Differences in Oncogenic Potency but Not Target Cell Specificity Distinguish the Two Forms of the BCR/ABL Oncogene

MICHELLE KELLIHER, AMY KNOTT, JAMI McLAUGHLIN, OWEN N. WITTE, and NAOMI ROSENBERG

Departments of Pathology, and Molecular Biology and Microbiology and the Immunology Graduate Program, Tufts University School of Medicine, Boston, Massachusetts 02111, and Department of Microbiology and Molecular Genetics, and Howard Hughes Medical Institute, University of California--Los Angeles, Los Angeles, California 90024

Received 6 May 1991/Accepted 24 June 1991

Two forms of activated BCR/ABL proteins, P210 and P185, that differ in BCR-derived sequences, are associated with Philadelphia chromosome-positive leukemias. One of these diseases is chronic myelogenous leukemia, an indolent disease arising in hematopoietic stem cells that is almost always associated with the P210 form of BCR/ABL. Acute lymphocytic leukemia, a more aggressive malignancy, can be associated with both forms of BCR/ABL. While it is virtually certain that BCR/ABL plays a central role in both of these diseases, the features that determine the association of a particular form with a given disease have not been elucidated. We have used the bone marrow reconstitution leukemogenesis model to test the hypothesis that BCR sequences influence the ability of activated ABL to transform different types of hematopoietic cells. Our studies reveal that both P185 and P210 induce a similar spectrum of hematological diseases, including granulocytic, myelomonocytic, and lymphocytic leukemias. Despite the similarity of the disease patterns, animals given P185-infected marrow developed a more aggressive disease after a shorter latent period than those given P210-infected marrow. These data demonstrate that the structure of the BCR/ABL oncprotein does not affect the type of disease induced by each form of the oncogene but does control the potency of the oncogenic signal.

The Philadelphia chromosome is a pathognomonic marker associated with chronic myelogenous leukemias (CMLs), a significant number of acute lymphocytic leukemias, and rare cases of acute myelogenous leukemia (reviewed in reference 6). As a consequence of the translocation that generates this marker, ABL proto-oncogene sequences are fused to BCR sequences (13, 19), and an activated protein tyrosine kinase, the BCR/ABL protein, is produced (30). This molecule exists in two forms, reflecting the fact that some translocations occur in the first BCR intron while others occur within the originally designated breakpoint cluster region of BCR (15, 19, 25). Both BCR/ABL proteins contain identical ABL-derived sequences, but one form, called P210, contains 927 BCR-derived amino acids, while the second, P185, contains only the first 436 of these BCR-specified residues (16, 20, 25, 40). The presence of these latter sequences is sufficient to activate the transforming potential of the BCR/ABL protein and the tyrosine kinase activity of the molecule (39, 41). Direct comparisons of P185 and P210 suggest that the additional residues present in P210 moderate both in vitro transforming potential (34, 38) and protein tyrosine kinase activity (34, 39, 41).

Although both P185BCR/ABL and P210BCR/ABL transform similar types of cells in tissue culture systems (34, 38), the human leukemias associated with BCR/ABL proteins are distinct in that they arise in different cell types and have different clinical courses (reviewed in references 2 and 45). Analyses of large numbers of patient samples have left the impression that the differences in BCR-derived sequence between P210 and P185 either preferentially target the oncogene to specific hematopoietic cell types or affect the potency of the oncogenic signal or both (reviewed in references 6 and 49). For example, CML, almost always associated with the P210 form (19, 28, 29), is initially an indolent disease of hematopoietic stem cells characterized by the overproliferation and differentiation of myeloid lineage cells (2, 18). The P185 form is most commonly associated with acute lymphocytic leukemia (3, 7, 31, 53) and acute myelogenous leukemia (4, 32), aggressive malignancies of early lymphoid and myeloid precursors, respectively. However, rare cases of P185-positive CML have been documented (50, 52), and perhaps as many as half of all Philadelphia chromosome-positive acute lymphocytic leukemias express P210 (3, 15, 48, 51).

