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v-*abl* causes hematopoietic disease distinct from that caused by *bcr–abl*

(Abelson murine leukemia virus/bone marrow/chronic myelogenous leukemia/lymphocyte transformation)

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ABSTRACT **v-*abl*, the oncogene transduced by Abelson murine leukemia virus, was first characterized by its ability to transform lymphoid cells. *bcr–abl*, the oncogene formed by a *t*(9;22) translocation thought to occur in human hematopoietic stem cells, is detectable in almost all cases of chronic myelogenous leukemia (CML), a malignancy of granulocytic cells. *bcr–abl* also causes a CML-like syndrome in mice whose bone-marrow cells are infected with a retrovirus transducing the gene. More recent reports have suggested that v-*abl* can, however, cause a disease similar to CML. We demonstrate here that v-*abl*, when transduced in a helper virus-containing system, causes disease similar to, but distinct from, the CML-like syndrome induced by *bcr–abl*. Animals whose bone marrow has been infected by v-*abl* virus develop modest splenomegaly, marked granulocytosis, and malignant disease of several hematopoietic cell types. Unlike animals with CML-like disease resulting from *bcr–abl*, the polymorphonuclear leukocytes from animals infected with a v-*abl* construct do not contain the v-*abl* provirus at a significant frequency. Histopathologic analysis also shows significant differences between the diseases caused by v-*abl* and *bcr–abl*.**

Activated variants of the c-*abl* protooncogene code for proteins that transform a wide range of hematopoietic cells as well as other cell types. v-*abl*, the gene transduced by Abelson murine leukemia virus (A-MuLV), originated in a lymphoid tumor (1). The v-*abl* gene product is a 160-kDa tyrosine kinase (p160) whose N-terminal domain consists of MuLV core protein (*gag*) sequences; its 1003 C-terminal amino acids are homologous to c-*abl* (2). Under commonly used infection conditions, v-*abl* readily transforms B-lymphoid cells. The disease caused by intraperitoneal injection of susceptible newborn mice is characterized by a short latent period (1–3 mo), paraspinous masses of lymphoid cells, and a distinctive cranial involvement ("caput medusa"). v-*abl* can also transform fibroblasts, pre-B cells, T cells, mast cells, macrophages, and erythroid cells, but these lineages are not commonly involved *in vivo* (3). More recently, Kelliher *et al.* (4) and Chung *et al.* (5) have suggested that v-*abl* can cause a disease reminiscent of chronic myelogenous leukemia (CML).

In humans, the *t*(9;22) translocation giving rise to the Philadelphia chromosome appends 5′ exons from an unrelated cellular gene, *bcr*, to the second exon of c-*abl* and gives rise to a fusion gene, *bcr–abl*. Production of the corresponding chimeric abl protein (p210 or p190) results in the leukemias of mature granulocytes (CML) or lymphoid series cells (Philadelphia chromosome-positive acute lymphocytic leukemia, ALL) (for review, see ref. 6). Several studies have attempted to recapitulate CML-like disease in a murine model system by retroviral transduction of the *bcr–abl* gene (4, 7, 8). The malignancies induced by these protocols have varied somewhat, but a wide range of hematopoietic cell types are typically involved. Central features of the CML-like syndrome observed by Daley *et al.* (8) included massive splenic enlargement secondary to infiltration with mature granulocytes, a marked peripheral blood leukocytosis, and the presence of a *bcr–abl* provirus in the granulocytes of diseased animals. This syndrome is different from the malignancies of monocytic cells described by Kelliher *et al.* (4); these most closely resemble human histiocytic lymphomas not typically associated with the Philadelphia chromosome. More distinct still are the tumors of macrophage and erythroid derivation characterized by Elefanty *et al.* (7).

To compare the abilities of v-*abl* and *bcr–abl* to cause CML-like disease, we placed the gene in the vector described by Daley *et al.* (8), created a virus stock of high titer through use of helper virus, and employed the same infection protocol as in the previous study. We show here that v-*abl* induces a range of malignancies similar, but not identical, to those caused by *bcr–abl*.

MATERIALS AND METHODS

Viral Stocks and Infections. A 4.7-kilobase (kb) *Bam*HI fragment containing the v-*abl* sequence coding for p160 protein was subcloned into the pGD vector. The 5′ long terminal repeat of this vector is derived from Moloney MuLV; the 3′ long terminal repeat is a composite of the myeloproliferative sarcoma virus promoter and MuLV enhancer (8). The B2 mutation (9) and internally promoted *neo* markers are as described (8). The construct was cotransfected with pZAP [a M-MuLV clone; (10)] into NIH 3T3 cells, and a transformed producer cell population was isolated. Titers for both transformation (as assayed by focus formation on NIH 3T3 cells) and *neo* resistance from viral supernatants were $>10^6$ per ml.

