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Blast crisis in a murine model of chronic myelogenous leukemia

(*bcr/abl*/Abelson murine leukemia virus/hematopoietic stem cell)

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ABSTRACT **The $P210^{bcr/abl}$ protein is produced in cells from patients with Philadelphia chromosome-positive chronic myelogenous leukemia (CML). Retroviral transfer of the gene encoding $P210^{bcr/abl}$ into murine bone marrow induces a granulocytic leukemia that models the chronic phase of human CML. We have transferred the leukemic clone to syngeneic animals, albeit with surprising inefficiency, and have observed CML and clonally related acute leukemias of lymphoid or myeloid phenotype in some transplant recipients. These data show that murine CML can result from retroviral transfer of the *bcr/abl* gene into pluripotent hematopoietic stem cells, that infected clones repopulate poorly after adoptive transfer, and that these clones can give rise to acute leukemia, reflecting evolution to a phase resembling blast crisis in the human disease.**

In the initial, chronic phase of the human myeloproliferative disease chronic myelogenous leukemia (CML), the leukemic myeloid progenitors continue to differentiate into mature granulocytes (1). This feature distinguishes the chronic phase of CML from acute myeloid or lymphoid leukemia, which typically involves the accumulation of primitive hematopoietic cells arrested at an early stage of differentiation. Chronic-phase CML is unstable, and the disease ultimately progresses to a terminal stage called blast crisis, which resembles an acute leukemia.

The Philadelphia chromosome (Ph^1) is the cytogenetic hallmark of CML (2). It can be detected in all hematopoietic lineages and is evident in leukemic cells of both chronic and acute phase, implying that the reciprocal translocation that gives rise to the Ph^1 occurs in a stem cell (3, 4). The translocation leads to an aberrant juxtaposition of chromosome 22 *bcr* sequences with c-*abl* sequences from chromosome 9 (5) and expression of a 210-kDa *bcr/abl* fusion protein ($P210^{bcr/abl}$) (6). $P210^{bcr/abl}$ can transform a variety of hematopoietic cell types *in vitro* (7–11) and in animal model systems (12–15). However, only retroviral transfer of the $P210^{bcr/abl}$ gene into murine bone marrow induces a granulocytic leukemia that closely resembles the chronic phase of human CML (13, 15). Mice with the CML-like granulocytic leukemia succumb to their disease, probably from overwhelming granulocytosis, and our limited attempts to control the tumor load with cytotoxic chemotherapy have not been able to prolong survival (unpublished data). Thus, we have been unable to observe whether the CML-like granulocytic leukemia in primary diseased mice resembles human CML in its tendency to progress to acute leukemia.

To further study the leukemic clone, we have transplanted bone marrow from primary animals with CML into irradiated syngeneic recipients. A small number of secondary recipients have developed either CML or acute leukemias of lymphoid or myeloid phenotype. By analysis of the site of proviral integration, we demonstrate that the leukemic cells in the secondary animals derive from the clone that gave rise to CML in the primary animal. In one case, the clone marked by the *bcr/abl* provirus produced myeloid and lymphoid disease in separate secondary animals, thus verifying that the target of the initial retroviral infection was a pluripotent hematopoietic stem cell. These experiments establish that *bcr/abl* induces a CML-like syndrome in mice that recapitulates the distinctive biological features of both the chronic and acute phases of the human disease.

MATERIALS AND METHODS

Animals. Primary animals with murine CML were generated as described (13). Bone marrow for initial retroviral infection was derived from female mice, and all transplant recipients were male BALB/c mice between 6 and 12 weeks of age. The leukemic tissue in DR-1 and FR-1 was shown to be of female origin by the inability to detect Y chromosome-specific sequences in tumor samples (unpublished data). For adoptive transplantation of leukemia, leukemic cells from various sources were injected intravenously into recipient mice that had received either 900 cGy of γ-irradiation in two doses separated by 3 hr (lethal conditioning) or a single dose of 450 cGy of γ-irradiation (sublethal conditioning), as described in Fig. 1.

Histopathology and Immunohistochemistry. Histological preparation and staining of tissue specimens from diseased mice were as described (13). For immunohistochemical staining of thymus tissue from mouse HR-2, a frozen section of tissue was incubated with a rat monoclonal antibody against murine Thy-1.2 (Becton Dickinson) and then with alkaline phosphatase-conjugated mouse anti-rat IgG (Jackson ImmunoResearch) and developed with naphthol AS MX phosphate and fast blue BB (Sigma) to give a dark-blue specific reaction product (16). The nuclei were counterstained with 0.5% methyl green.