The issues of lineage specificity and potency of transforming signal can best be addressed by direct comparison of P185 and P210 in an experimental system that recapitulates features of the human BCR/ABL-associated leukemias. In the work presented here, we have used such a system, the murine bone marrow reconstitution leukemogenesis model (12, 14, 23, 26, 27). Our analysis of mice whose depleted bone marrow was reconstituted with stem cell populations infected with either P185- or P210-expressing retrovirus vectors reveals a similar spectrum of disease, including granulocytic, myelomonocytic, and lymphocytic leukemias. However, the animals given P185BCR/ABL-infected progenitors develop a more aggressive disease after a shorter latency period than animals treated with P210-infected cells. These data demonstrate that the differences in BCR sequences that distinguish P210 and P185 control the oncogenic potential of these two proteins but do not directly determine the types of hematopoietic cells that can be transformed by BCR/ABL.

MATERIALS AND METHODS

Cells and viruses. Bone marrow cells were prepared from mice treated with 5-fluorouracil (5-FU), infected, and used
TABLE 1. Disease induction by P185BCR/ABL and P210BCR/ABL

<table>
<thead>
<tr>
<th>Form (no. of animals)</th>
<th>Disease</th>
<th>No. with diseasea</th>
<th>Leukocytes/μL</th>
<th>Avg differentialb</th>
<th>Other tumorsd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Avg</td>
<td></td>
</tr>
<tr>
<td>P185(26)</td>
<td>Granulocytic leukemia</td>
<td>5</td>
<td>12,500–192,000</td>
<td>54,340</td>
<td>15 11%L 75%M 26%G</td>
</tr>
<tr>
<td></td>
<td>Myelomonocytic leukemia</td>
<td>3</td>
<td>10,000–28,000</td>
<td>16,800</td>
<td>18 55%L 26%M 0/3 1/3 2/3</td>
</tr>
<tr>
<td></td>
<td>Lymphocytic leukemia</td>
<td>4</td>
<td>13,200–44,000</td>
<td>27,900</td>
<td>80 &lt;1%L 17%M 0/4 3/4 0/4</td>
</tr>
<tr>
<td>Pre-B lymphoma</td>
<td>11</td>
<td>1,600–17,000</td>
<td>5,600</td>
<td>45 2%L 54%M 0/4 1/4 1/4</td>
<td></td>
</tr>
<tr>
<td>Macrophage tumor</td>
<td>2</td>
<td>3,600–8,250</td>
<td>5,900</td>
<td>45 2%L 54%M NA 1/2 0/2</td>
<td></td>
</tr>
<tr>
<td>P210(23)</td>
<td>Granulocytic leukemia</td>
<td>4</td>
<td>17,500–84,000</td>
<td>51,400</td>
<td>9 8%L 92%M 0/4 1/4 1/4</td>
</tr>
<tr>
<td></td>
<td>Myelomonocytic leukemia</td>
<td>2</td>
<td>11,800–25,000</td>
<td>18,400</td>
<td>36 38%L 26%M 0/2 1/2 0/2</td>
</tr>
<tr>
<td></td>
<td>Lymphocytic leukemia</td>
<td>3</td>
<td>12,500–18,500</td>
<td>14,500</td>
<td>71 9%L 19%M 0/3 2/3 0/3</td>
</tr>
<tr>
<td>Pre-B lymphoma</td>
<td>13</td>
<td>3,000–7,000</td>
<td>4,800</td>
<td>67 8%L 26%M 1/13 1/13 1/13</td>
<td></td>
</tr>
</tbody>
</table>

a Lethally irradiated mice were given bone marrow from 5-FU-treated mice that had been infected with retrovirus stocks expressing P185BCR/ABL or P210BCR/ABL cDNAs. Animals were killed when signs of tumor were evident. Data for eight of the animals included in the P210 series were reported previously (27). Diagnosis was based on examination of the blood data and other pathology in hemato logic tissues.

