Induction of Chronic Myelogenous Leukemia in Mice by the P210$^{bcr/abl}$ Gene of the Philadelphia Chromosome

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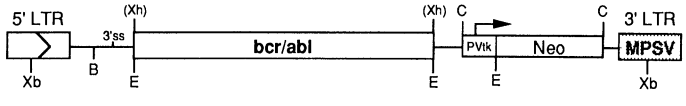
In tumor cells from virtually all patients with chronic myelogenous leukemia, the Philadelphia chromosome, a fusion of chromosomes 9 and 22, directs the synthesis of the P210$^{bcr/abl}$ protein. The protein-tyrosine kinase activity and hybrid structure of P210$^{bcr/abl}$ are similar to the oncogene product of the Abelson murine leukemia virus, P160$^{gag/v\text{-}abl}$, which induces acute lymphomas. To determine whether P210$^{bcr/abl}$ can induce chronic myelogenous leukemia, murine bone marrow was infected with a retrovirus encoding P210$^{bcr/abl}$ and transplanted into irradiated syngeneic recipients. Transplant recipients developed several hematologic malignancies; prominent among them was a myeloproliferative syndrome closely resembling the chronic phase of human chronic myelogenous leukemia. Tumor tissue from diseased mice harbored the provirus encoding P210$^{bcr/abl}$. These results demonstrate that P210$^{bcr/abl}$ expression can induce chronic myelogenous leukemia. Retrovirus-mediated expression of the protein provides a murine model system for further analysis of the disease.

HUMAN CHRONIC MYELOGENOUS LEUKEMIA (CML) IS A multilineage hematologic malignancy that progresses in distinct stages. The cardinal features of the initial, "chronic phase" of CML include elevation in the peripheral granulocyte count, and splenomegaly attributable to granulocytic infiltration (*1*). Although immature myeloid cells appear in the peripheral blood in CML, the capacity for the leukemic clone to differentiate into mature, functional granulocytes distinguishes the chronic phase from an acute leukemia, in which the leukemic cells remain immature. The remarkable granulocytic accumulation may cause the death of patients if they are left untreated in the chronic phase of CML, but if it is controlled by cytotoxic chemotherapy, the chronic phase may last several years. The chronic phase is invariably followed by a terminal stage, called "blast crisis." Like acute leukemias, it involves the accumulation of immature blast cells of either the myeloid or the lymphoid cell lineage (*2*).

The Philadelphia chromosome is cytogenetically evident in virtually all cases of CML (*3, 4*). It is generated in a pluripotent hematopoietic stem cell by a reciprocal translocation between chromosomes 9 and 22, and appears in myeloid, erythroid, megakaryocytic, and lymphoid cell types of CML patients. The translocation juxtaposes the coding sequence for a gene of unknown function on chromosome 22, denoted *bcr*, with coding sequence for the c-*abl* gene on chromosome 9 (*5, 6*).

The c-*abl* gene is the cellular homologue of v-*abl*, the oncogene of the Abelson murine leukemia virus (*7*). The hybrid *bcr/abl* gene of the Philadelphia chromosome encodes a 210-kD phosphoprotein (P210$^{bcr/abl}$), which resembles v-*abl* in that both are fusion proteins with disregulated protein-tyrosine kinase activity (*8, 9*). Like the v-*abl* protein, the P210$^{bcr/abl}$ protein will transform a variety of hematopoietic cell types in vitro, including established factor-dependent lymphoid and myeloid cell lines (*10, 11*). While the v-*abl* protein is responsible for the capacity of Abelson virus to induce lymphoid malignancies in susceptible mouse strains (*12*), the role of P210$^{bcr/abl}$ in myeloid leukemogenesis is less clear. Attempts to express P210$^{bcr/abl}$ in primary bone marrow culture have yielded only lymphoid transformants in vitro, even under conditions that favor myeloid cell proliferation (*13, 14*). Transgenic strains of mice carrying a *bcr*/v-*abl* fusion gene driven by an immunoglobulin enhancer or retroviral promoter develop lymphoid malignancy (*15*). To date, no system exists that models the apparent role of P210$^{bcr/abl}$ in the biology of myeloid leukemia, raising questions about the centrality of this protein in the disease.

To determine whether P210$^{bcr/abl}$ would induce CML, we attempted to express P210$^{bcr/abl}$ in the hematopoietic stem cells of



pGD210

Fig. 1. The retroviral construct used to transduce the P210$^{bcr/abl}$ gene. The construct pGD210 was made from a derivative of pZIPNeoSV(X) that had been modified to include the 3′ long terminal repeat (LTR) of the myeloproliferative sarcoma virus (*16*) and the B2 mutation (*38*) shown to enhance retroviral expression in undifferentiated embryonal carcinoma cell types [pSV(X)B2-MPSV] (*39*); the *neo* gene of pSV(X)B2-MPSV was deleted, and replaced by the 172/215 *bcr/abl* cDNA (*19, 40*), such that the *bcr/abl* gene sequences would be expressed from the spliced subgenomic proviral mRNA; the promoter element and *neo* gene sequences of the pMC1Neo plasmid (Stratagene) were inserted at the Cla I site. The pGD210 plasmid construct was used to make a helper-free retroviral producer cell line. Enzyme abbreviations: B, Bam HI; C, Cla I; E, Eco RI; Xb, Xba I; Xh, Xho I.

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mice through retrovirus-mediated gene transfer. A myeloproliferative syndrome with features of human CML resulted from reconstituting irradiated mice with marrow that had been infected with a retrovirus encoding $P210^{bcr/abl}$, demonstrating that this hybrid protein can cause myeloid cell proliferation. Tumors of a macrophage cell type and several instances of acute lymphoid leukemia resulted as well, suggesting that $P210^{bcr/abl}$ can transform several hematopoietic cell types after bone marrow infection.