Bone-Marrow Infections. Male BALB/c donor mice (8–12 weeks of age) were injected with 5-fluorouracil (5 mg each or ≈200 mg/kg) and sacrificed 6 days later. Marrow cells from tibia and femur were pooled, washed once with phosphate-buffered saline, and layered onto a cushion of Ficoll-Paque (Pharmacia) at a maximum of 10^7 nucleated marrow cells per ml of Ficoll solution. After centrifugation at 1500 × *g* for 30 min, the mononuclear cells at the Ficoll–phosphate-buffered saline interface were washed twice in phosphate-buffered saline. Nearly confluent virus-producing NIH 3T3 cells in 100-mm plates were killed by γ irradiation (5000 R; 1 R = 0.258 mC/kg); each plate was then overlaid with 2–5 × 10^6 mononuclear marrow cells in 10 ml of Dulbecco's modified Eagle's medium/10% calf serum/20% WEHI-3B-conditioned medium/Polybrene at 0.4 μg/ml. After 60–72 hr co-

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Abbreviations: CML, chronic myelogenous leukemia; J_H, heavy-chain joining region; A-MuLV and Mo-MuLV, Abelson and Moloney murine leukemia virus, respectively. MPSV, myeloproliferative sarcoma virus.

cultivations, nonadherent marrow cells were harvested by gently squirting the monolayer several times. Nonadherent cells thus obtained were washed once in phosphate-buffered saline and injected into lethally irradiated (two doses of 450 R γ irradiation, separated by 3–6 hr) female recipients at 1.0–2.5 $\times$ 10^5 cells per animal.

Establishment of Cell Lines. Cells from the resulting tumor masses and bone marrow of each animal were seeded in RPMI medium/20% fetal calf serum/penicillin/streptomycin/50 μM 2-mercaptoethanol at 2–5 $\times$ 10^6 cells per ml. After 4–8 days, medium was replaced, and nonadherent cells were passed 1:2 into fresh medium. The procedure was repeated until nonadherent cells became capable of growth with a doubling time of 24–48 hr.

Examination of Diseased Animals. Animals from three separate transplants (total 22 animals) were included in this study. Total and differential white blood cell counts were obtained by retroorbital bleeding of anesthestized mice at 2-week intervals beginning ≈4 weeks after transplant. One hundred percent of animals reconstituted with infected marrow developed disease. Those animals for which data is not shown died within 16 weeks of transplant but were too long dead to allow complete autopsy and analysis. For the remainder, 0.5–1.0 ml of peripheral blood was obtained at sacrifice and fractionated on Ficoll/Paque gradients as above. Cells pelleting through the gradient were >95% polymorphonuclear granulocytes, as assayed by Wright–Giemsa staining. Permanent (formalin fixed) sections were made from liver, spleen, thymus, lymph node, and any visualized tumor mass. Cells from spleen or tumor masses were cultured as described; seven cell lines from different animals were obtained.

Hybridization and Protein Studies. DNA was prepared from peripheral blood granulocytes, liver, spleen, nodes, and tumor masses per standard protocols. After digestion with specified enzymes, DNAs were blotted to nylon membranes. Cytoplasmic RNAs were also obtained from cell lines, electrophoresed, and blotted by standard techniques onto supported nitrocellulose filters (11). Gel-purified fragments used as probes included the 1.2-kb G418-resistance cassette (*Cla* I digest) from the pGD vector, a 2.5-kb c-*abl*-specific fragment (*Hin*cII digest) from pRS-2 (12), and a 2-kb mouse β-globin (*Hin*dIII–*Bam*HI) fragment excised from plasmid pUCMβ_{maj}, which contains the mouse β-globin major locus described by Konkel *et al.* (13) and was provided by M. Baron (Harvard University). Also used was a mixture of Zfx- and Zfy-specific DNA fragments [(pDP1055 and pDP1193, provided by E. M. Simpson and D. C. Page (Whitehead Institute); (14)] to determine the sex of origin of tumor cell lines. A probe containing the coding region of the murine erythropoietin receptor (15) was supplied by G. Longmore, Whitehead Institute. Probes to chicken β-actin and *Rag-1*, one of the genes central to variable-diversity joining region recombination in pre-B cells (16), were obtained from D. Schatz (Whitehead Institute). A 3.4-kb *Bam*HI–*Eco*RI genomic clone, pJH1-4, encompassing J_{1-4} of the immunoglobulin μ locus was provided by M. Schlissel (Whitehead Institute).