DNA Hybridization Analysis. DNA (5 μg) from tumor tissue samples was digested with *Eco*RI (or with *Bgl* II, where indicated), separated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with a relevant probe. The probe for the neomyocin-resistance gene was a 1.2-kilobase *Cla* I fragment derived from the pGD210 retroviral vector, as described (13). The probe for the murine T-cell-receptor β-chain locus was a mixture of Jβ1 and Jβ2 sequences, supplied by Astar Winoto (University of California, Berkeley, CA). The probe for the murine immunoglobulin μ-heavy-chain locus included J1–4 sequences, supplied by Mark Schlissel (The Johns Hopkins University, Baltimore).

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Abbreviations: CML, chronic myelogenous leukemia; Ph^1, Philadelphia chromosome.

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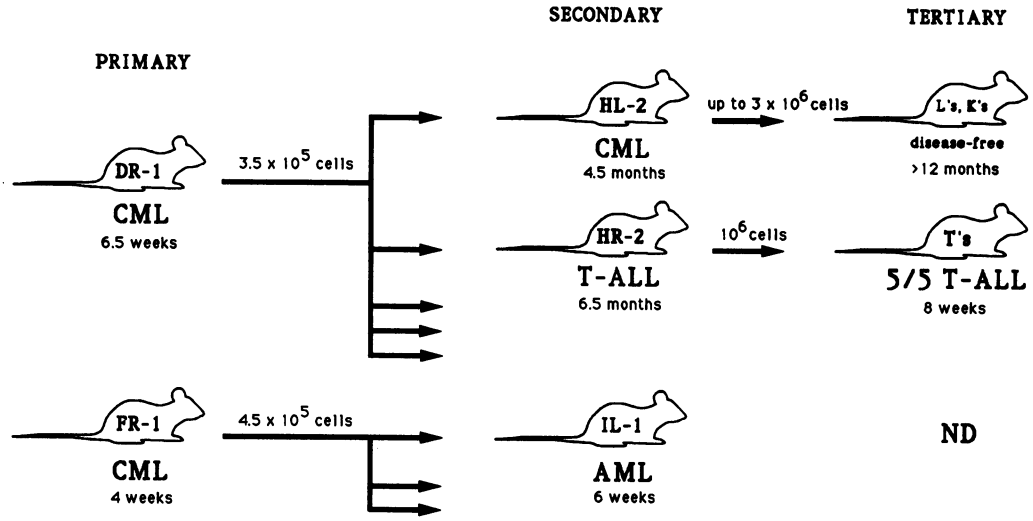

FIG. 1. Adoptive transplantation of leukemia. For the transplantation of bone marrow from DR-1, 3.5×10^5 nucleated marrow cells per mouse were injected intravenously into five mice that had received sublethal conditioning. For FR-1, 4.5×10^5 nucleated cells per mouse were injected into three mice that had received lethal conditioning. A mixture of bone marrow and spleen cells was transplanted from HL-2 into recipient animals; 3×10^6 nucleated cells per mouse were injected into three mice that had received lethal conditioning (K cage) and 1.5×10^6 nucleated cells per mouse were injected into five mice that had received sublethal conditioning (L cage). For the transplantation of tumor cells from HR-2, 1×10^6 cells from the thymic mass per mouse were injected into five unirradiated recipients. ND, transplantation of mouse IL-1 marrow into tertiary mice was not done. Disease latency (time after transplant until development of significant morbidity or death) is listed below the pathologic diagnosis for each animal. T-ALL, acute lymphoid leukemia of T-cell origin; AML, acute myeloid leukemia.