Bone marrow infection with the *bcr/abl* retrovirus. The retroviral construct used for these experiments (Fig. 1) contains a complementary DNA (cDNA) sequence encoding *bcr/abl* expressed under the control of the promoter elements of the myeloproliferative sarcoma virus (MPSV) (*16*). The construct was transfected into a retroviral packaging cell line, and a high-titer retroviral producer cell line (Ψ-P210-36) was derived (*17–19*). The Ψ-P210-36 retroviral producer cell line, as well as NIH 3T3 cell lines infected by the produced virus, expressed the 210-kD *bcr/abl* phosphoprotein specified by the retroviral construct, as determined by immunoprecipitation (*20*).

Cells for infection were harvested from the marrow of the long bones of young BALB/c mice and cocultivated with the Ψ-P210-36 retroviral producer cell line (*21–23*). Three separate marrow cocultivation experiments were performed, and infected marrow was transplanted into 30 lethally irradiated syngeneic recipients. Of the transplanted mice, 43 percent (13 of 30) developed hematologic disease within 5 months after reconstitution with infected marrow. Three distinct disease processes were observed in the mice (Table 1): (i) a CML-like myeloproliferative syndrome with a mean latency of approximately 9 weeks; (ii) acute lymphoblastic leukemia with a mean latency of approximately 14 weeks; and (iii) a tumor of a macrophage cell type with a mean latency of approximately 16.5 weeks. In some mice, the macrophage tumor occurred together with the myeloproliferative syndrome or in association with acute lymphoblastic leukemia. A detailed pathologic analysis of each of these disease processes is presented below.

CML-like disease. Examination of the abdomens of mice with the CML-like myeloproliferative syndrome revealed massive splenomegaly characteristic of CML. The spleen, palpable in the live animals, was grossly enlarged and diffusely white at necropsy. Cytospin preparations from dissociated spleen tissue demonstrated a predominance of mature granulocytes. The peripheral white blood cell counts of these mice were 15,000 to 500,000 cells/mm^3 (compared to the normal 5,000 cells/mm^3) and showed a differential increase in the granulocyte lineage (Fig. 2A and Table 1). Peripheral blood was fractionated on density gradients to remove erythrocytes and granulocytes. Cytospin preparations showed several abnormal elements not typically found in peripheral blood, including mitotic figures, hypogranulated basophils, granulated early myeloid precursors, and an abundance of metamyelocytes (doughnut cells). Histopathologic analysis of the spleen and liver from these mice showed extensive infiltration with granulocytes and immature cells of the granulocyte lineage. The normal splenic architecture (Fig. 2B) was obliterated, and replaced with granulocytes (Fig. 2, C and D). In the liver, granulocyte infiltrates and numerous foci of extramedullary erythropoiesis were present in the perivascular areas, portal tracts, and sinusoids (Fig. 2E). The bone marrow from these mice was hypercellular, with a predominance of myeloid cells at all stages of maturation. The myeloproliferative process in these mice clearly has many of the cardinal features of human CML.

Analysis of DNA from the spleens of mice with the CML-like myeloproliferative syndrome demonstrated the presence of the *bcr/abl* provirus (Fig. 3A, lanes 2 to 7). The provirus in leukemic tissue was comparable in structure to that in a control NIH 3T3 cell line that had been infected with the pGD210 retrovirus and shown by immunoprecipitation to express the $P210^{bcr/abl}$ protein (Fig. 3A, lane 1). In two mice (BR-1 and BR-2), the leukemic cells also contained altered proviruses (Fig. 3A, lanes 6 and 7); these altered proviruses have deleted the *abl* coding region as shown by their size