b Number of animals with the particular disease of the total number of animals (26 or 23) available for evaluation. Five animals given P185BCR/ABL-infected marrow failed to survive the 2-week postreconstitution period and were excluded from the analysis; eight animals given P210-infected cells and seven given P185-infected cells died during the experiment but were not recovered in a condition suitable for examination. One animal given P210-infected cells was lost, and one died from an infection during the course of the study. Data for these animals were also excluded from the tabulation.

c Differential counts were performed on smears stained with Wright-Giemsa stain: %L, percent lymphocytes; %M, percent monocytes and macrophages; %G, percent granulocytes. Leukocyte types not listed in the table were present in the samples at a frequency of 1% or less.

d Some animals contained multiple tumor types. Mac, macrophage tumors; pre-B, pre-B lymphomas.

NA, not applicable.

for reconstitution as described previously (27). The cells were infected with stocks of either -447P185/M, a retrovirus containing a P185BCR/ABL cDNA (38), or -447P210/M, a retrovirus containing a P210BCR/ABL cDNA (11). Both stocks contained Moloney murine leukemia virus. Mock-infected animals and animals infected with Moloney virus have been evaluated earlier in our laboratory (27). As described previously (38, 41), the titer of the BCR/ABL retrovirus stocks was monitored by Southern analysis of NIH 3T3 or RAT-1 cells that had been infected for the same period of time as the bone marrow cultures with equal volumes of the two virus stocks. The relative intensity of the internal EcoRI fragment from the P210 and P185 proviruses was revealed by probing Southern blots with an ABL probe (40).

Evaluation of mice. Complete peripheral blood cell counts were performed on the marrow-reconstituted mice biweekly, beginning 2 weeks postreconstitution. Animals were monitored daily and killed when signs of disease, such as splenomegaly, lymphadenopathy, cachexia, ruffled fur, or other evidence of ill health, were noted. Gross pathologic observations were recorded, and blood, spleen, marrow, liver, lymph node, and any other tissue showing enlargement or macroscopic evidence of tumor was processed for histologic examination. Leukocytes were purified from the blood samples by Ficoll (Pharmacal Chemical Co., Piscataway, N.J.) gradient centrifugation and frozen for subsequent analysis; samples of other tissues were frozen directly.

DNA analysis. DNAs were prepared from frozen tissues by grinding the tissue into a fine powder with a mortar and pestle in a dry-ice bath. Lysis buffer (10 mM NaCl, 10 mM Tris [pH 7.5], 25 mM EDTA, 1% sodium dodecyl sulfate) was added, and the mixture was treated with 625 μg of pronase per ml for at least 2 h at 37°C. Purified leukocyte pellets were lysed directly as described above. DNAs were extracted, digested with appropriate restriction enzymes, and analyzed by Southern blotting as described previously (44). The Nytran membrane (Schleicher & Schuell, Keene, N.H.) blots were hybridized to DNA probes labeled by the random priming method (17) according to the manufacturer's suggestions. The 1-kb PstI fragment from pJW5 (40) was used as an ABL probe; the 1.9-kb ClaI fragment from pMV6TKneo (35) was used as a neo probe; the 2-kb BamHIEcoRI fragment from pJ11 was used as an immunoglobulin heavy-chain gene probe (37); the 2.7-kb HindIII fragment from pCae1 (36) was used to probe the T-cell receptor β gene; and the 1.4-kb EcoRI-SalI fragment from py2 was used as a Y chromosome probe (33).