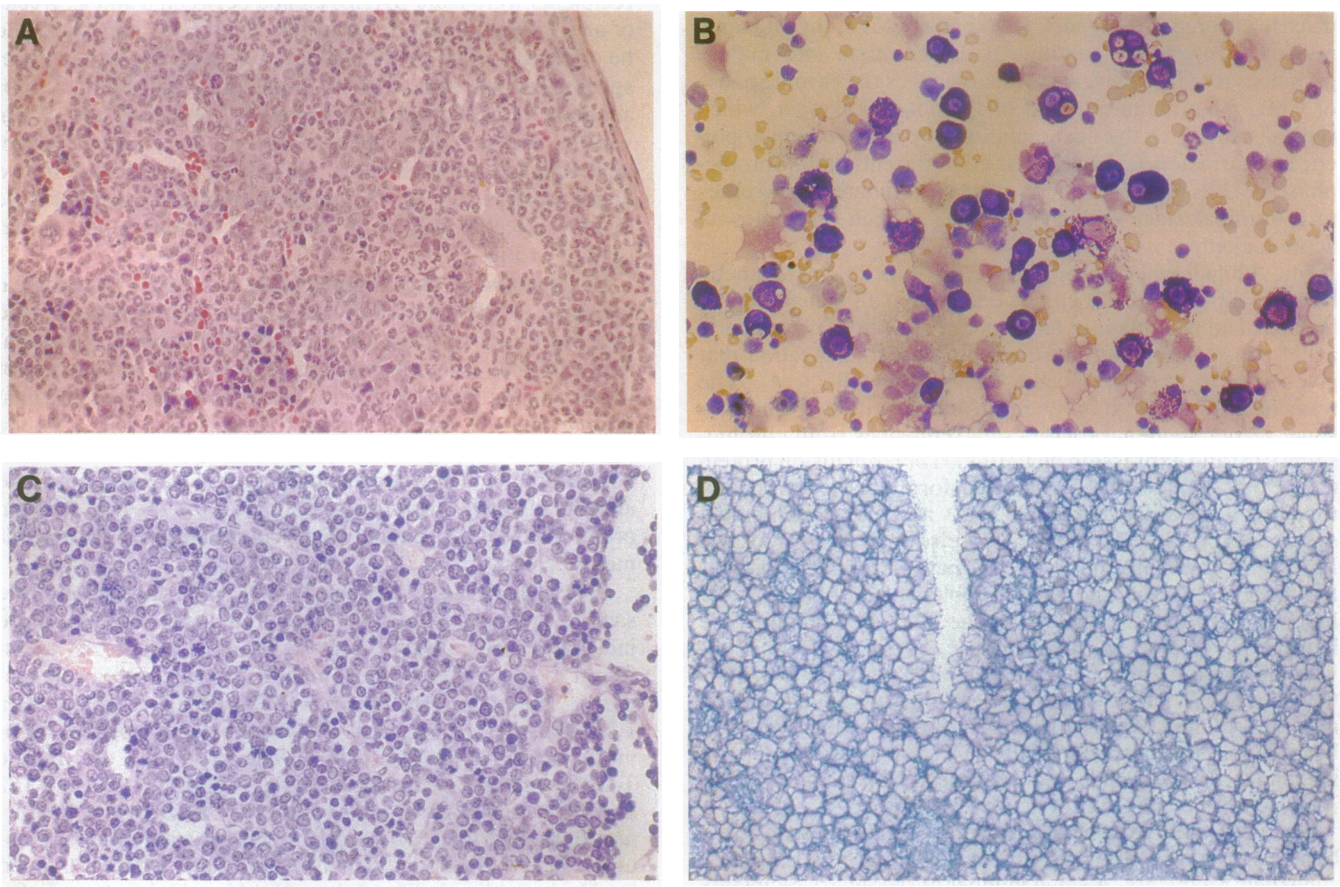

FIG. 2. Histopathologic and immunologic characterization of the tumor tissue in diseased mice. (*A*) Spleen from mouse HL-2, stained with hematoxylin and eosin, demonstrating loss of normal follicular architecture and extensive infiltration with granulocytes. (*B*) Wright–Giemsa stain of a cytospin preparation of bone marrow from mouse IL-1, showing predominance of immature basophils. (*C*) Thymic mass from mouse HR-2, stained with hematoxylin and eosin, demonstrating destruction of thymic architecture and replacement with homogenous mononuclear cell type. (*D*) Immunohistochemical stain of thymic mass from mouse HR-2 with Thy-1 antisera. The presence of the Thy-1 antigen at the cell surface is indicated by the deep-blue reaction product.