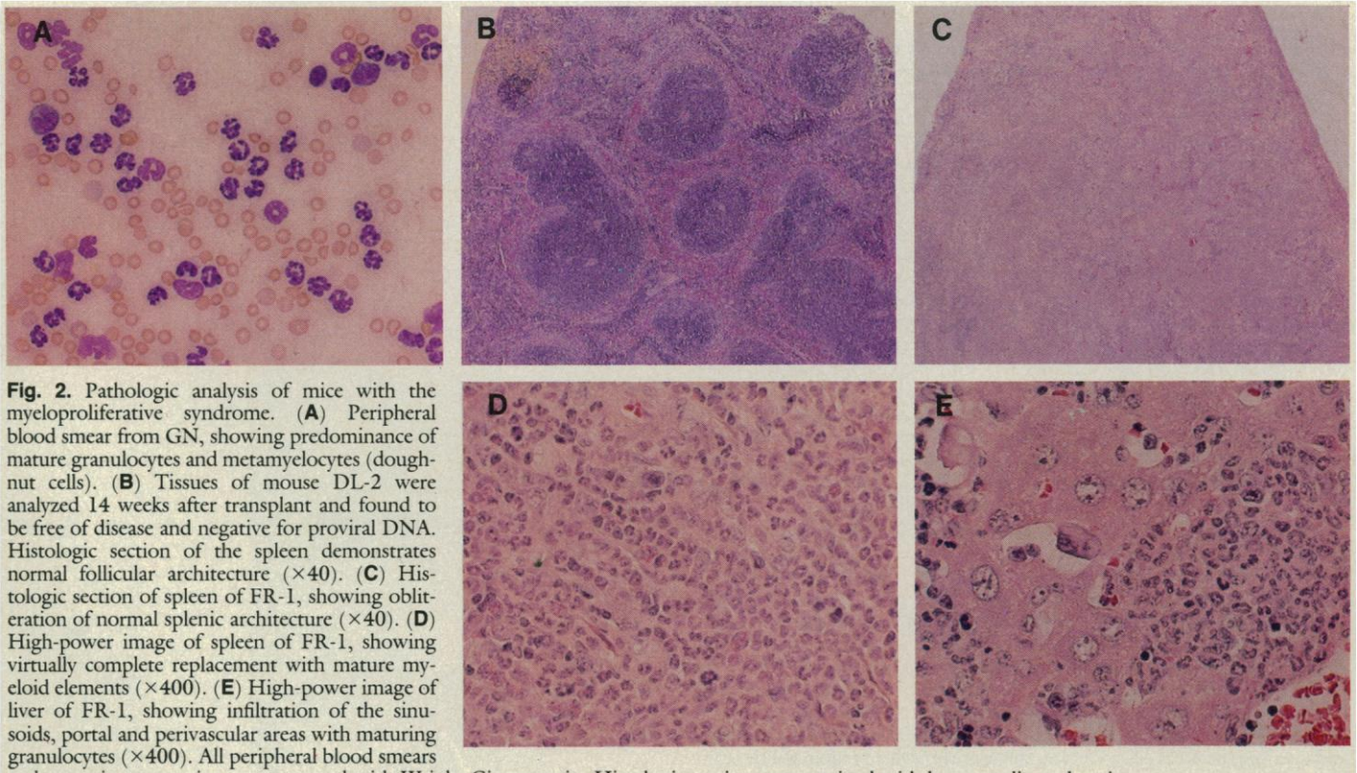

Fig. 2. Pathologic analysis of mice with the myeloproliferative syndrome. (**A**) Peripheral blood smear from GN, showing predominance of mature granulocytes and metamyelocytes (doughnut cells). (**B**) Tissues of mouse DL-2 were analyzed 14 weeks after transplant and found to be free of disease and negative for proviral DNA. Histologic section of the spleen demonstrates normal follicular architecture (×40). (**C**) Histologic section of spleen of FR-1, showing obliteration of normal splenic architecture (×40). (**D**) High-power image of spleen of FR-1, showing virtually complete replacement with mature myeloid elements (×400). (**E**) High-power image of liver of FR-1, showing infiltration of the sinusoids, portal and perivascular areas with maturing granulocytes (×400). All peripheral blood smears and cytospin preparations were treated with Wright-Giemsa stain. Histologic sections were stained with hematoxylin and eosin.

and inability to hybridize to *abl* DNA probes (*20*). To enumerate the proviruses present in the tissue samples, DNA was digested with Eco RI and probed with the *neo* gene (Fig. 3B). Three animals (DR-1, FR-1, and GN) showed a single proviral integrant, suggesting that the myeloproliferative disease arose from the progeny of a single infected cell (Fig. 3B, lanes 2, 3, and 4). Two animals (DR-2 and BR-1) harbored multiple proviruses, with most proviral integrants at levels comparable to a single copy of each per genome, suggesting that the disease tissue arose from a single cell that had sustained multiple retroviral infections (Fig. 3B, lanes 5 and 6). The less intensely hybridizing bands (one for DR-2, two for BR-1) imply lesser contributions from independently infected clones, or may constitute contamination with macrophage tumor cells (see below). None of the tumor DNA's showed multiple retroviral integrants at less than a single copy equivalent, which would have suggested that the tumor derived from many independently infected clones of cells.

Bone marrow from one of the affected mice (FR-1) was transplanted into a lethally irradiated secondary recipient at dilutions that allowed for isolation of individual spleen colonies at day 14. Day 14 spleen colonies were dissected, and genomic DNA was analyzed for the provirus. Five spleen colonies of mixed myeloid type harbored the same proviral integrant as the FR-1 donor (Fig. 3C, lanes 2 to 6). No provirus was detected in two other colonies that were analyzed (Fig. 3C, lanes 7 and 8), implying that uninfected progenitors coexist with infected progenitors in diseased mice. Thus, the cell type that was infected by the retrovirus encoding $P210^{bcr/abl}$ to give rise to the myeloproliferative syndrome in FR-1 was a primitive multipotential progenitor cell that either is or can give rise to the spleen colony forming unit (CFU-S).

Attempts to immunoprecipitate the $P210^{bcr/abl}$ protein from the spleens of mice with the myeloproliferative syndrome were unsuccessful because of the high protease content of granulocytes, as shown by mixing the granulocytes with the CML cell line K562 prior to the preparation of cell extracts (*20*). However, the $P210^{bcr/abl}$ protein could be detected in spleen tissue by immunoblotting of tissue lysates (*24*). Moreover, *abl* protein expression in early myeloid cells and metamyelocytes could be detected by a sensitive indirect immunofluorescence assay with antisera to *abl* determinants (Fig. 4). Expression of the endogenous c-*abl* proteins is too low to detect by this method. Therefore, the provirus appears to direct a high level of expression of the $P210^{bcr/abl}$ protein in myeloid cells.

Other malignancies. Three mice developed acute leukemias. Peripheral blood smears showed a predominance of immature blast cells (Fig. 5A). Leukemic blasts infiltrated spleen, lymph nodes, and bone marrow, and in one case formed a large paraspinal tumor (GR-