RESULTS

Animals given P185- or P210-infected 5-FU-treated marrow develop an identical disease spectrum. Lethally irradiated animals were given bone marrow from 5-FU-treated mice that had been infected with matched retrovirus stocks expressing either P185BCR/ABL or P210BCR/ABL and were then monitored for disease. Autopsy and histologic analyses revealed that 26% of the animals given P210BCR/ABL-infected marrow and 39% of those treated with P185BCR/ABL-infected cells developed diseases involving cells of the myeloid lineage (Table 1). Included in this group are animals that showed total peripheral leukocyte counts of greater than 10,000 cells per μl and differential counts demonstrating the presence of abnormal numbers of mature granulocytes or macrophages. Most of the animals given P185-infected bone marrow in this group displayed marked splenomegaly, primarily reflecting the presence of large macrophage tumors in this organ (Fig. 1A and B), a feature that was less prominent in the P210-infected animals with granulocytic disease. Marked granulocytic proliferation and trilineage proliferation and differentiation, hallmark of the granulocytic disease induced by P210BCR/ABL (12, 27), were also prominent in the spleens of these mice (Fig. 1C). Animals in both groups also showed evidence of lymphoblastoid tumor cells in the peripheral lymph nodes. In addition to diseases with an obvious leukemic component, a small number of animals in both groups developed splenic tumors containing macrophages either as the only obvious pathology or coincident with pre-B lymphoma.

A significant number of animals developed diseases in which the dominant pathology involved lymphoid cells. While a disease identical to typical Abelson virus-induced pre-B lymphoma (46) was observed in many of these ani-
mals, some animals in both groups developed lymphocytic leukemias (Table 1). Unlike typical pre-B lymphomas induced by activated ABL genes, in which tumor cells are rarely observed in the circulation, animals with this disease had elevated numbers of lymphoblasts in the peripheral blood (Fig. 1D). Histologic examination of tissues from most of these mice revealed a picture similar to that observed in pre-B lymphoma, with large numbers of lymphoblasts in the spleen, lymph node, and bone marrow (Fig. 1E and F and not shown). The thymus was spared in all cases. Consistent with this picture, analysis of DNA prepared from the leukemic cells with probes for the immunoglobulin heavy-chain locus and the T-cell receptor β locus indicated that they were related to the B-lymphocyte lineage (data not shown).

The pathologic examinations revealed that both forms of BCR/ABL induced the same spectrum of hematologic diseases (Table 1). Although classically associated with leukemias involving lymphoid precursors (45), the P185 form of BCR/ABL actually induced a slightly higher frequency of myeloid disease than the P210 form in the group of animals studied. In addition, the pattern of lymphoid disease observed in animals given P210- and P185-infected bone marrow was similar, with pre-B lymphomas and lymphocytic leukemias occurring in both groups. Thus, the sequence differences that distinguish P185 and P210 do not modulate the type of disease each oncogene induces when they are tested in an identical setting.

P185BCR/ABL induces more rapid and aggressive leukemias than P210. Although the disease spectrum observed in the P210- and P185-reconstituted animals is similar, the latent period required for evidence of disease was not identical. Comparison of animals inoculated with matched stocks of the P210 and P185 retroviruses revealed that animals given P185BCR/ABL-infected marrow exhibited leukocytosis about 4 weeks earlier than those given P210-infected cells (data not shown). Most of these mice also developed disease sooner than the P210-treated animals (Fig. 2A), with 50% of the animals succumbing by day 49 postreconstitution, compared with 71 days for the P210-reconstituted animals. The difference in latent period was particularly striking for the granulocytic leukemias, which developed during a much narrower time window than the other diseases in both sets of mice (Fig. 2B). Because the P210 and P185 stocks are matched for titre by assays that measure infectivity in rodent fibroblast cells, we also evaluated animals given bone marrow that had been infected with a 10-fold dilution of the P185 retrovirus stock. Even under these circumstances, the P185-treated mice developed disease more rapidly than those treated with P210-infected bone marrow (Fig. 2A).

Histologic examinations of tissues revealed a second difference between animals given P210- and P185-infected bone marrow. The animals given P185BCR/ABL-infected bone marrow developed more invasive tumors. This difference was particularly evident in the liver, where extensive granulocyte and macrophage infiltration was observed in 10 of 11 of the P185-treated animals with myeloid disease (Fig. 3A and B). In contrast, infiltration was observed in only one of six of the P210-treated animals with myeloid disease, and most animals had little evidence of tumor cells in the liver (Fig. 3C and D). These data, and those derived from the mortality curve, are consistent with the idea that P185BCR/ABL delivers a more potent transforming signal than the P210 form of the oncogene.