RESULTS

Histopathology. Twenty-two lethally irradiated animals were injected with bone marrow infected by v-*abl*-containing virus. All animals were deemed reconstituted insofar as they survived longer than 2 weeks after irradiation, but all died within 16 weeks of transplant. Data is shown for those mice sacrificed when moribund; the remainder were not well enough preserved to allow complete autopsy. Each recipient mouse examined had developed disease of multiple hematopoietic lineages.

Table 1 shows the results of peripheral blood studies and the disease histology ascertained by hematoxylin/eosin sections in individual animals. The disease latency of 4–10 weeks is comparable to that reported in previous studies using v-*abl* in a similar protocol (4) and to that observed for the *bcr–abl*-associated CML-like disease reported by Daley *et al.* (8). The mice carrying cells infected with v-*abl* under these conditions developed an abnormal peripheral blood granulocytosis suggestive of, but not by itself diagnostic of, a CML-like syndrome.

Beyond these similarities, significant differences between the diseases caused by v-*abl* and *bcr–abl* were evident. (*i*) The average white blood cell counts in our v-*abl* mice were substantially lower than in those mice that received *bcr–abl* virus by a similar protocol: the maximum of 61,000 reported here is nearly an order of magnitude lower than the maximum recorded in mice receiving marrow infected by *bcr–abl* and is comparable to the number reported by Kelliher *et al.* (4) in similar studies. (*ii*) The granulocytes of v-*abl*-infected mice were almost entirely mature cells, whereas animals with a true CML-like syndrome exhibited both mature and immature cells in their peripheral blood. (*iii*) The marked splenomegaly occurring in mice that have received p210-expressing marrow was not present in the v-*abl*-infected mice. Mean spleen weights of 0.60 ± 0.17 g noted in our previous study of *bcr–abl*-infected mice are significantly greater than the 0.30 ± 0.03 g observed here; spleen weights for untreated BALB/c mice of this age are ≈0.1 g. (*iv*) Hematoxylin/eosin staining of spleen sections from v-*abl*-infected animals revealed splenic disease very different from that occurring in *bcr–abl*-

Table 1. Histopathology of v-*abl*-infected mice

Mouse	Latency*, weeks	WBC, $\times$ $10^{-3}/\mu$l	Differential†			Spleen weight, g	Organ involvement‡			
			P	L	M		Spl	Liv	LN	EN
4/7 CN	10	36.5	90	5	4	0.15	L, M	M	+	PS
6/8 AN	6	24	64	10	16	0.28	L, M	L, M	–	T
6/8 AL-2	6	41	58	34	8	0.33	L, E	M, E	–	PS, T
6/20 AN	5	35	50	45	5	0.37	L, M, E	L, M	+	T
6/20 AR-2	4	58	68	20	12	0.35	L	L, E	+	PS, T
6/20 AL-1	4	25.5	47	45	8	0.28	L, M, E	L, M	+	–
6/20 BN	4	37	55	32	13	0.28	L, M, E	M	+	PS, T
6/20 CR-1	5	52	54	36	10	NA	L, M	L, M	+	T
6/20 CR-2	5	61	55	35	10	0.30	L, M	L	–	–
6/20 CL-1	5	39	69	17	14	0.37	L, E	L	+	PS, T

WBC, white blood cells; NA, not available.

*Time elapsed from transplant of v-*abl*-infected bone marrow to death of the animal.

†Differential WBC counts: P, polymorphonuclear granulocyte; L, lymphocyte; M, monocyte; no significant basophilia or eosinophilia were noted.

‡Spl, spleen; Liv, liver; LN, lymph node; EN, extranodal; L, lymphoid; M, monocytoid; E, erythroid; PS, paraspinous mass present; T, thymus involved.