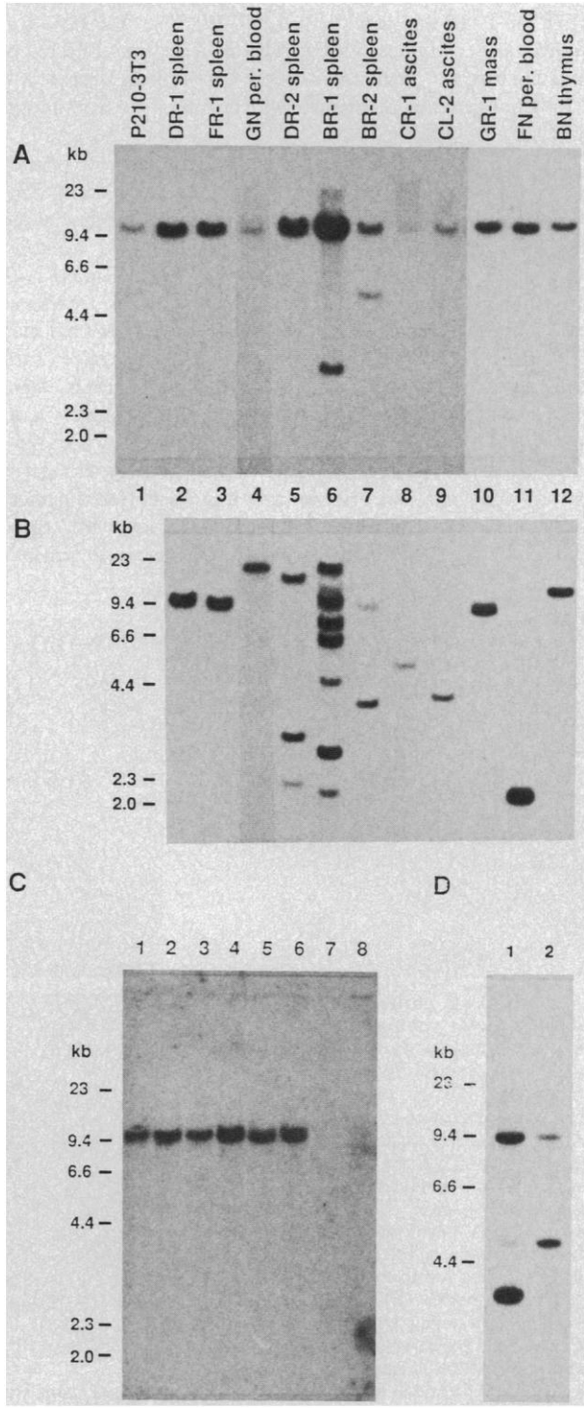

Fig. 3. Analysis of genomic DNA isolated from leukemic tissues. Genomic DNA was isolated, digested with restriction enzymes as noted below, separated by electrophoresis, transferred to a nylon membrane, and hybridized with a probe derived from the *neo* gene of the retroviral vector. (**A**) Demonstration of the provirus in tumor tissue of diseased mice. DNA (6 μg) was digested with the enzyme Xba I, which demonstrates the structure of the provirus because it cuts one time each in the 5′ and 3′ LTR's. (Lane 1) NIH 3T3 cells infected with retroviral supernatant from the Ψ-P210-36 retroviral producer cell line; (lane 2) DR-1 spleen; (lane 3) FR-1 spleen; (lane 4) GN peripheral blood; (lane 5) DR-2 spleen; (lane 6) BR-1 spleen; (lane 7) BR-2 spleen; (lane 8) CR-1 ascites; (lane 9) CL-2 ascites; (lane 10) GR-1 paraspinal mass; (lane 11) FN peripheral blood; and (lane 12) BN thymus. Lanes 4, 10, 11, and 12 derive from a separate experiment with a different preparation of the *neo* probe. (**B**) Enumeration of the proviral integrants in tumor tissue. DNA (6 μg) was digested with the enzyme Eco RI, which demonstrates the integration site for each provirus. Eco RI cuts 5′ to the *neo* gene sequences in the vector, and generates a specific fragment determined by the first Eco RI site encountered in cellular sequences flanking the 3′ LTR. Lanes are as in (A). (**C**) Detection of the provirus in day 14 spleen colonies in a secondary transplant recipient. Bone marrow from a mouse with the CML-like myeloproliferative syndrome (FR-1) was harvested and washed, and dilutions of cells were injected into secondary recipients that had received a lethal dose of irradiation (900 rads). Individual spleen colonies were dissected from secondary transplant recipients at day 14 after transplant, and genomic DNA was prepared. DNA (6 μg) was digested with Eco RI to demonstrate the proviral integration site. (Lane 1) FR-1 spleen DNA; (lanes 2 to 8) DNA from seven individual spleen colonies of mixed myeloid type. (**D**) Detection of different proviral integrants in the macrophage tumor and myeloproliferative tissue in mouse BR-2. DNA from spleen and liver was analyzed for the integration site of the provirus by digestion with Eco RI. Spleen was extensively replaced with granulocytes and maturing myeloid cells, whereas the liver showed a predominance of the macrophage tumor. (Lane 1) spleen; (lane 2) liver tumor nodule.

