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Induction of a chronic myelogenous leukemia-like syndrome in mice with v-*abl* and *BCR/ABL*

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ABSTRACT **The v-*abl* gene in Abelson virus induces pre-B-cell lymphoma in mice while the *BCR/ABL* oncogene is associated with chronic myelogenous leukemia and some cases of acute lymphocytic leukemia in humans. Understanding the mechanisms by which these oncogenes affect various cell types has been hampered by a paucity of experimental systems that reproduce the range of biological effects associated with them. We have developed an experimental system in which murine hematopoietic stem cell populations are infected with either v-*abl* or *BCR/ABL* retroviruses and are used to reconstitute lethally irradiated mice. Irrespective of the form of activated *abl*, >90% of the animals reconstituted with such cells develop tumors. About 50% of them develop a myeloproliferative syndrome that shares several features with the chronic phase of chronic myelogenous leukemia; the remaining animals succumb to pre-B-cell lymphomas. The myeloproliferative syndrome is characterized by large numbers of clonally derived, infected myeloid cells. This model will allow study of the mechanism by which activated *abl* genes affect hematopoietic precursors in chronic myelogenous leukemia. Furthermore, our results demonstrate that introduction of an activated *abl* gene into the appropriate target cell, not the structure of the gene, is the major determinant in myeloid cell specificity.**

Expression of activated *abl* genes is associated with a variety of malignant hematological disorders (reviewed in refs. 1 and 2). In mice, the v-*abl* oncogene of Abelson virus induces pre-B-cell lymphoma. In humans, *ABL* is activated via recombination with sequences from the *BCR* locus, an event that plays a key role in chronic myelogenous leukemia (CML) and in some cases of acute lymphocytic leukemia. In both cases, the activated gene is controlled by new promoter elements and encodes a protein with new amino-terminal residues introduced by the recombination (3, 4). As a consequence, the protein tyrosine kinases encoded by the activated genes bypass regulatory controls acting on the normal abl protein (5–7). Although secondary changes are important for full malignant transformation by activated abl (8–10), expression of the v-*abl*-encoded protein is required for both initiation and maintenance of transformation in the murine system (11, 12), and the BCR/ABL protein probably plays a similar role in the human diseases.

The v-abl and BCR/ABL proteins contain a large unrelated region. The amino terminus of the v-abl protein is specified by the *gag* gene of Moloney leukemia virus while that of the BCR/ABL protein is derived from BCR. The BCR sequences lack the myristoylation signal present in the *gag*-derived sequence that probably directs the v-abl protein to the membrane (3, 4, 6, 7). The acute course of v-*abl*-induced disease versus the complex, chronic course of CML (1, 2, 10), in which BCR/ABL stimulates clonal dominance of the affected stem cells (13), may reflect these differences. Consistent with the disease patterns, *in vitro* studies with murine bone marrow cells have demonstrated that v-abl rapidly transforms pre-B cells (14), while BCR/ABL stimulates clonal outgrowth of such cells after an extended period (15).

While these results are consistent with the indolent nature of CML, the fact that B cells are stimulated, irrespective of experimental conditions (15–17), has hampered dissection of the role of BCR sequences and promoter elements in mediating disease specificity. We have circumvented this problem by using bone marrow from mice treated with 5-fluorouracil (5-FU) as a target cell population. When these cells are infected with either v-*abl* or P210 *BCR/ABL* retroviruses and used to reconstitute lethally irradiated mice, >90% of the animals develop tumors. About 50% of the animals develop myeloproliferative disease and the others develop pre-B-cell lymphoma. The myeloproliferative syndrome is characterized by proliferation of virus-infected cells of the granulocytic and myelomonocytic lineage in the blood and spleens of the afflicted mice. This system allows study of the mechanisms by which activated *abl* genes alter the proliferative capacity of hematopoietic cells and provides an animal model in which to study human CML. Furthermore, these data demonstrate that expression of either the v-*abl* or *BCR/ABL* genes in an appropriate target cell population is the central feature controlling myeloid disease specificity.

