 0.16</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>DAC-2</td>
<td>0.19 ± 0.10</td>
<td>0.75 ± 0.16</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD of weight (g).

**Table 4** BALB/c mice and unique sensitivity to B-cell line tumorigenicity

<table>
<thead>
<tr>
<th>Host</th>
<th>BALB/c</th>
<th>BWF1</th>
<th>NZB</th>
<th>DBA/2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^6 cells</td>
<td>10^6 cells</td>
<td>10^6 cells</td>
<td>10^6 cells</td>
</tr>
<tr>
<td>BDL-2</td>
<td>3/5</td>
<td>2/10</td>
<td>ND*</td>
<td>0/5</td>
</tr>
<tr>
<td>DAC-1</td>
<td>4/5</td>
<td>3/10</td>
<td>0/10</td>
<td>0/5</td>
</tr>
<tr>
<td>DAC-2</td>
<td>3/5</td>
<td>1/10</td>
<td>0/20</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* ND, not determined.
* One animal found with a tumor at autopsy 95 days postinoculation.

**DISCUSSION**

To our knowledge, this is the first experimental system in which a series of spontaneous in vitro-derived B-cell lines have been isolated from normal murine lymphoid tissue with an immune-dependent, premalignant phenotype. Here, we discuss evidence supporting this conclusion and the host immune mechanisms which may be relevant to malignant progression in this system.

The Phenotype and Functional Properties of the B-Cell Lines Correspond to the Features of Premalignant Cells. Several features of these cell lines reflect properties of the incompletely malignant c-myc B-cells common to immunodeficient hosts. (a) They are analogous to B-lymphomas of immunocompromised hosts with regard to activation of their c-myc oncogene, aberrant or absent immunoglobulin, preferential rearrangement of the λ light chain locus, and pattern of tissue invasion and tumor histology. (b) They are more rapidly and completely tumorigenic in BWF1 mice (Table 3) and cells that were recovered after host passage were tumorigenic with a reduced mean latency (e.g., at 1 x 10^6 cells, 42 versus 83 days) but with an insignificant difference in limiting tumor dose (Fig. 4). In contrast, host passage of parental DAC-1 cells in BALB/c mice only modestly increased their tumorigenicity in BALB/c (Table 5). In fact, host passage of parental DAC-1 cells in BALB/c mice only slightly increased compared with parental DAC clones (Table 5). In contrast, host passage of parental DAC-1 cells in BALB/c mice only modestly increased compared with parental DAC clones (Table 5). Finally, titration of MV-1 into BALB/c revealed a limiting tumorigenic dose ~10^5 lower than the parent DAC-1 cell line (Fig. 4). Therefore, DAC-1 malignant progression required selection by a fully nonpermissive host.

MV-1 and MV-2 tumors, but not MV-3, were moderately tumorigenic in BWF1 mice (Table 5). Tumorigenicity of MV-1 was unchanged following serial passage in BWF1 (data not shown) or after prolonged (2-mo) in vitro culture. However, tumorigenicity of the MV clones in BALB/c was dramatically increased compared with parental DAC clones (Table 5). In contrast, host passage of parental DAC-1 cells in BALB/c mice only modestly increased their tumorigenicity in BALB/c (Table 5). Finally, titration of MV-1 into BALB/c revealed a limiting tumorigenic dose ~10^5 lower than the parent DAC-1 cell line (Fig. 4). Therefore, DAC-1 malignant progression required selection by a fully nonpermissive host.
to 10^4-fold increase in tumorigenicity (limiting cell dose) and by novel tumorigenicity in nonpermissive H-2^d hosts.

Other experimental systems concerning B-cell lymphomagenesis feature a population of premalignant B-cells. In particular, transgenic animals bearing activated oncogenes (c-myc or bcl-2) initially display a hyperplastic but nonmalignant B-cell population. The hyperplastic B-cells are nontumorigenic when transferred into normal hosts and lack immortal growth characteristics in vitro (8–11); occurrence of subsequent B-cell malignancy is uniform, but only after significant latency. However, these systems have thus far failed to reveal the molecular or biological basis for acquisition of a fully malignant phenotype.

Similarly, retrovirus-mediated transfer of activated c-myc or bcl-2 into normal B-lymphocytes can promote in vitro survival and growth, but does not generally result in a malignant phenotype unless subsequent activated oncogenes are introduced into these cells (35, 36). In spontaneous lymphomagenesis involving c-myc and bcl-2 activation, a substantial delay in the ultimate expression of a malignant phenotype has also been inferred (3). A similar example of naturally occurring premalignant B-cells has recently been reported in humans. The transfer of human blood lymphocytes from healthy EBV+ donors at high doses (5 x 10^7) into CB-17-scid hosts results in malignant outgrowth (37); in contrast, this lymphocyte population fails to undergo tumorigenic growth in BALB/c-nu/nu hosts.