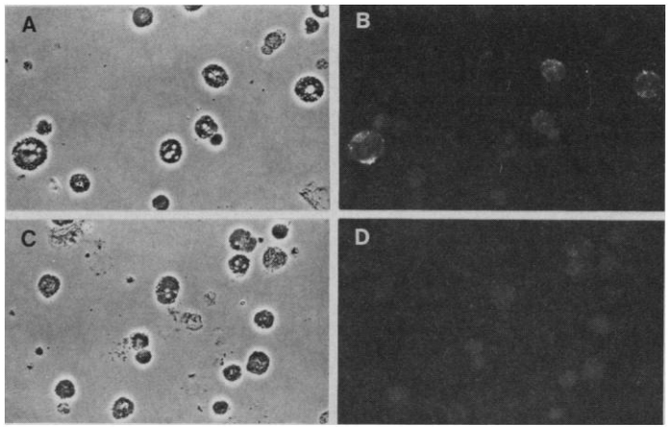

Fig. 4. Detection of $P210^{bcr/abl}$ protein expression by immunofluorescence. Individual day 14 spleen colonies were isolated from an irradiated mouse which had received bone marrow from a mouse with the myeloproliferative syndrome (FR-1). Cytospin preparations of dispersed cells were fixed in 4 percent paraformaldehyde and processed for indirect immunofluorescence localization of *abl* proteins as described (*41*). Genomic DNA was prepared from a portion of each colony, and the presence or absence of the provirus was determined by DNA hybridization (Fig. 3C). (**A** and **C**) Phase-contrast image; (**B** and **D**) immunofluorescence image; (B) indirect immunofluorescence of cells from a provirus-positive spleen colony, with antiserum to the *abl* protein; (D) indirect immunofluorescence of provirus-negative spleen colony, using antiserum to the *abl* protein. Control immunofluorescence experiments performed with serum from an unimmunized animal appear as in (D). Positively staining cells correspond to early granulated myeloid precursor cells and metamyelocytes (doughnut cells) on phase contrast. Expression is not detected in mature granulocytes by this method, suggesting that either the protein is not expressed in this cell type, or the antigen is poorly preserved because of their high protease content.

1) (Fig. 5B). Although we were unable to document a preexisting chronic myeloproliferative phase in these mice, one (GR-1) showed evidence of granulocytic infiltration of the spleen, suggesting that this might represent a case of lymphoid blast crisis. Tumor cells from these mice were analyzed for expression of lineage-restricted surface antigens by cytofluorimetry. Cells from two of the mice (GR-1 and FN) stained positive for the B-lineage restricted marker, B220, and were shown to be actively rearranging their immunoglobulin genes (*25, 26*). Therefore, these represented pre–B cell leukemias, the same cell type most frequently transformed by the Abelson murine leukemia virus (*12*). The third mouse with acute leukemia (BN) had a greatly enlarged thymus (0.45 g). Leukemic cells from this mouse stained positive for the T cell marker Thy-1, and thus represented an acute leukemia of T cell origin. Tumor cell DNA of each of the three acute leukemias harbored a single proviral integrant at levels comparable to a single copy per genome (Fig. 3, lanes 10 to 12).

Several of the mice displayed a tumor infiltration of a macrophage cell type. Mice with this tumor developed macroscopic tumor nodules in the liver and mesentery. Histologically, the liver showed focal nodules of a pale-staining tumor infiltrate (Fig. 6A). At high power, the pale-staining tumor cells had small, irregular nuclei with prominent nucleoli that easily distinguished them from the neighboring normal hepatocytes (Fig. 6B). In two cases (CR-1 and CL-2), mice with nodular tumor infiltrates developed a malignant ascites, which facilitated analysis of the tumor cell type. The ascites cells were heavily vacuolated, indicating active phagocytic activity (Fig. 6C). Few mitotic figures were seen, suggesting that the proliferative activity of the population was low, consistent with the long latency of the disease (mean of 16.5 weeks). The cells were positive in a histochemical assay for α-naphthyl acetate esterase activity, an enzyme detected in the macrophage-monocyte lineage (Fig. 6D). Moreover, the cells were positive for the myeloid surface antigen Mac-1 when analyzed by cytofluorimetry. These data place the cell type in the macrophage lineage. DNA from the ascites cells

Table 1. Hematologic parameters of diseased mice. Conditions for the three separate bone marrow cocultivation experiments A, B, and C are given (*21*). Disease latency was scored as the number of weeks after transplant until development of significant morbidity, or until time of spontaneous death. Mouse DL-2 appeared normal and was found to be disease free on histopathologic analysis. The white blood cell counts were determined before the animals were killed, except where noted (†). These represent the peak white cell count (WBC) reached before death. The differential white blood count was performed on 200 cells. Differential white blood cell counts for sham transplant controls and normal uninfected BALB/c mice varied widely, but typically showed a predominance of lymphocytes, in the range of 46 to 68 percent. P, granulocytes (polymorphonuclear cells) including early cells of the maturational series; L, lymphocytes; M, monocytes; E, eosinophils; B, basophils. ND, number not determined because blood sample could not be obtained.

Mouse	Transplant	Disease latency (weeks)	Spleen weight (g)	WBC (per μl)	Differential				
					P	L	M	E	B
DL-2	B	*	0.098	4,050	60	38	2		
				Chronic myelogenous leukemia					
DR-1	B	6.5	0.84	500,000	84	5	7	1	3
FR-1	C	4	0.84	111,500	75	17	6	1	
GN	C	8	0.81	43,500	67	28	5		
				CML + macrophage tumor					
DR-2	B	6.5	1.2	40,000†	80	16		4	
BR-1	A	11	0.49	71,500†	85	13	1		1
BR-2	A	9	0.3	70,500	93	4	3		
CL-2	B	12.5	0.23	93,500	93	5	2		
CR-1	B	14	0.31	15,000	65	32	3		
				Macrophage tumor alone					
CL-1	B	20.5	0.086	ND					
DL-1	B	12.5	0.144	<2,000	Pancytopenia				
				Acute lymphoblastic leukemia					
GR-1	C	9.5	0.11	26,500	30	70	(blasts)		
FN	C	13	0.82	167,000	<1	>99	(blasts)		
				Acute lymphoblastic leukemia + macrophage tumor					
BN	B	20.5	0.49	51,750	35	64	(blasts)		

*No disease.

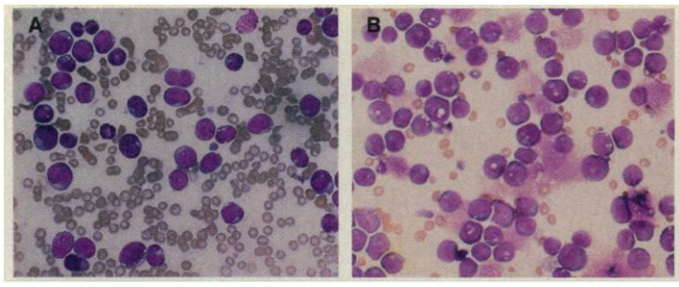

Fig. 5. Morphology of acute lymphoid leukemia cells. (**A**) Peripheral blood smear of mouse FN, showing predominance of immature blast cells. (**B**) Cytospin preparation of tumor cells from large paraspinal mass of mouse GR-1. This same immature blast cell type was detected in cytospin preparations from liver and spleen as well.

of these mice harbored the input provirus (Fig. 3, lanes 8 and 9). The P210$^{bcr/abl}$ gene therefore appears able to induce the proliferation of a macrophage cell type.