MATERIALS AND METHODS

Infection of Hematopoietic Stem Cells. Bone marrow from BALB/cByJ mice treated 6 days earlier with 5-FU (150 mg per kg of body weight) was infected *in vitro* with Abelson virus (18); JW-RX, a retrovirus expressing P210 BCR/ABL (15); or Moloney virus (13), or it was mock-infected in the presence of recombinant interleukin 3 (20 units/ml) (gift of James Ihle, St. Jude's Childrens Research Hospital) and Polybrene (4 μg/ml). Two days later, syngeneic mice of the sex opposite that of the donor cells were irradiated with 600 R followed by 300 R 3 hr later (1 R = 0.258 mC/kg), and each animal was injected with 1×10^5 cells. Animals were bled bimonthly to assess the peripheral blood picture, and they were monitored for signs of disease.

Examination of Cells and Tissues. Tissues were processed for histological examination and were used to prepare DNA (19). Peripheral blood leukocytes were centrifuged through Ficoll before extracting the DNA. DNAs were digested with restriction enzymes, fractionated through 0.8% agarose, and transferred to Nytran membranes. Hybridizations with pJ11 (20), pv-*abl* (21), pABL (3), and pY2 (22) were as described (19).

Bone marrow and spleen were cultured in RPMI 1640 medium containing 20% fetal calf serum and 50 μM 2-

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Abbreviations: CML, chronic myelogenous leukemia; 5-FU, 5-fluorouracil; Ab-MLV, Abelson murine leukemia virus.

Table 1. Disease induction by activated *abl* oncogenes

Gene	Diagnosis	Incidence	Mean latent period, days	Cell lines derived		
				Pre-B	Myeloid	Both
BCR/ABL	Myelomonocytic leukemia	2/12	70	2/2	0/2	0/2
	Granulocytic leukemia	2/12	63	0/2	0/2	0/2
	Pre-B-cell lymphoma	4/12	78	3/4	0/4	0/4
	Unavailable for examination	3/12				
v-*abl*	Myelomonocytic leukemia	6/18	33	1/6	0/6	3/6
	Pre-B-cell lymphoma	7/18	45	6/7	0/7	0/7
	Unavailable for examination	5/18				

Diagnosis was based on gross and microscopic pathological assessment. Cells were cultured *in vitro* as described in the text and were classified as myeloid or lymphoid based on morphological, histochemical, and differentiation antigen profiles. Cell lines classified as lymphoid expressed the B-cell lineage markers B220 and J11D and were negative for the myeloid markers Mac-1, Mac-2, and Mac-3. These cells did not stain with α-naphthyl acetate esterase or toluidine blue. Cells classified as myeloid were negative for B220, J11D, and Mac-3 and did not stain with toluidine blue. These cells expressed Mac-1 and Mac-2 and were positive with α-naphthyl acetate esterase stain. Unavailable for examination indicates that the animals died of tumors before they could be examined.

mercaptoethanol, in alpha MEM containing 10% fetal calf serum or in soft agar as described (14). No specific lymphokines or growth factors were used. Cell lines were stained with antibodies against B220 (23); J11D (24); Mac-1 (25), -2 (26), and -3 (27); and isotype-matched control reagents and were analyzed with a fluorescence-activated cell sorter.

RESULTS

Tumors in Mice Reconstituted with abl-Infected Stem Cell Populations. Bone marrow from 5-FU-treated mice was infected *in vitro* with either Abelson murine leukemia virus (Ab-MLV) or the BCR/ABL virus and injected into lethally irradiated mice. Unlike unreconstituted controls, which died 3–6 days after irradiation, the reconstituted animals remained healthy at early time points. All 19 mice that received Ab-MLV-infected cells succumbed 4–10 weeks later and 11 of 12 animals that received BCR/ABL-infected cells died 9–12 weeks postreconstitution. Gross and histologic examination and tissue culture studies (see below) were used to categorize the tumors. Two disease patterns were observed in the mice reconstituted with Ab-MLV-infected cells: myelomonocytic leukemia and pre-B-cell lymphoma (Table 1). Mice reconstituted with BCR/ABL-infected cells developed myelomonocytic leukemia, granulocytic leukemia, and pre-B-cell lymphomas. Control animals reconstituted with mock-