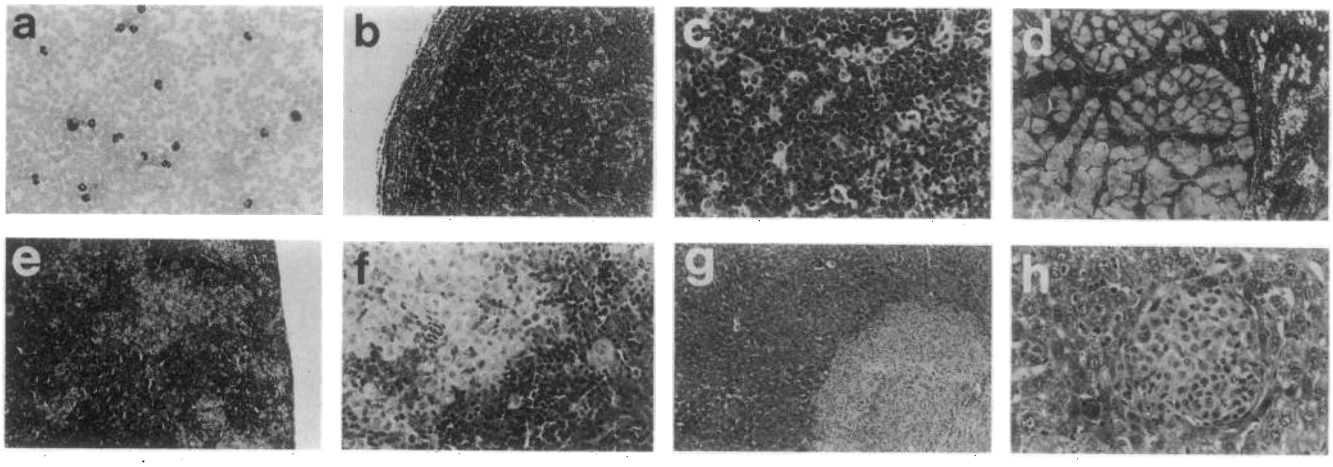

FIG. 1. Histochemical staining of tissues from a representative mouse (4/7 CN). (*a*) Wright–Giemsa stain of peripheral blood, demonstrating numerous polymorphonuclear cells. (*b*) Hematoxylin/eosin staining of lymph node (×85) showing effacement of usual architecture and capsular invasion. (*c*) Higher magnification (×350) of lymph node, showing lymphoblasts. (*d*) Cross section of paraspinous tumor mass, in which muscle bundles are invaded by lymphoma cells. (*e*) Hematoxylin/eosin staining of spleen (×85) demonstrates macrophage, erythroid, and lymphoid cells with minimal involvement by polymorphonuclear cells. (*f*) Nodule of macrophage-like cells with cells from other hematopoietic lineages present at higher magnification (×350) of spleen. (*g*) Hematoxylin/eosin staining of liver tissue; normal hepatocytes surround a nodule of macrophage-like cells. (*h*) Nodule of macrophage-like cells at higher magnification (×350) of liver tissue.

infected mice. In contrast to the complete obliteration of splenic architecture by sheets of mature granulocytes in p210/CML mice, spleens from v-*abl*-infected mice showed cells from several major lineages (erythroid, lymphoid, and macrophage) but exhibited minimal numbers of mature granulocytes. The patterns of disease also differed at other sites. Similar to previous reports, we found that ≈50% of autopsied animals bore paraspinous masses of lymphoma cells characteristic of more classical A-MuLV disease. In addition, the occurrence of lymphoid disease at other sites was nearly universal in mice receiving v-*abl*-infected marrow (Fig. 1, Table 1) but rare in mice receiving *bcr–abl*. The extent and frequency of malignant lymphoid involvement was such that it appeared to be the primary cause of death in nearly all of these animals.

Localization of Provirus. A central feature of the previously reported CML-like disease in mice was the presence of the activated *abl* genome at near single-copy number in the granulocytes of peripheral blood (8). Analysis of DNA from the Ficoll/Paque purified granulocyte population of v-*abl* mice revealed that the viral genome was undetectable in these cells. Hybridization of a *neo* probe to the *Xba* I-digested DNAs from several mice failed to detect the gene in granulocytes (Fig. 2); hybridization with a c-*abl* probe showed only endogenous c-*abl* sequences (data not shown).

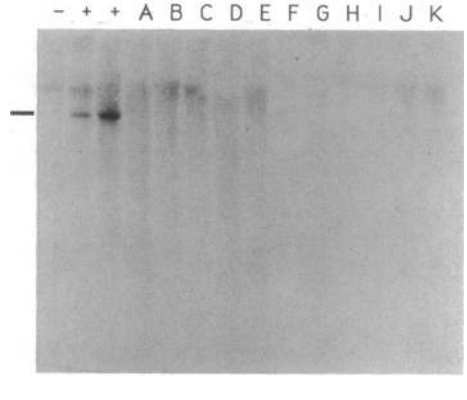

FIG. 2. *Xba* I-digested primary granulocyte DNAs (2.5–5.0 μg of samples, lanes A–K) from Ficoll-pelleted cells from peripheral blood of 11 primary animals. The DNAs have been hybridized with a *neo*-specific probe. −, Digestion of NIH 3T3 DNA; +, +, 2.5-μg and 5.0-μg samples of DNA from a cell line (GR-1) known to contain a single copy of the *neo*-resistance gene from pGD. Bar shows hybridization to the predicted *neo*-containing fragment.