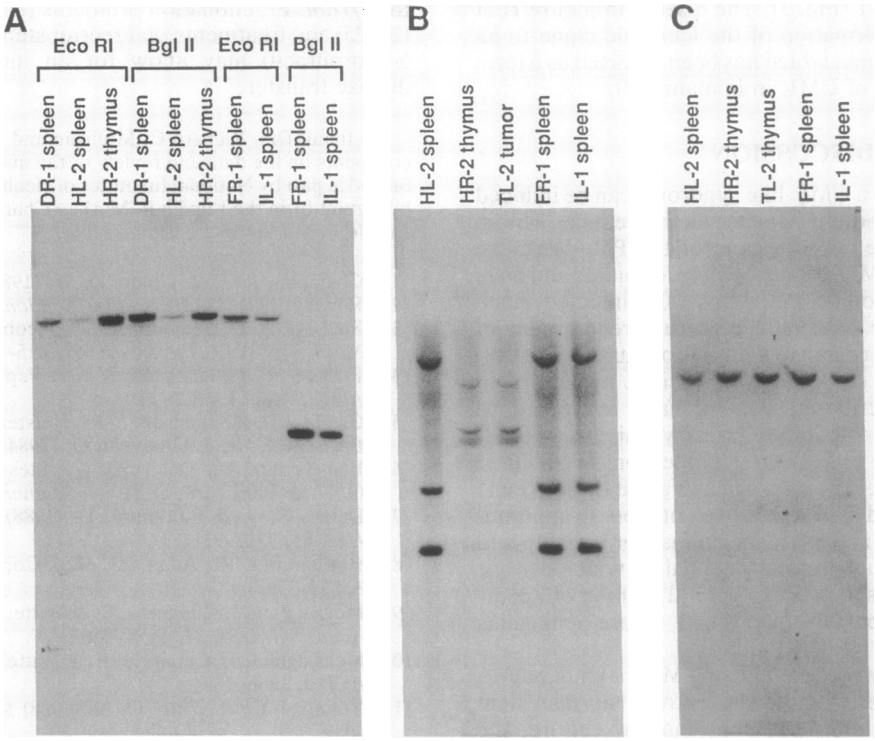

FIG. 3. DNA hybridization analysis of tumor tissue. Tumor DNA samples are as listed above each lane. (*A*) Determination of the site of proviral integration in tumor tissue. The blot was hybridized with a probe derived from neomyocin-resistance gene sequences. *Eco*RI generates a specific fragment for each site of proviral integration. Identity of the integration site was verified by a second enzyme, *Bgl* II. (*B*) Determination of the rearrangement status of the murine T-cell-receptor β-chain locus. The probe was a mixture of Jβ1 and Jβ2 sequences and detects rearrangements in tumor samples HR-2 and TL-2. (*C*) Determination of the rearrangement status of the murine immunoglobulin μ-heavy-chain locus. The probe included J1–4 sequences and verified germ-line status in all samples.

RESULTS

The scheme for transplanting marrow from primary animals with CML is shown in Fig. 1. Mouse DR-1 was diagnosed with CML on the basis of hematologic and pathologic criteria (13). Bone marrow was transplanted into five syngeneic recipients pretreated with a sublethal dose of irradiation (450 cGy). Recipient mice were monitored by periodic sampling of peripheral blood and examined for clinical signs of disease (e.g., splenomegaly, malaise). After several months of apparently normal hematopoiesis, mouse HL-2 became moribund 4.5 months after transplant. At autopsy, this mouse had an elevated leukocyte count (26,250 cells per mm^3) with a differential increase in granulocytes and immature myeloid forms and marked splenomegaly (0.54 g). Histopathologic analysis of the spleen (Fig. 2), liver, and bone marrow showed extensive accumulations of granulocytes and immature myeloid cells, consistent with a pathologic diagnosis of CML. After an initial period of apparently normal hematopoiesis, mouse HR-2 developed palpable splenomegaly and a mild leukocytosis (15,000 leukocytes per mm^3, with 50% immature cells and 40% granulocytes) 3.5 months after transplant. Over time, immature blast cells came to predominate in the peripheral blood, and HR-2 was discovered dead 6.5 months after transplant. At autopsy, HR-2 was found to have a large thymic mass (0.49 g) and leukemic blasts in the bone marrow. The leukemic cells in this animal stained positive for the T-cell marker Thy-1 (Fig. 2) and showed rearrangements at the β locus of the T-cell-receptor gene and germ-line configuration of the immunoglobulin μ-heavy-chain gene (Fig. 3). These findings are consistent with a diagnosis of acute lymphoid leukemia of T-cell origin. The remaining three mice later died with tumors of host-cell origin that did not carry the *bcr/abl* retrovirus.

