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In vitro transformation of immature hematopoietic cells by the P210 *BCR/ABL* oncogene product of the Philadelphia chromosome

(chronic myelogenous leukemia/protein tyrosine kinase)

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ABSTRACT **The Philadelphia chromosome [t(9;22)-(q34;q11)] is the cytogenetic hallmark of human chronic myelogenous leukemia. RNA splicing joins sequences from a gene on chromosome 22 (*BCR*) across the translocation breakpoint to a portion of the *ABL* oncogene from chromosome 9, resulting in a chimeric protein (P210) that is an active tyrosine kinase. Although strongly correlated with this specific human neoplasm, and implicated as an oncogene by analogy to the gene product of the Abelson murine leukemia virus, the P210 gene had not been tested directly for oncogenic potential in hematopoietic cells. We have used a retroviral gene-transfer system to express P210 in mouse bone marrow cells. When infected bone marrow is plated under conditions for long-term culture of cells of the B-lymphoid lineage, cells expressing high amounts of P210 tyrosine kinase dominate the culture and rapidly lead to clonal outgrowths of immature lymphoid cells. Expression of P210 is growth-stimulatory but not sufficient for full oncogenic behavior. Some clonal lines progress toward a fully malignant phenotype as judged by increased cloning efficiency in agar suspension and frequency and rapidity of tumor induction in syngeneic mice. Such *in vitro* systems should be useful in evaluating the sequential and perhaps synergistic involvement of the P210 gene and other oncogenes as models for the progressive changes observed in human chronic myelogenous leukemia.**

Human chronic myelogenous leukemia (CML) is a progressive disorder originating from an abnormal clone of a pluripotent hematopoietic stem cell (1). During the chronic phase, myeloid elements predominate in the marrow and peripheral blood, but cells of other lineages are also affected. Patients progress toward a more aggressive disease culminating with a blast crisis of a single lineage—usually immature myeloid or pre-B lymphoid cell types (2). Progeny of the affected stem cell at all stages of the disease routinely contain the Philadelphia chromosome [Ph[1], t(9;22)(q34;q11)] (3, 4). During blast crisis, secondary cytogenetic alterations, including duplication of Ph[1], are commonly observed (2). Ph[1] likely provides an essential growth-promoting change in the affected stem cell and its progeny that is inductive, permissive, or complementary for further genetic changes required for tumor progression resulting in a blast crisis.

A dramatic molecular consequence of Ph[1] is the structural alteration and activation of the *ABL* oncogene and its tyrosine kinase activity. The translocation creates a chimeric gene between a specific subset of the exons of a gene of unknown function on chromosome 22 called *BCR* (breakpoint-cluster region) and a majority of the exons of the cellular *ABL* oncogene from chromosome 9 by the process of mRNA splicing (5, 6). A large [>8.5-kilobase (kb)] chimeric mRNA with 5′ BCR-ABL 3′ structure results that encodes a chimeric 210-kDa protein, called P210 BCR/ABL, with intrinsic tyrosine kinase activity derived from the *ABL* gene segment (7, 8). The similarity of the *BCR/ABL* gene product to the chimeric *gag/abl* protein expressed by the Abelson murine leukemia virus (Ab-MuLV) suggests that P210 is a key determinant of pathogenesis in CML. The viral *gag/abl* gene product is capable of acute transformation of established rodent fibroblast cell lines (e.g., mouse NIH 3T3 cells) and a wide range of hematopoietic growth stimulation and transformation (reviewed in ref. 9).

To test the hypothesis that *BCR/ABL* is an active oncogene, we have cloned cDNA copies of the chimeric message that encodes the complete P210 gene product (10) and used them to prepare murine retroviral vectors that express P210 at high levels following transfection or infection of susceptible cells. In contrast to Ab-MuLV, the P210-expressing retrovirus was not capable of transforming NIH 3T3 cells unless the amino-terminal portion of the *BCR* segment was recombined with the Moloney murine leukemia virus (Mo-MuLV) *gag* gene during cotransfection procedures (11). However, by combining retroviral infection with a long-term murine bone marrow culture system selective for growth and differentiation of the early phase of the B-lymphoid lineage (12, 13), we can demonstrate the growth-promoting effects of the P210 gene product on immature hematopoietic elements. This system should be useful for future efforts to discern the cellular specificity of P210 growth stimulation and interactions with other oncogenes.