In several mice, the macrophage tumor occurred in association with either the myeloproliferative syndrome or acute lymphoblastic leukemia (Table 1). The macrophage tumor may not arise from the same infected cell clone as the other leukemic cell types. In most animals with a mixture of tumor types, it was impossible to harvest tumor tissue of one cell type free of the other. However, in one mouse (BR-2), DNA from the liver, which contained the macrophage tumor as well as some granulocytic infiltrates, was compared to DNA from the spleen, which was composed primarily of granulocytes with little evidence of the macrophage tumor cells. DNA hybridization analysis detected different predominant proviral integration sites in the two tissues (Fig. 3D), implying that the macrophage disease in BR-2 arose from infection of a different cell from that which gave rise to the myeloproliferative tissue. One mouse (CL-2) with a high peripheral white blood cell count in association with the macrophage tumor displayed proviral DNA in both the liver tumor nodule and ascites. However, provirus was not detected in the peripheral blood, despite the presence of elevated numbers of granulocytes. The granulocytosis in this animal may have been a reactive process not driven by the P210$^{bcr/abl}$ protein.

In these experiments, a retrovirus directing the expression of the P210$^{bcr/abl}$ protein was used to infect murine bone marrow in an attempt to introduce an analogue of the Philadelphia chromosome into the hematopoietic stem cells of mice. A myeloproliferative syndrome with features resembling the human disease CML was the principal pathologic feature in 8 of the 13 diseased mice. As in the human disease, these mice developed granulocytosis and marked splenomegaly. Human CML may also be characterized by basophilia, as well as abnormalities of platelets which can lead to bleeding or thrombosis. Some of the mice exhibited increased numbers of hypogranulated basophils and large, irregular platelets in the peripheral blood, further strengthening the resemblance to CML. Although we did not obtain data on platelet number and function, we did observe that mice with the myeloproliferative syndrome had a high incidence of thrombotic complications, including splenic infarctions, and apical necrosis of the ears and tail. With perhaps one exception (GR-1), the mice with the CML-like myeloproliferative disease have not survived long enough for us to determine whether their disease would progress from "chronic phase," where differentiation of the leukemic clone is maintained, to "blast crisis," marked by unrestrained growth of immature cells. The chronic phase of human CML would be fatal were it not for chemotherapy, suggesting that treatment of mice with an antiproliferative chemotherapeutic agent may control the CML-like disease long enough to examine whether progression to acute leukemia will occur.

The retroviral construct pGD210 incorporates the LTR (long terminal repeat) of the myeloproliferative sarcoma virus, raising the possibility that the MPSV elements caused the disease, rather than P210$^{bcr/abl}$. However, MPSV sequences themselves do not induce hematologic disease in the absence of the *mos* oncogene, which is responsible for the transforming activity of the virus (*27*). Also, a

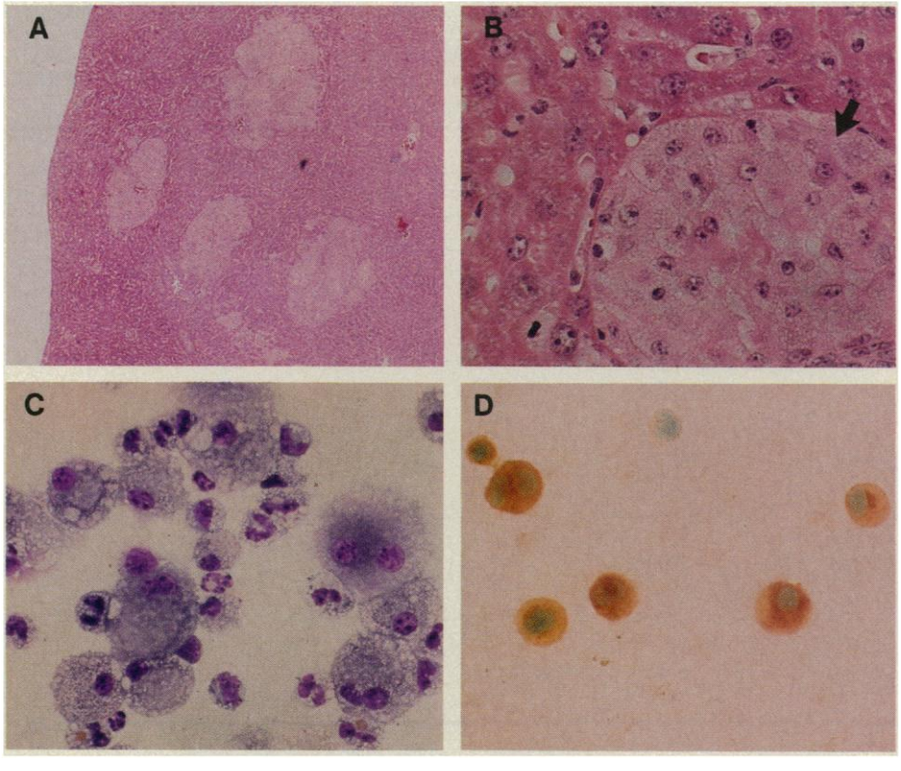

Fig. 6. Pathologic analysis of mice with tumors of macrophage cells. (**A**) Histologic section of the liver of mouse DR-2, demonstrating the pale-staining nodular infiltrates of tumor cells (×40). (**B**) High-power image of (A) showing the border area between normal hepatic tissue and the pale-staining tumor infiltrate, marked by arrow (×400). (**C**) Cytospin preparation of cells in the ascites of mouse CL-2, showing the highly vacuolated cytoplasm. (**D**) Histochemical stain of ascites cells for α-naphthyl acetate esterase, showing the red-brown reaction product characteristic of the monocyte-macrophage lineage.