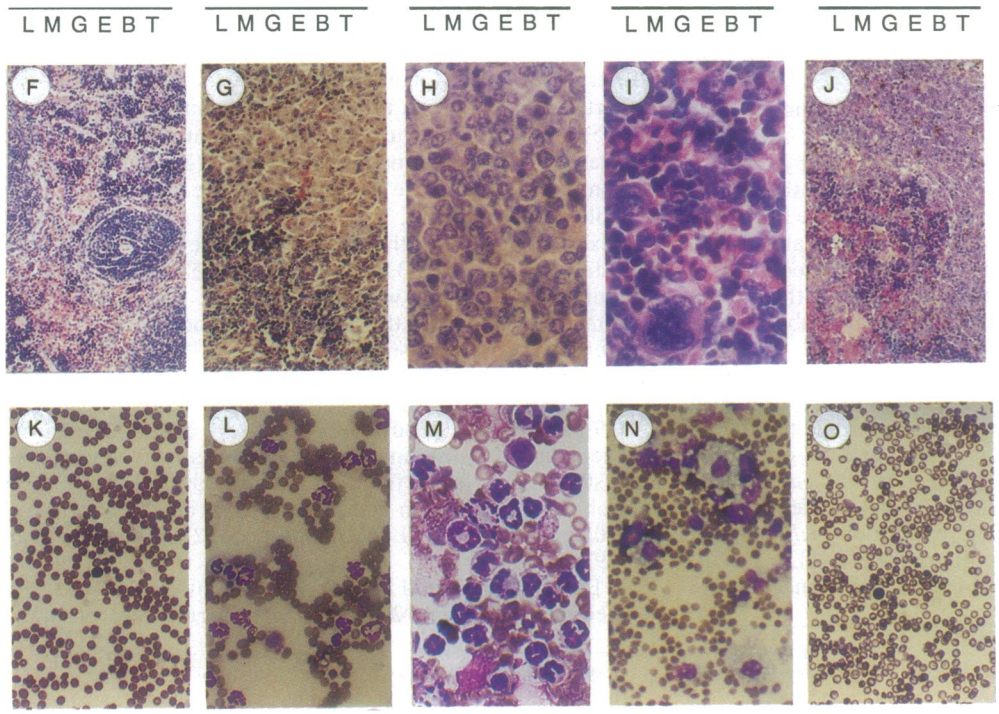

FIG. 1. Histopathologic examination of reconstituted mice. (*A–E*) Peripheral blood samples were obtained prior to sacrifice and total leukocyte and differential counts were performed. The percentage of lymphocytes (L), macrophages (M), granulocytes (G), eosinophils (E), and basophils (B) observed in stained smears is indicated by the bars. The total leukocyte count (T) is also shown. In this case, the value on the ordinate indicates cells per μl × 10^3. (*F–J*) Representative sections of spleen stained with hematoxylin and eosin. (*K–O*) Representative examples of peripheral blood smears are shown. *A*, *F*, and *K* are from an animal reconstituted with mock-infected cells; *B*, *G*, and *L* are from a v-*abl*-reconstituted mouse with myelomonocytic disease; *C*, *H*, and *M* are from a *BCR/ABL* reconstituted mouse with granulocytic disease; *D*, *I*, and *N* are from a *BCR/ABL* reconstituted mouse with myelomonocytic disease; *E*, *J*, and *O* are from BCR/ABL mice with pre-B-cell lymphoma. (*F*, ×80; *G*, *J–L*, *N*, and *O*, ×160; *H*, *I*, and *M*, ×320.)

infected cells remained healthy and those receiving Moloney murine leukemia virus-infected cells developed thymic lymphomas after >90 days (data not shown).

Many of the Reconstituted Animals Develop Myeloproliferative Disease. Total leukocyte and differential counts were performed on peripheral blood from diseased animals. About 50% of the animals displayed leukocyte counts that were 5–20 times higher (Fig. 1 *B–D*) than those in the mock-reconstituted animals (Fig. 1*A*). Examination of Wright–Giemsa-stained smears revealed that the elevated leukocyte counts reflected abnormally high percentages of mature and immature granulocytes and myelocytes but few myeloblasts (Fig. 1 *L* and *M*) in most cases. In some animals, α-naphthyl acetate esterase positive macrophages and other less-differentiated monocytic cells were prominent in the peripheral blood (Fig. 1*N*; data not shown).