Cells from other tissues, however, contained v-*abl* at a readily detectable copy number. A c-*abl* probe was hybridized to DNA from liver, spleen, and lymph node or tumor tissue in representative mice. *Eco*RI cleaved the integrated provirus in the *neo* cassette, yielding fragments the sizes of which varied depending upon integration site. *Xba* I cuts within the long terminal repeat sequences of the integrated provirus, giving a fragment independent in length of the integration site. In each case, primary lymphoid or macrophage tumor tissue contained a limited number (1–3) of integrated proviruses detected either by *neo* or *abl* probes (Fig. 3). These proviral genomes were present at significant copy number (≈0.5–1.0 copy per genome, as estimated from the ratio of intensity of integrated provirus to endogenous c-*abl* bands) in the liver, spleen, or tumor tissue of all animals tested. These data established that v-*abl* was present in the malignant cells in recipient animals.

Cell Lines. Tumor cells and bone marrow were harvested from each autopsied animal and cultured. Cell lines were established from seven independent tumors. These lines were analyzed by Wright–Giemsa staining, antibody staining for selected lineage markers, and Southern blotting to assay provirus (c-*abl* and *neo* probes), immunoglobulin heavy-chain gene configuration (J_H probe), and origin (male donor or female recipient). We also used Northern (RNA) blotting to determine lineage and immunoprecipitation with anti-abl sera to establish the presence of p160 in the cell lines. Results of these analyses are shown in Table 2.

Wright–Giemsa staining revealed different morphologies among the cell lines, suggesting transformation of lymphoid and macrophage lineages. Nonspecific esterase staining as well as staining with monoclonal antibodies to pre-B cell (B220), thymocyte (Thy 1.2), and macrophage (Mac-1) antigens largely confirmed our initial impressions. Although the majority of cultured cells were clearly of pre-B origin ($B220^+$ and positive for immunoglobulin heavy-chain rearrangement at both alleles), two Mac-1-positive cell lines and an apparently lineage-negative line were obtained. The two Mac-1-positive lines had, however, rearranged their μ loci. They are apparently similar to lines reported by Kelliher *et al.* (4) and may represent a more primitive, multipotential population of cells. The last line, although erythroid in appearance, did not clearly stain with benzidine and failed to express mRNA

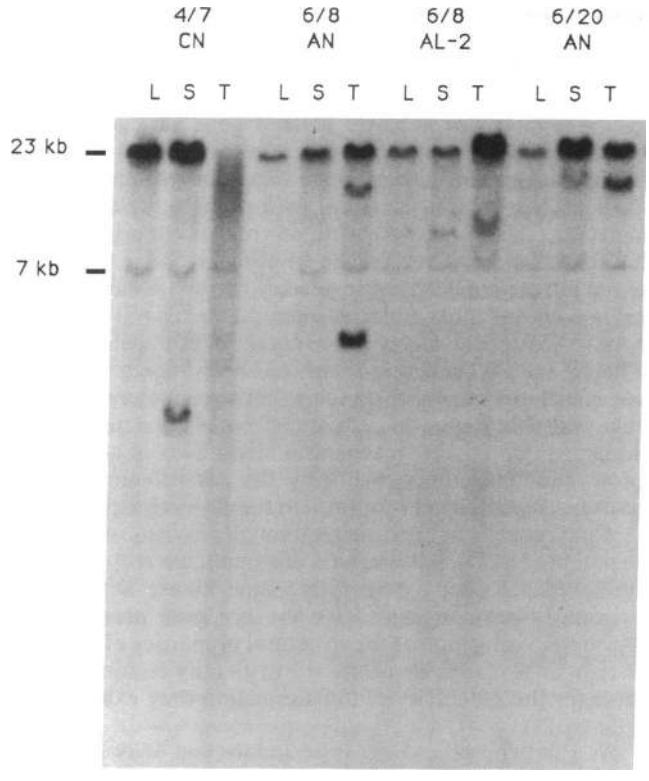

FIG. 3. *Eco*RI-digested primary DNAs from representative tissues of four mice, hybridized with a c-*abl*-specific probe. The major band at 23 kb and the minor band at 7 kb represent endogenous c-*abl* sequences, whereas other bands are virally transduced *abl* sequences. L, liver; S, spleen; T, tumor or node DNAs.