retroviral construct similar to pGD210 expressing the DHFR gene from the MPSV LTR promoter has been used to infect bone marrow in numerous experiments without the development of hematologic abnormalities in mice receiving transplanted marrow (*28*). The promoter elements of the virus may enhance expression in the myeloid lineage, but are not lineage-specific, as shown by the lymphoid leukemias that arose in these mice. Furthermore, an analogue of Abelson virus, encoding $P160^{gag/v\text{-}abl}$ with LTRs derived from MPSV, causes Abelson disease upon intraperitoneal injection of newborn mice in a manner indistinguishable from wild type Abelson virus (*20*). Thus, the MPSV LTR sequences might facilitate retroviral gene expression in hematopoietic cells, but their influence on the disease processes is clearly ancillary to the central role of $P210^{bcr/abl}$.

In these experiments, most of the tumors harbored a single retroviral integrant and thus were monoclonal. Monoclonal disease could result if a second mutation has conferred a selective advantage on a subclone from a population of infected cells, or if only one or a few productively infected stem cells were transferred per animal. Although we cannot exclude the occurrence of secondary mutations, the latter explanation is more likely for two reasons. First, small numbers of bone marrow cells (2×10^5) were transplanted in these experiments, and therefore relatively few stem cells were transplanted per animal. Experiments using greater numbers of transplanted cells (10^6) still show a tendency for repopulation of animals with the progeny of at most a few stem cells (*29*). Second, the bone marrow cocultivations were not highly efficient. Several mice received dilutions of cells from the primary bone marrow infections that allowed for the isolation of individual spleen colonies after 14 days. The provirus was not detected in DNA from 13 colonies dissected from two spleens at day 14 after transplant, but was detected at significantly less than a single copy per genome in DNA extracted from whole spleen at day 14. This suggests that few transplanted bone marrow cells were productively infected. Monoclonal disease would result if the few infected bone marrow progenitor cells have a selective advantage over uninfected cells. This is likely because a high proportion of the transplanted animals developed disease. Healthy animals may not have received stem cells that were productively infected.

The secondary transplant data for the mouse FR-1 is evidence that infection of a primitive multipotential stem cells (CFU-S or its progenitor) gives rise to the myeloproliferative disease, establishing that CML can be induced by the expression of $P210^{bcr/abl}$ in early progenitor (stem?) cells of mice. The CML-like myeloproliferative syndrome has been successfully transferred to irradiated secondary recipients with the use of bone marrow from diseased primary animals, thereby establishing that the disease is transplantable (*20*). The secondary transplant recipients develop a pure CML syndrome without evidence of the macrophage tumor, suggesting that the macrophage tumor does not arise from infection of a stem cell. The prevalence of the macrophage tumor in the mouse system may reflect the fact that mouse macrophages or their progenitors are mitotically active and susceptible to transformation by the $P210^{bcr/abl}$ retrovirus, as evidenced by their capacity to be transformed by a v-*abl*–containing virus (*30*). A similar mechanism may underlie the generation of acute lymphoid disease. For Abelson disease in mice, infection of a committed lymphoid progenitor cell appears responsible for generating acute lymphoid leukemia (*31*). In human disease, some $P210^{bcr/abl}$-positive acute lymphoid malignancies appear to be restricted to the lymphocyte lineage (*32*). Thus, the cases of acute lymphoid leukemia described here may have resulted from the infection of a cell committed to the lymphoid lineage. Fractionation protocols which enrich for antigenically defined hematopoietic progenitor populations, including the hematopoietic stem cell (*33*), should help to define the target cells which yield particular hematologic diseases when infected with a retrovirus encoding $P210^{bcr/abl}$.

The $P210^{bcr/abl}$ protein is responsible for inducing several distinct hematologic malignancies when expressed in bone marrow cells of mice, including a myeloproliferative syndrome with the clinical and pathological features of CML. A similar myeloproliferative disease is induced in mice by retroviral transduction of the genes for v-*fms*, v-*src*, GM-CSF, or interleukin-3 into mouse bone marrow (*34–37*), but these genes have not been implicated in the pathogenesis of human CML. The introduction of the $P210^{bcr/abl}$ retrovirus into bone marrow stem cells of mice reflects the etiology and recapitulates the pathogenesis of human CML, and effectively models the consequences of the translocation that gives rise to the Philadelphia chromosome in the stem cells of humans. This model system may prove useful in studying the mechanisms involved in progression of the chronic phase of the disease to blast crisis, and should provide a system for testing therapeutic regimens.