Autopsy revealed that the animals with elevated leukocyte counts displayed marked splenomegaly with spleen weights 5–10 times normal. Histologic examination of the spleens revealed an expanded red pulp and normal white pulp with extensive hematopoietic trilineage proliferation and differentiation in the red pulp (Fig. 1*I*). Areas infiltrated with chloroacetate esterase-positive cells (data not shown) in all stages of granulocytic differentiation were prominent in some of these animals as were undifferentiated hematopoietic progenitors (Fig. 1*H*). In addition to these features, the spleens of the v-*abl* reconstituted mice displayed multiple white nodules (3–5 mm) of disorganized macrophage proliferation (Fig. 1*G*). Macrophage infiltration was also observed in the portal sinuses of the liver in some of these animals (data not shown). Slight lymphadenopathy and proliferation of blasts indistinguishable from those found in Abelson pre-B-cell lymphoma was observed occasionally and probably reflects the mixed disease picture evident from the cell expansion studies (Table 1).

About 50% of animals developed a disease similar to typical Abelson lymphoma with lymphadenopathy, spinal tumors, and slight splenomegaly. As expected (28, 29), these animals had a normal blood picture (Fig. 1 *E* and *O*). Histologic examination of the spleens revealed expansion of lymphoblastoid cells in the white pulp (Fig. 1*J*), a feature that is usually not observed in typical Abelson disease (28, 29). This difference may reflect the fact that the lymphoma developed during hematopoietic reconstitution in an irradiated host.

The Tumor Cells Contain abl. Two approaches were used to confirm that the tumors in the reconstituted mice contained the virus used to infect the stem cell populations. For v-*abl* reconstituted mice, cell lines were derived from 10 animals. The presence of Ab-MLV in these cells was confirmed by Southern analysis with a v-*abl* probe (21). In all cases, including those shown (Fig. 2*A*), between one and six copies of the Ab-MLV genome were detected. The v-*abl* protein was detected in cellular extracts from all the cell lines (data not shown). A similar analysis, using DNA from tissues of the *BCR/ABL* reconstituted mice, detected the BCR/ABL retrovirus (Fig. 2*B*). To confirm that the tumors arose from the cells injected into the mice, DNA from the v-*abl*-derived cell lines was probed with a male-specific probe (22). The characteristic 15-kilobase (kb) Y chromosome band observed in all of the tumor samples (Fig. 2*C*) is diagnostic of male and donor origin of the cells. Taken together, these data demonstrate that the tumors arise from infection with viruses expressing activated v-*abl* and *BCR/ABL* oncogenes and that the tumors arise from donor cells.

Some v-abl Tumors Contain Myeloid and Lymphoid Cells Derived from the Same Infected Cell. Two types of cell lines were isolated from the v-*abl* reconstituted mice, one composed of lymphoblastoid cells and a second made up of large vacuolated adherent cells. Characterization with a panel of