homologous to β-globin, the erythropoietin receptor, or to *Rag-1* [a gene central to variable/diversity/joining region recombination in pre-B cells (16)]. However, these cells exhibited J_H rearrangements at both alleles (Fig. 4). In four of five $B220^+$ lines, the mRNA for Rag-1 was readily detected, and both J_H alleles had undergone rearrangement, similar to the pattern predicted from previously characterized A-MuLV-transformed lines (17). All cells contained either one or two copies of provirus detected by hybridization to *neo*-specific probes, were of donor (male) origin by hybridization to Zfx/Zfy probes, and contained the characteristic v-*abl* (p160) gene product, as assayed by immunoprecipitation and autophosphorylation reactions (18).

DISCUSSION

On the basis of these and previous results, v-*abl* and *bcr–abl* apparently differ in the spectrum of hematopoietic disease they induce. The *bcr–abl* gene product likely initiates CML in humans. A CML-like syndrome has been seen in nearly half of the diseased mice transplanted in our laboratory with bone-marrow cells that contain a retrovirally transduced *bcr–abl* cDNA (8). This syndrome is characterized by massive splenic enlargement with total obliteration of splenic architecture by mature granulocytes. These animals also exhibit white blood cell counts similar to those in the human disease. Notably, the provirus coding for p210 is present at single-copy levels in these granulocytes.

v-*abl* causes a disease that differs by several criteria. (*i*) Involvement of lymph node, spleen, or paraspinous tissues by masses of lymphoblasts is nearly universal in animals transplanted with v-*abl*-infected marrow. (*ii*) Modest splenomegaly with much less extensive replacement of spleen cells by abnormal granulocytes is present. (*iii*) Mice infected with

Table 2. Characteristics of cell lines derived from tumors or bone marrow of v-*abl*-infected animals

	Antigen			mRNA		DNA
Cell line	B220*	Mac-1*	Thy-1.2	Globin†	RAG-1	μ‡
4/7 CN	+	−	−	−	−	+/+
6/8 AN	−	+	−	−	−	+/+
6/20 AN	+	−	−	−	+	+/+
6/20 AR-2	+/−	+/−	−	−	+	+/+
6/20 BN	−	−	−	−	−	+/+
6/20 BL-1	+	−	−	−	+	+/+
6/20 CL-1	+	−	−	−	+	+/+

*+/− denotes a mixed population of cells, positive for either one antigen or the other, as detected with simultaneous staining.
†Result was identical for erythropoietin-receptor mRNA.
‡−, germ-line IgM configuration; +, rearrangement as assayed by hybridization to a J_H-region probe.

a v-*abl*-containing virus also exhibit a more modest granulocytosis in peripheral blood and do not harbor the provirus at significant copy number in their granulocytes. In those tissues where provirus is detectable, histology confirms that the number of granulocytes is not sufficient to account for the observed signal. Given these findings, we believe that the granulocytes in these mice are not descended from the malignant clone and are probably not malignant cells. A potential explanation for this phenomenon is suggested by the molecular analysis of Elefanty *et al.* (7), who found that a number of their p210-transformed macrophage cell lines produced biologically active granulocyte/macrophage colony-stimulating factor. Elaboration of a granulocyte-stimulating factor by the frequently observed macrophage nodules in our v-*abl*-infected mice might well account for the peripheral blood picture described above.

Still, significant similarities in the diseases caused by v-*abl* and *bcr–abl* are notable. As described in both our work above and in previous studies by Kelliher *et al.* (4), mice infected with marrow producing either gene product can develop lymphoid and macrophage-like neoplasms. Cells from these