Tumor tissue from the diseased secondary mice (HL-2 and HR-2) was transplanted into tertiary animals. Fourteen months after transplant, eight recipients of cells from HL-2 were free of hematologic disease and thus apparently failed to reconstitute with the leukemic clone. In contrast, each of five recipients of lymphoblasts from HR-2 rapidly developed an acute lymphoblastic leukemia similar to that in HR-2. Thus, the leukemic clones in the secondary animals HL-2 and HR-2 not only differ in phenotype but also differ upon transplantation, with that in HR-2 exhibiting markedly enhanced transplantability perhaps as a result of additional genetic alterations.

DNA from the tumor tissue of diseased mice was analyzed to determine the integration site of the *bcr/abl* provirus (Fig. 3). Leukemic cells in primary (DR-1), secondary (HL-2 and HR-2), and tertiary animals (TL-2) have the same retroviral integration, establishing that they are derived from a single infected cell. We showed previously that the retrovirally marked clone in primary mice with CML gives rise to day 14 spleen colonies (splenic colony-forming units) in secondary transplant recipients (13) and thus represents a multipotential hematopoietic progenitor cell. By repopulating both myeloid and lymphoid cell lineages (in HL-2 and HR-2, respectively), the marked clone in mouse DR-1 has properties of a pluripotent hematopoietic stem cell.

Three recipients of bone marrow from another primary animal with CML (FR-1) (13) had normal peripheral leukocyte counts at 4 weeks, but died 6 weeks after transplant. The only animal suitable for pathologic examination (mouse IL-1) was morbidly anemic (hematocrit of 5%) and pancytopenic at autopsy. On examination, the bone marrow was hypercellular, with a predominant immature basophilic or mast cell type (Fig. 2). The spleen lacked evidence of follicular structure and showed extensive replacement with basophils. The proviral integration site in DNA isolated from mouse IL-1 was

identical to that in FR-1 (Fig. 3). The disease in mouse IL-1 thus represents transformation of the leukemic clone to an acute basophilic leukemia, which has been reported as a form of myeloid blast crisis of CML in humans (17).

DISCUSSION

In mice, it appears that a CML-like syndrome can be induced by infection of a pluripotent hematopoietic stem cell with a retrovirus carrying the *bcr/abl* gene of the Ph^1. We have transplanted murine CML into secondary recipients and have observed transformation of the retrovirally infected clone from chronic to acute leukemia, a feature reminiscent of human CML. However, transplantation of murine CML to secondary recipients is surprisingly inefficient. In addition to the two animals described here, we have attempted to transfer disease from two other primary animals without success. After passage to HL-2, the clone from DR-1 failed to repopulate tertiary recipients, suggesting that the leukemic clone was not expanded by serial transplantation. In addition, the latency of disease in secondary animals is variable and can be longer than for any primary animal, in which disease occurs soon after transplant (13). Several months of apparently normal hematopoiesis intervened before leukemias appeared in HL-2 and HR-2.

The Ph^1-positive stem cell in human CML may not have a proliferative or competitive advantage over normal stem cells. When grown in long-term bone marrow culture, the Ph^1-positive clone is selectively lost in many cases and is overgrown by normal clones (18). In a few cases, chronic-phase CML patients treated with intensive chemotherapy alone or in combination with autologous marrow transplantation have achieved cytogenetic remission for significant periods (19, 20). These data demonstrate that normal stem cells exist in the bone marrow of CML patients. The accumulation of cells in human CML patients apparently occurs in more differentiated compartments (21), leading to large numbers of Ph^1-positive committed progenitor cells in the marrow. The proliferative or competitive advantage of the Ph^1-positive clone may not be manifest within the stem cell compartment itself.

In the mouse model system, the *bcr/abl*-infected stem cell may represent only a minority of stem cells, making transplantation inefficient. Marrow transplantation may reset or "reshuffle" the stem cell compartment, enabling normal clones to repopulate secondary animals. Early after bone marrow transplantation in mice, only a limited number of stem cells contribute to peripheral blood formation, while the rest presumably remain quiescent (22, 23). CML occurs early after the transfer of infected marrow into primary animals because the *bcr/abl* retrovirus infects cycling stem cells; however, disease latency in secondary animals is unpredictable. Secondary animals may not develop disease until an infected stem cell is recruited into cycle and only then would the $P210^{bcr/abl}$ protein exhibit its leukemogenic function, perhaps in a myeloid progenitor that proliferates in response to *bcr/abl*. Fractionation protocols that enrich for stem cells (23–25) and treatments that recruit stem cells into cycle (e.g., 5-fluorouracil) may allow for an increased efficiency of disease transfer.

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