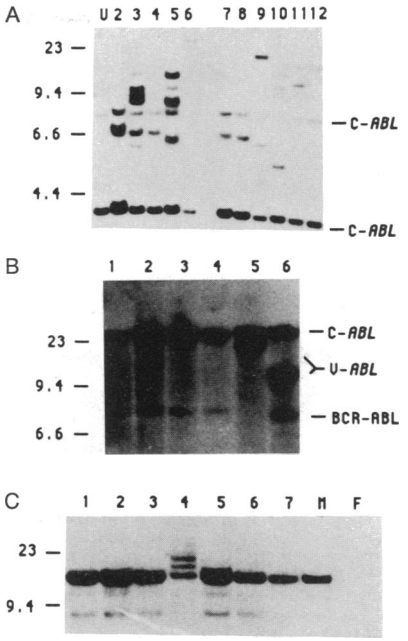

FIG. 2. Tumors in reconstituted mice arise from infected donor cells. (*A* and *B*) *abl* sequences were visualized by probing with a v-*abl* probe (21). In *A*, the DNAs from uninfected cells (lane U) and cell lines derived from the v-*abl* reconstituted mice U (lanes 2–4), R (lane 5), L (lane 6), J (lanes 7–9), F (lane 10), and E (lanes 11 and 12) were digested with *Hin*dIII, resulting in detection of viral sequences linked to cellular DNA. In *B*, the DNAs from the tumors arising in four different *BCR/ABL* reconstituted mice (lanes 1–4) were digested with *Eco*RI, resulting in detection of an internal fragment of the BCR/ABL virus. DNAs from an Ab-MLV-transformed cell line (lane 5) and an Ab-MLV-transformed cell clone that also contains a single integrated copy of BCR/ABL (lane 6) were used as controls. In *C*, DNAs from v-*abl* tumor cell lines derived from mouse U (lane 1), mouse R (lanes 2 and 3), and mouse J (lanes 4–7) were digested with *Bam*HI and analyzed with the male-specific probe pY2 (22). DNAs from normal male (lane M) and female (lane F) mice were used as controls. Numbers on left are kb.

histochemical and cell-surface markers confirmed that the lymphoblastoid cells were related to pre-B lymphocytes and that the adherent cells were similar to macrophages (Table 1). Because both types of cells were isolated from three animals, the relationship of the myeloid and lymphoid lineage cells could be assessed by the Ab-MLV integration sites as a marker (Fig. 3*B*; data not shown). Although identical patterns were not always observed in the cell lines from a single mouse, integrations shared between one of the myeloid and at least one of the lymphoid cell lines were observed in all cases (lanes J-B2 and J-M1; lanes R; lanes U-M2 and U-B1; data not shown), indicating that these cell lines arose from a common infected cell.

Analysis of the heavy-chain immunoglobulin genes in the cell lines related by proviral integration site revealed three patterns of rearrangement (Fig. 3*A*; data not shown). In one case, the lymphoid and myeloid cells shared identical heavy-chain gene rearrangements on both alleles (Fig. 3, lanes R), indicating that differentiation into the myeloid lineage occurred after heavy-chain gene rearrangement. In a second case, the myeloid and lymphoid cells shared one allele but differed at the second allele (Fig. 3, lanes J-B2 and J-M1), suggesting that these two cells diverged early in the course of heavy-chain variable region assembly. In the third case, distinct rearrangements were observed in the related lymphoid and myeloid cells (Fig. 3, lanes U-B1 and U-M2). Here, Ab-MLV infection probably occurred in a precursor cell that had not yet begun rearrangement. Rearrangement of immunoglobulin genes in myeloid cells was not anticipated; how-

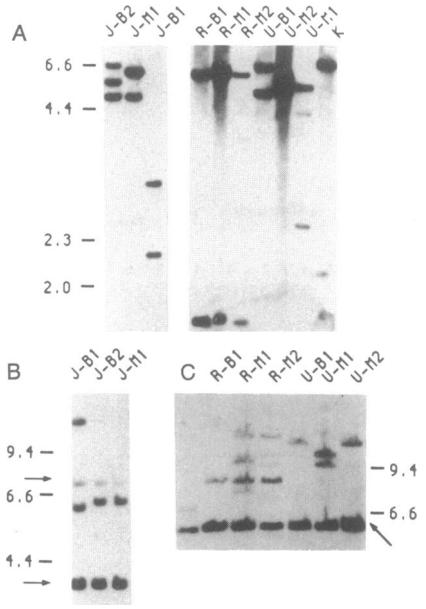

FIG. 3. Clonal relationship between myeloid and lymphoid cells from the v-*abl*-reconstituted mice. Proviral integration sites were analyzed by using cell lines from the v-*abl*-reconstituted mice. DNAs digested with *Hin*dIII (*B, Left*) or *Bam*HI (*B, Right*) were probed with a v-*abl* probe (21). The immunoglobulin heavy-chain variable region structure was analyzed after *Eco*RI digestion with the pJ11 probe (20) (*A*). Lanes are designated by the name of the cell line, using a code in which the first letter refers to the mouse from which the cells were isolated, the second letter indicates the myeloid (M) or pre-B cell (B) nature of the cell line, and the number signifies the independent derivation of each of the cell lines. Kidney DNA (*A*, lane K) and DNA from an uninfected cell line (*B*, lane C) were used as controls. The c-*abl* bands are identified by arrows. Numbers on left are kb.