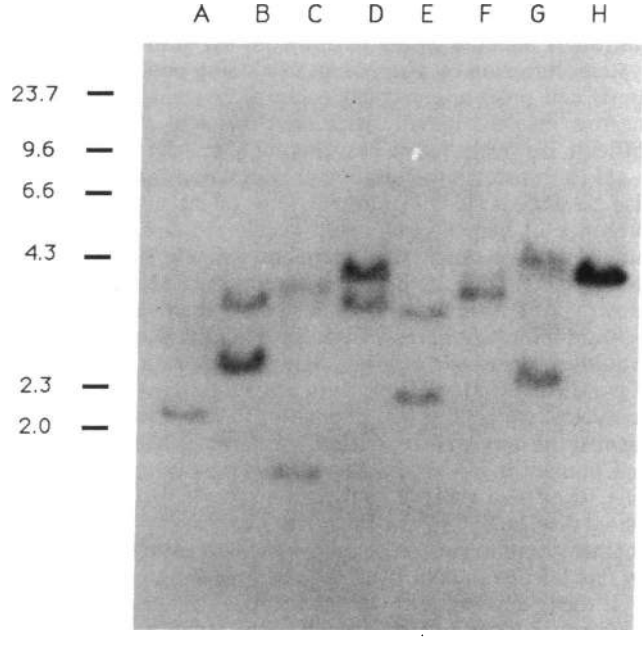

FIG. 4. *Eco*RI-digested DNAs from indicated cell lines hybridized with probes detecting J_H (μ rearrangement). Lane designations correspond to cell lines derived from the indicated mice or cell lines: A, 4/7 CN; B, 6/8 AN; C, 6/20 AR-2; D, 6/20 BN; E, 6/20 BL-1; F, 6/20 CL-1; G, GR-1, a $B220^+$ pre-B cell line transformed by p210; H, NIH 3T3 DNA (unrearranged). Numbers at left represent kb.

masses readily give rise to cell lines that produce p160 and that exhibit cell-surface markers characteristic of lymphoid or myeloid lineages. Other early cells may be involved as well, as evidenced by our isolation of one lineage-negative cell line. The presence of nests of erythroid proliferation on the histologic sections suggests, but does not prove, that involvement of the erythroid lineage is possible in this host/vector system using v-*abl*.

The phenotype of disease in animals infected by viruses containing activated c-*abl* variants is determined by a number of factors. One is the route of administration. Demonstration of a pre-B cell tumor phenotype in animals receiving A-MuLV i.v. or intraperitoneally was followed by the demonstration that the virus could, after intrathymic injection, transform T cells, even in mouse strains previously thought resistant to Abelson virus transformation (19, 20). As we have used identical infection protocols for *bcr–abl* and v-*abl* here, other explanations for the difference in phenotype must be sought. A second possibility is that infection of different target-cell populations occurred. The lymphoid precursor cell that A-MuLV transforms to yield pre-B cell tumors has been relatively well characterized (21). Hematopoietic stem cells are apparently involved in human disease (22), while preliminary studies (G.Q.D., R.A.V., and D.B., unpublished work) suggest that the murine CML target is a pluripotent cell also. Although the data above do not clearly demonstrate stem-cell infection, our isolation of Mac-1-positive cell lines that have rearranged both immunoglobulin μ alleles suggests transfer of v-*abl* to an early cell capable of differentiating along both myeloid and lymphoid pathways. As we have used viruses of similar titer under identical infection conditions in both our *bcr–abl* and v-*abl* studies, it seems likely that we are initially introducing these activated *abl* genes into similar populations of cells.

A third potentially important variable in determination of the phenotype of disease is the nature of the retroviral vector system used to introduce activated *abl* variants into marrow cells. Although the vector used in our v-*abl* and *bcr–abl* experiments is identical, helper virus was in the producer cell population used in the v-*abl* experiments but not in the previous study. This may account, at least in part, for the frequency of lymphoid disease observed in mice whose marrow is infected with v-*abl*. In contrast to the helper-free system, infection by v-*abl* of an expanding pool of maturing pre-B cell progenitors could occur after transplantation of marrow into the lethally irradiated recipient animals. It is difficult, however, to see how this could restrict the ability of v-*abl* to transform the target-cell population that could give rise to a CML-like syndrome. One might argue that the presence of helper virus could accelerate disease onset in other lineages, such that recipient animals would die of different malignancies before exhibiting the CML phenotype. However, the latency of CML-like disease in mice receiving *bcr–abl*-infected marrow was sufficiently short that one would expect to see at least some animals with that disease if v-*abl* behaved identically with respect to transformation of a myeloid progenitor cell population. Yet another argument against the importance of helper virus comes from the results of Chung *et al.* (5), who used a helper-free v-*abl* producer cell line. They also observe malignant macrophage-like and lymphoid disease but do not document massive expansion of the mature granulocyte compartment or the presence of provirus in this lineage *in vivo*.