P185BCR/ABL, like P210, induces clonal tumors that can arise from hematopoietic progenitor cells. The histologic and latent period assessments suggest that P185BCR/ABL delivers a more potent oncogenic signal than P210. However, this interpretation is based on the assumption that the P185 provirus is

FIG. 1. Histopathological examination. (A) Representative hematoxylineosin-stained sections from a mouse given P185BCR/ABL-infected bone marrow that developed granulocytic leukemia illustrate the expansion of the red pulp and the presence of a tumor mass. (B) The morphology of macrophages in the tumor mass and (C) the extensive trilineage expansion and differentiation observed in animals with granulocytic and myelomonocytic leukemia, demonstrated by the presence of large numbers of mature granulocytes, megakaryocytes, and erythroid cells. (D) Representative Wright-Giemsa-stained peripheral blood smear and (E) a hematoxylin-eosin-stained spleen section showing expanded white pulp, reflecting lymphoblastoid cell proliferation (F) from an animal with lymphocytic leukemia. Magnification: (A and E) × 100; (B, C, D, F) × 400.
FIG. 2. P185<sub>BCR/ABL</sub>-treated mice develop tumors more rapidly than P210-treated mice. Lethally irradiated adult BALB/c mice were given 10<sup>7</sup> cells infected with matched stocks of P185 (○) and P210 (□) retroviruses or a 10-fold dilution of the P185 stock (●). Animals were monitored daily and killed when disease was evident. (A) Each point represents a single animal, and the percent tumoried is calculated from the total number of animals that could be evaluated in each experiment, as detailed in Table 1, footnote b. Including data for mice found dead after the initial reconstitution phase does not alter the shape of the curves (not shown). (B) Only animals diagnosed with granulocytic leukemias (P185 [○] or P210 [□] and pre-B lymphomas (P185 [●] or P210 [■]) are represented. The data are pooled from six separate experiments with undiluted P185 virus, four separate experiments with P210 virus, and two separate experiments with diluted P185 virus.

Southern blot analysis of EcoRI-digested DNAs with a neo probe (35), which reveals the provirus in the context of flanking cellular sequences. In addition, the intensity of the proviral bands was compared with a standard dilution series to give an indication of the frequency of cells in the tumor sample that contained the provirus. These studies, similar to those reported for animals given P210<sub>BCR/ABL</sub>-infected marrow (27), revealed that all four samples of peripheral blood from animals with myeloid leukemias contained a dominant fragment of an intensity similar to that observed for one copy of <i>BCR/ABL</i> in a standard cell line (Fig. 4B). Analysis of DNAs from tumor tissue and peripheral blood revealed that the tumor cells present at different sites often arose from a single clone of cells (Fig. 4C). In several of these cases, samples from different tissues contained different types of hematopoietic tumor cells. For example, in two cases of granulocytic leukemia, peripheral blood samples contained predominantly granulocytes, while macrophage tumors were present in the liver and spleen. Analysis of the proviral integration sites in these tissues revealed that all of the tumor tissue and the peripheral blood contained the same single dominant <i>BCR/ABL</i>-infected clone (Fig. 4C). Thus, these two distinct tumor types arose from an infected cell that, at a minimum, could differentiate into macrophages and granulocytes.