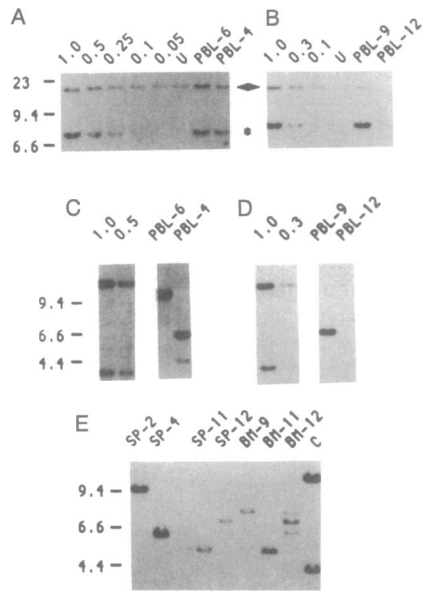

FIG. 4. Clonal relationship of the BCR/ABL tumor cells. DNAs prepared from myeloid cells in the peripheral blood were analyzed with an ABL probe (15) after digestion with *Eco*RI (*A* and *B*), resulting in detection of an internal fragment of the BCR/ABL retrovirus. The same filters were reprobed with a *neo* probe that detects a single band for each BCR/ABL provirus in the context of cellular sequences flanking the integration site (15) (*C* and *D*). In *A* and *B*, samples from the peripheral blood of BCR/ABL mice 6, 4, 9, and 2 (*A*, lanes PBL-6 and PBL-4; *B*, lanes PBL-9 and PBL-12) were compared with a dilution series of DNA from a cell line containing two copies of BCR/ABL. These lanes are designated by the genome equivalents loaded in each lane. Lanes U contain DNA from normal, uninfected cells. The 25-kb c-*abl* band is designated by the diamond and the BCR/ABL band is designated by the asterisk. The samples shown in *C* correspond to those in the lanes with the identical designations in *A*; those in *D* correspond to the lanes with the identical designations in *B*. (*E*) *Eco*RI-digested DNAs from the spleens (SP) and bone marrow (BM) of BCR/ABL mice analyzed with the *neo* probe, which detects the size of the proviral integration fragment. The numbers in the lane designations correspond to the number of the animal from which the tissue was derived. DNA prepared from a cell line carrying two copies of the BCR/ABL retrovirus (lane C) served as a control. Numbers on left are kb.

ever, similar rearrangements have been noted in human myeloid tumor cells (30) and in murine myeloid cells transformed by the *raf* oncogene (31).

Clonality of the BCR/ABL-Induced Disease. Unlike the v-*abl* reconstituted mice, myeloid lines could not be derived from the *BCR/ABL* reconstituted animals (Table 1). To assess the role of BCR/ABL infection in myeloid proliferation, peripheral leukocytes were analyzed for the presence of the BCR/ABL retrovirus. In the four cases in which sufficient cells were present to allow for analysis of DNA, the BCR/ABL retrovirus was readily detected (Fig. 4 *A* and *B*). Comparison with a standard dilution series prepared with DNA from a cell line carrying two copies of the BCR/ABL provirus showed that one haploid genome equivalent of BCR/ABL was present in the peripheral blood samples. Analysis of the same filter with a probe that detects a single, unique fragment for each BCR/ABL provirus revealed the presence of a dominant clone in all four cases (Fig. 4 *C* and *D*, lanes PBL-6, -4, -9, and -12). These results demonstrate that the myeloproliferation in the peripheral blood is a consequence of BCR/ABL infection and that a single or small number of clones contributes to the disease. A similar analysis of DNAs prepared from the spleens and bone marrow of these mice revealed the presence of a dominant infected clone or several clones (Fig. 4*E*). In some cases, the dominant clone present in the tissues was identical to that observed in the peripheral blood (Fig. 4*E*, SP-4; Fig. 4*C*, PBL-4). In other instances, the clone dominating the peripheral blood was distinct from that most prominent in the tissues (Fig. 4*E*, BM-9; Fig. 4*D*, PBL-9). These results suggest that more than one clone can be stimulated to proliferate after infection with the BCR/ABL retrovirus. Further studies will be required to determine the relationship of the different clones to the disease process.