If these explanations do not account for the difference in diseases induced by *bcr–abl* and v-*abl*, structural differences between the two proteins are a potential explanation. Several structural features determining the transforming properties of activated c-*abl* variants have been previously defined. Motifs necessary for transformation in all cell types include the SH2 region and kinase domains, whereas the presence of a myristoylation site near the N terminus of activated proteins appears required for fibroblast transformation (23, 24). Although deletion of regions such as SH3 can activate transforming ability, the most common naturally occurring mutations involve appending additional sequence information to the N terminus of a partly deleted gene product. These include addition of retroviral *gag* sequences (in A-MuLV or Hardy Zuckerman feline sarcoma virus) or *bcr* sequences (in the Philadelphia translocation) to a partially deleted c-*abl* molecule. The role of these additions remains poorly defined, but several mechanisms can be envisioned. Steric hindrance of the interaction between regulatory regions normally present in c-*abl* and other molecules may occur. The SH3 domain appears to function as a negative regulator of transformation, whereas the SH2 domain recently has been shown to interact specifically with several proteins that have undergone tyrosine phosphorylation in cells transformed by activated *abl* variants (25). Another mechanism might involve acquisition of an additional functionality by the *abl* protein. *gag* sequences, in part, determine protein stability in lymphoid cells (26) and subcellular distribution through provision of a myristoylation site (27). Although no enzymatic activity has been proposed for *gag*, a serine/threonine kinase activity has previously been suggested for the *bcr* gene product (28). Further investigation of the structural properties of these *abl* variants will clearly be necessary to identify regions responsible for the specificity of transformation they exhibit.

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1. Abelson, H. T. & Rabstein, L. S. (1970) *Cancer Res.* **30**, 2213–2222.
2. Reddy, E. P., Smith, M. J. & Srinivasan, A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3623–3627.
3. Rosenberg, N. & Witte, O. N., eds. (1988) in *Advances in Virus Research*, (Academic, New York), pp. 39–81.
4. Kelliher, M. A., McLaughlin, J., Witte, O. N. & Rosenberg, N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6649–6653.
5. Chung, S. W., Wong, P. M. C., Durkin, H., Wu, Y. S. & Petersen, J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1585–1589.
6. Champlin, R. E. & Golde, D. W. (1985) *Blood* **65**, 1039–1047.
7. Elefanty, A. G., Hariharan, I. K. & Cory, S. (1990) *EMBO J.* **9**, 1069–1078.
8. Daley, G. Q., Van Etten, R. A. & Baltimore, D. (1990) *Science* **247**, 824–830.
9. Barklis, E., Mulligan, R. C. & Jaenisch, R. (1986) *Cell* **47**, 391–399.
10. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22**, 777–785.
11. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
12. Jackson, P. & Baltimore, D. (1989) *EMBO J.* **8**, 449–456.
13. Konkel, D. A., Tilghman, S. M. & Leder, P. (1978) *Cell* **15**, 1125–1132.
14. Mardon, G., Luoh, S., Simpson, E. M., Gill, G., Brown, L. G. & Page, D. C. (1990) *Mol. Cell. Biol.* **10**, 681–688.
15. D'Andrea, A. D., Lodish, H. F. & Wong, G. G. (1989) *Cell* **57**, 277–285.
16. Schatz, D. G., Oettinger, M. A. & Baltimore, D. (1989) *Cell* **59**, 1035–1048.
17. Schlissel, M. S. & Baltimore, D. (1989) *Cell* **58**, 1001–1007.
18. Konopka, J. B. & Witte, O. N. (1985) *Mol. Cell. Biol.* **5**, 3116–3123.
19. Cook, W. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2917–2921.
20. Scott, M. L., Davis, M. M. & Feinberg, M. B. (1986) *J. Virol.* **59**, 434–443.
21. Tidmarsh, G. F., Heimfeld, S., Whitlock, C. A., Weissman, I. L. & Muller-Sieburg, C. E. (1989) *Mol. Cell. Biol.* **9**, 2665–2671.
22. Fialkow, P. J., Jacobson, R. J. & Papayannopoulou, T. (1977) *Am. J. Med.* **63**, 125–130.
23. Prywes, R., Foulkes, J. G., Rosenberg, N. & Baltimore, D. (1983) *Cell* **34**, 569–579.
24. Prywes, R., Foulkes, J. G. & Baltimore, D. (1985) *J. Virol.* **54**, 114–122.
25. Mayer, B., Jackson, P. K. & Baltimore, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 627–631.
26. Prywes, R., Hoag, J., Rosenberg, N. & Baltimore, D. (1985) *J. Virol.* **54**, 123–132.
27. Van Etten, R. A., Jackson, P. & Baltimore, D. (1989) *Cell* **58**, 669–678.
28. Li, W. J., Kloetzer, W., Gale, R. P. & Arlinghaus, R. B. (1989) *Oncogene* **4**, 127–138.