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17. The pGD210 plasmid was linearized by digestion with a restriction enzyme at a single site in the plasmid backbone. The plasmid (10 μg) was transfected into the Ψcre ecotropic retroviral packaging cell line (*18*). After 2 days in culture, transfected cells were split into selective medium containing G418 at 1 mg/ml. Individual colonies were picked after 14 days, expanded, and screened for high titer production of retrovirus by assessing the amount of virion RNA in cell culture supernatants (*19*). The Ψ-P210-36 producer cell line was further shown to pass a titer of retrovirus equivalent to 10^6 G418-resistant colony-forming units per milliliter.
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21. Young (6- to 8-week-old) female BALB/c mice from the National Cancer Institute repository were injected intravenously (lateral tail vein) with 5 mg of 5-fluorouracil 6 days before we harvested their femoral and tibial marrow. The BALB/c strain was selected because it carries dominant sensitivity alleles that define genetic susceptibility to Abelson virus–induced malignancy (*22*). Bone marrow was washed once in phosphate-buffered saline (PBS), layered on a cushion of Ficoll-Paque (Pharmacia), and centrifuged at 1500*g* for 30 minutes to remove erythrocyte and high-density, differentiated myeloid elements. Cells in the low-density mononuclear fraction were harvested, washed twice in PBS, and seeded at 2×10^5 cells per 10-cm dish onto subconfluent retroviral producer cells. Conditions for the three bone marrow cocultivation experiments were as follows: (A) Cocultivation for 3 days in the presence of 20 percent conditioned medium of the WEHI-3B cell line as a source of interleukin-3 (*23*), and Polybrene at 0.4 μg/ml; (B) conditions as for (A), but with addition of interleukin-6 at 10 units/ml (Genetics Institute); (C) cocultivation for 2 days under the medium conditions for (A). After cocultivation, bone marrow was harvested, washed once with PBS, and resuspended in Hanks balanced salt solution (HBSS). Transplant recipients were young (6- to 8-week-old) male BALB/c mice that received 900 rads of γ-irradiation in two doses split by 3 hours prior to intravenous injection with 2×10^5 cultured bone marrow cells.
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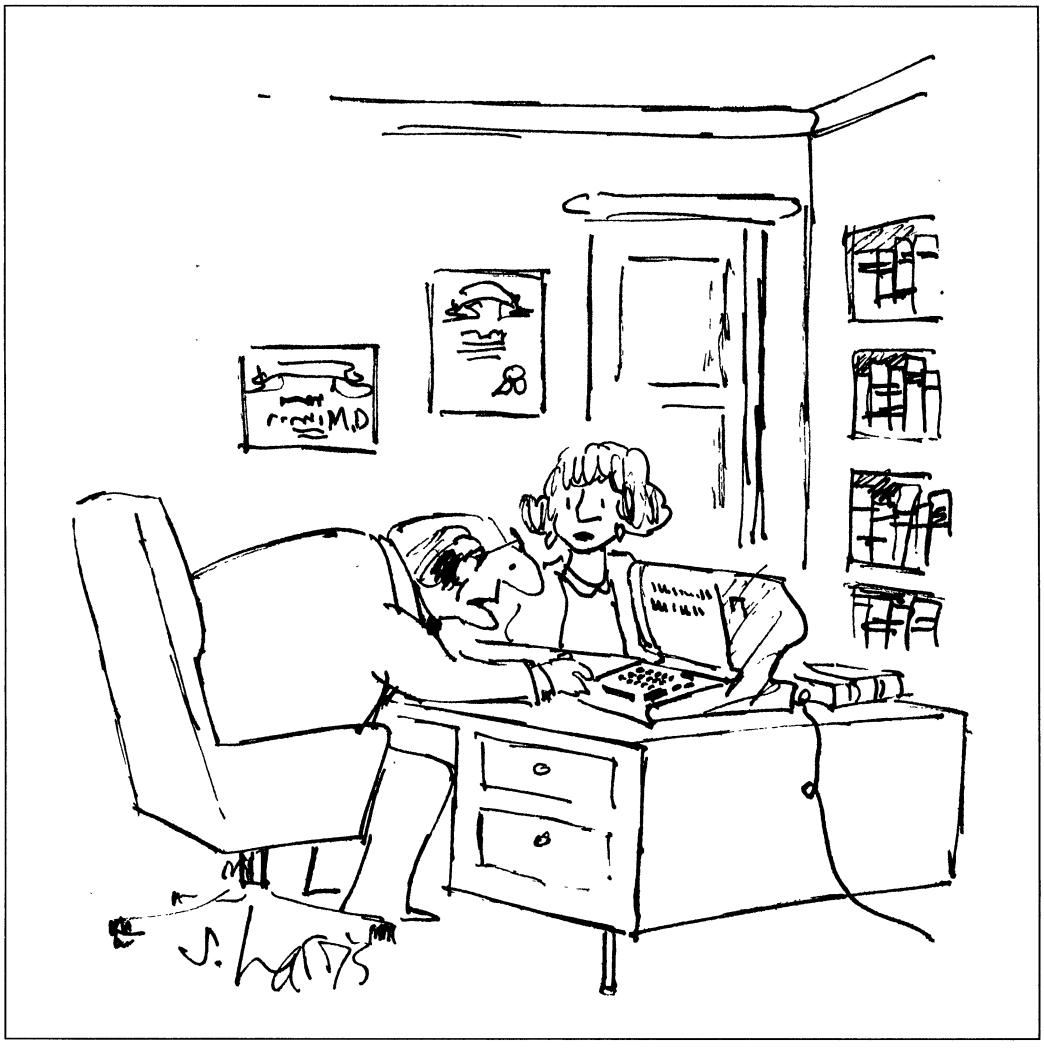
42. We thank M. Schlissel for the analysis of the immunoglobulin gene rearrangements in the acute lymphoid leukemia cells; A. Sharpe and P. Chassler for assistance and advice in the preparation of the histopathologic specimens; P. K. Jackson, L. Spain, M. Finer, and R. Mulligan for helpful suggestions, and P. K. Jackson and L. Spain for reviewing the manuscript. Supported by NIH grant CA38497; a Cancer Research Scholar Award (23010-1) from the American Cancer Society, Massachusetts Division (R.A.V.); and PHS Research Service Award 2T 32 GM07753-07 (G.Q.D.) from the National Institute of General Medical Sciences.

8 November 1989; accepted 19 January 1990



" So, you want a second opinion? Let's see what the computer says this time."