DISCUSSION

Our central finding is that expression of an activated *abl* gene under the appropriate conditions induces myeloproliferative disease in mice. Previous work has focused on the possible significance of the major differences in both the structure of v-*abl*- and *BCR/ABL*-encoded proteins and their promoters. Indeed, while this work was in progress, Daley and coworkers (32) used a similar protocol and the identical BCR/ABL cDNA expressed from a myeloid cell-specific promoter to induce myeloproliferative disease. Similar diseases were observed in both studies, suggesting that both BCR/ABL constructs stimulate the same cells. Although neither study directly demonstrates abl-driven stem cell proliferation, our results demonstrating infection of progenitors that differentiate into myeloid and lymphoid cells and theirs (32) demonstrating BCR/ABL provirus in CFU-S suggest that undifferentiated progenitors are involved in the disease. In addition, we have shown that both BCR/ABL and murine v-*abl* expressed from an efficient but not lineage-specific promoter induce myeloproliferative disease. Therefore, neither BCR sequences nor a myeloid cell promoter element is required for

myeloproliferative disease. Indeed, the ability of *fms* to induce a similar syndrome (33) suggests that *abl* is not alone in its ability to stimulate myeloid proliferation. In all of these cases, infecting the appropriate target cell and providing a favorable environment for expansion of the infected cells seems to be the major requirement for induction of the diseases.

Although the disease that develops in the v-*abl* and *BCR/ABL* reconstituted mice is similar, subtle differences exist. In particular, tumor cells of both the lymphoid and myeloid lineages could be readily expanded from the v-*abl* mice, while only pre-B-cell lines were obtained from the *BCR/ABL* reconstituted mice. This feature may reflect the absence of large macrophage tumors in the spleens of the *BCR/ABL* mice. Also, the latent period is significantly shorter in animals reconstituted with v-*abl*-infected stem cell populations, a feature most likely reflecting the different transforming potencies of *BCR/ABL* and v-*abl* (14, 15, 34).

The variations in disease pattern observed in the mice probably stem from the structural differences between BCR/ABL and v-abl proteins, features known to play key roles in transformation of at least some cell types *in vitro* (6, 7, 34, 35). These differences include the unrelated nature of the *BCR*- and *gag*-derived sequences of the two transforming proteins and the absence of a myristoylation signal in BCR/ABL (3, 4). The SH3 (*src* homologous 3) domain, present only in the BCR/ABL protein and involved in regulating the kinase activity of normal c-abl proteins (6, 7), may affect disease induction by BCR/ABL in a subtle way. Because some mouse strains develop other BCR/ABL-mediated hematopoietic proliferative disorders after reconstitution with 5-FU marrow (36), host factors may also influence the disease.

The induction of pre-B-cell lymphoma by the P210 *BCR/ABL* retrovirus was unanticipated. This virus induces clonal outgrowth of pre-B cells in long-term B-cell cultures (15, 34), but direct BCR/ABL-mediated transformation of pre-B cells *in vitro* or *in vivo* occurs at a very low frequency. The transformed pre-B cells present in the reconstituted animals may arise from precursors distinct from those involved in typical Ab-MLV-induced pre-B-cell lymphoma. Alternatively, the microenvironment present during hematopoietic reconstitution may facilitate the transformation of pre-B cells by BCR/ABL.

The animals with myeloproliferative disease share several features with the chronic phase of human CML. In particular, the presence of large numbers of clonally derived BCR/ABL-infected granulocytic cells in the peripheral blood is similar to the picture that characterizes the early stages of the human disease as is the granulocytic infiltration prominent in the spleens of these animals (5, 37). The extensive hematopoietic differentiation of megakaryocytic, erythroid, and granulocytic cells in the spleens of the diseased animals is another shared feature (5, 37). The ability to detect clonal or pauciclonal expansion of BCR/ABL-infected cells in these spleens suggests that the gene stimulates the apparently normal differentiation of all of these elements. Because different dominant BCR/ABL-infected clones expand in the spleens and peripheral blood of some of the mice, it is possible that either microenvironmental influence or stochastic variations in the proliferation of different BCR/ABL-infected progenitor clones influence the disease process. In either case, the mouse model will allow study of the role of activated *abl* genes in the elevated, but seemingly normal, hematopoiesis found in the early phases of CML.

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