The pattern of tissue involvement by these cell lines was notable for the peritoneum and marginal zone of the splenic white pulp. These are favored anatomical sites of localization of CD5+ B-cells (24, 25), perhaps involving a distinctive homing mechanism (38); however, areas such as the marginal zone are also important sites of nonspecific lymphatic filtration and trapping (39).

A Model for the Augmented B-Cell Neoplasia of Immunodeficient States. The present study and several of the cited examples support the hypothesis that premalignant B-cells bearing activated oncogenes (e.g., c-myc) exist in normal hosts but are not malignant due to suppressive mechanisms of the intact host immune system. Outgrowth of c-myc B-cells in the predictable consequence of environments in which relevant immune components are deficient: in hosts with certain genetic or acquired immunodeficiencies and, perhaps, in long-term tissue culture due to the attrition of the normal lymphoid milieu (a scenario for the establishment of the present cell lines). Consequently, the development of fully malignant lymphomas in immunocompetent individuals should involve the acquisition of additional transforming events which allow the cells to overcome these host obstacles. Lymphomas arising in normal versus immunocompromised settings should be distinguishable by resistance or sensitivity to relevant host mechanisms, respectively (34).

This model of lymphomagenesis is supported by studies of Burkitt's lymphoma. EBV+ lymphomas, but not conventional EBV+ lymphoblastoid cell lines, are resistant to EBV-specific cytolytic T-cells, a phenotype correlated with a selective down expression of certain major histocompatibility complex Class I genes (40) or EBV antigens (41). However, escape from viral specific cytotoxic T-lymphocytes is clearly not the exclusive mechanism of B-cell malignant progression. EBV infection is a uniform feature of B-cell hyperplasia, but not lymphoma in AIDS (42). Moreover, B-cell-transforming infections equivalent to EBV are unknown in the mouse.

This model has many features of the controversial theory of immune surveillance (43), usually presented in the context of all types of cancer. However, in the setting of immunodeficiency, B-cell neoplasia is particularly common (13, 44). Hence, while immune mechanisms are of uncertain relevance to naturally occurring cancer in general, they appear to be a critical obstacle to B-cell lymphomagenesis. This restricted notion of immune surveillance seems to merit further study.

The nature of the additional transforming events in B-cell lymphomagenesis remains ill defined. Experimentally, acceler-
Fig. 4. Tumorigenicity of limiting cell numbers of parental versus host-passaged B-cell lines. Parent or BALB/c-passaged BDL-2 were injected i.v. into untreated BALB/c; parent or BWF₁-passaged (MV-1) DAC-1 were injected i.p. into untreated BALB/c. A, effect of cell number on tumor formation: \(10^7\) (•); \(10^6\) (□); \(10^5\) (●); \(10^4\) (○); \(10^3\) (▲) cell per animal. B, tumorigenicity of parent (●) versus host-passaged (□) cell lines after 200 (BDL-2) or 60 (DAC-1) days. The fraction of tumor-free animals was calculated from A.

Table 5 Malignant progression of DAC-1 following host passage

One \(10^7\) DAC-1 cells were inoculated i.v. into BALB/c mice. Cells recovered after host passage (i.e., from tumors) were re-inoculated into 5 BALB/c or BWF₁ mice i.v., \(10^7\) per animal. MV-1 and MV-2, malignant variants of DAC-1 origin, and MV-3, a malignant variant of DAC-2 origin, were inoculated i.p. into BALB/c and BWF₁ mice in groups of 5 animals at a dose of \(1 \times 10^6\) cells per animal.

<table>
<thead>
<tr>
<th>Host passage</th>
<th>BALB/c</th>
<th>BWF₁</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency (days)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>In vitro</td>
<td>32 ± 7.4 *</td>
<td>28 (9/32)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>26 ± 2.9</td>
<td>40 (6/15)</td>
</tr>
<tr>
<td>BWF₁ (MV-1)</td>
<td>10 ± 0.5</td>
<td>100 (5/5)</td>
</tr>
<tr>
<td>NZB (MV-2)</td>
<td>18 ± 2.2</td>
<td>100 (5/5)</td>
</tr>
<tr>
<td>CBA/Ca (MV-3)</td>
<td>23 ± 1.6</td>
<td>100 (5/5)</td>
</tr>
</tbody>
</table>

* Mean ± SD.

grown lymphomas in the setting of myc activation can be produced by the additional introduction of activated ras genes (35, 45). However, no role for ras or several other complementing oncogenes has been observed in most naturally occurring lymphomas (46), with rare exceptions (47, 48). Instead of

growth regulation, it appears that other functions such as evasion of host defenses may be the locus of additional events in natural lymphogenesis. These possibilities are not mutually exclusive: the capacity for host evasion may contribute to the activation of common or novel protooncogenes by increasing the pool of cells at risk for additional genetic change. Further analysis of the present experimental system may allow dissection of the nature of these host mechanisms (34) and characterization of the corresponding molecular events which permit host evasion resulting in B-cell malignant progression.

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REFERENCES

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