DISCUSSION

Our work represents the first instance in which the oncogenic potential of P210<sub>BCR/ABL</sub> and P185<sub>BCR/ABL</sub> has been compared directly under conditions that favor development of a broad range of hematologic malignancies. Because both forms induce a similar spectrum of disease in the bone marrow reconstitution leukemogenesis model, the <i>BCR</i> sequences that distinguish them do not control the selection of particular types of hematopoietic cells. The idea that the precise structure of activated abl genes does not control the
outcome of the cell-virus interaction is supported by a wide variety of in vitro experiments. For example, virtually all forms of activated ABL genes tested transform pre-B lymphoid cells in vitro (1, 38, 47) and abrogate the requirement for certain interleukins in other tissue culture cells (8–10, 21, 42, 43). In addition, infection of bone marrow from 5-FU-treated mice with Abelson virus or retroviruses expressing either form of BCR/ABL induces growth of both lymphoid colonies and granulocyte-macrophage colonies in vitro (27a). This parallel may extend to the v-abl form of the oncogene in the reconstitution model, where diseases with a presentation similar to those observed with BCR/ABL have been documented (27). However, tests for v-abl provirus integrations in peripheral blood granulocytes have not yet been conducted, and the granulocytosis observed may result from indirect effects of the disease process (14). Indeed, both the fms and src oncogenes induce multiple hematologic malignancies in the reconstitution leukemogenesis model (23, 26). However, only those associated with fms appear to be similar to the spectrum of disease associated with BCR/ABL.

The human malignancies associated with BCR/ABL expression are distinct in clinical course and probably involve different target cells. P185 expression is almost always associated with human leukemias involving proliferation of early hematopoietic progenitors that fail to differentiate (45). In contrast, P210 is most commonly found in a chronic leukemia initially characterized by quasi-normal differentiation (2). However, our results demonstrate that both P210 and P185 expression can stimulate maturation of clonal populations of myeloid cells. Furthermore, both oncogenes can also induce proliferation of B-lineage precursors that are probably arrested in their differentiation. A likely explanation for these apparently contrasting results is that the impact of BCR/ABL expression on growth and differentiation depends in large part on the cell in which the oncogene is expressed. The ability of v-abl to stimulate either malignant transformation or normal differentiation, depending upon the cell type in which it is expressed (54), lends experimental support to this point of view, as do results obtained with transgenic animals expressing various forms of ABL under the control of different promoters (22, 24).

The exact target cells involved in the diseases studied here remain a subject of intense investigation. In some animals with multiple tumor types, analyses of provirus integration patterns suggest that the lymphoid malignancies arise from clones distinct from those contributing to myeloid disease (unpublished data). The same analyses reveal that the CML-like disease arises in a cell that is at least a bipotential myeloid progenitor. Indeed, the fact that the trilineage proliferation and differentiation seen in some animals involve a single clone of BCR/ABL-infected cells suggests that an even earlier precursor gives rise to the malignant clone in some cases. Such an interpretation is consistent with several lines of indirect evidence supporting infection of multipotential cells in the bone marrow reconstitution leukemogenesis model (5, 12, 27).

P185BCR/ABL is most commonly associated with highly aggressive, acute leukemias in humans (6). Consistent with this association, the animals given P185-infected bone marrow developed disease after a shorter latent period than those given P210-infected cells. This difference is particularly striking for the granulocytic leukemias that resemble
chronic-phase CML. The results of the dilution experiment demonstrate that this difference is not a function of the titer of the infecting virus stock. Therefore, the P185 protein must deliver a more potent transforming signal to the cell. This interpretation is consistent with the ability of P185 to transform cells more readily in a variety of in vitro cultures systems (34, 38). This property of P185*BCR/ABL* may stem from its having a higher protein tyrosine kinase activity than P210 (34). Alternatively, P185 and P210 may differ in their ability to interact effectively with important cellular substrates. Either of these features might increase the probability that cells stimulated with P185 would continue to cycle, providing additional opportunities for secondary events that are important in the tumorigenic process.

ACKNOWLEDGMENTS

We thank John Coffin and David Schenkein and the members of our laboratories for suggestions on the manuscript and Michael Thursby for preparing the figures.

This work was supported by Public Health Service grants CA 33771 (N.R.) and CA 27507 (O.N.W.) from the National Institutes of Health. O.N.W. is an Investigator of the Howard Hughes Medical Institute.

REFERENCES