MATERIALS AND METHODS

Viral Stocks and Cell Culture Conditions. Details of the construction of pJW-RX from the parent retroviral vector pMCV-6 (14) and *BCR/ABL* cDNA clones 172/215 (10) have been described (11). Briefly, recombinant cDNA clones representing the complete coding sequence for P210 and approximately 150 base pairs (bp) of 5′ untranslated region were inserted into the *Eco*RI site of retroviral vector pMV-6(TK-neo), provided by B. Weinstein (Columbia University). This results in a Mo-MuLV-derived long terminal repeat acting as the transcriptional control for the *BCR/ABL*-containing genome-length mRNA and an internal herpes virus-derived thymidine kinase gene (*TK*) promoter segment driving a bacterial neomycin gene (*neo*) to confer resistance to antibiotic G418 in mammalian cells. Viral stocks were rescued by cotransfection with Mo-MuLV DNA into NIH 3T3 cells or direct transfection into the packaging line ψ-2 (15). G418-resistant colonies were selected and screened for high-level virus production by RNA dot blots and titer on NIH 3T3 cells, followed by tyrosine kinase activity (11). Stocks with effective titers $>2 \times 10^6$ were used in all experiments. Infection of freshly explanted mouse bone

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Abbreviations: CML, chronic myelogenous leukemia; Ab-MuLV, Abelson murine leukemia virus; Mo-MuLV, Moloney murine leukemia virus; Ph[1], Philadelphia chromosome.

marrow and establishment of long-term B-cell-lineage cultures were described (13, 16–18). Marrow was infected for 3 hr at 2×10^6 cells per ml with P210-expressing or control virus and plated in RPMI 1640 medium supplemented with 5% fetal bovine serum and 50 μM 2-mercaptoethanol at 37°C. Cultures were aspirated and fed twice weekly.

Protein and Nucleic Acid Analysis. Extraction, immunoprecipitation, autophosphorylation with [γ-^{32}P]ATP, $NaDodSO_4$/polyacrylamide gel electrophoresis, and autoradiography of P210 and control Ab-MuLV-encoded protein P160 from cultured cells was as previously described (7), using site-directed rabbit anti-abl sera pEX-5 (19) or anti-abl exon 2 (A. Pendergast, A. Mes-Masson, Y. Ben-Neriah, D. Baltimore and O.N.W., unpublished data). Cells were incubated with [^{35}S]methionine, extracted, immunoprecipitated, and analyzed with rabbit anti-mouse immunoglobulin for analysis of μ heavy chain production (16) or with rabbit anti-serum to terminal deoxynucleotidyltransferase (20) as previously described. All probes for DNA or RNA analyses were labeled by nick-translation (21) with [α-^{32}P]dATP. Preparation and analysis of cytoplasmic RNA and high molecular weight DNA was as described (18). Probes and restriction enzyme digestions are described in the figure legends.

Growth in Soft Agar and Assay of Tumorigenicity. Cell suspensions were seeded in soft agar medium at 10^3 cells per dish either with or without a stromal feeder underlayer as described (17, 18) and evaluated 12–14 days later for macroscopic colonies. Cells for assay of tumorigenicity were washed with serum-free medium. Cells (2×10^6) were injected intraperitoneally into 4-week-old BALB/c mice. Animals were observed for 12 weeks and were killed if visible or palpable tumor mass at the site of injection or in lymphoid organs was observed. Tumors were extracted for DNA analysis to monitor the position and structure of their integrated retroviral genomes.

RESULTS

P210-Expressing Cells Dominate Lymphoid Bone Marrow Cultures. Fresh femoral bone marrow from 3- to 4-week-old BALB/c mice was infected with a P210-expressing retrovirus construct rescued with replication-competent Mo-MuLV or released from the helper-virus-free packaging cell line ψ-2 (ref. 15; Fig. 1). The P210 coding sequences are expressed from a Mo-MuLV long terminal repeat that is transcriptionally active in both fibroblastic cell lines and a wide variety of hematopoietic cell types including immature B cells (9, 22). Infected or control uninfected marrow specimens (10^6 cells per ml) were plated in the long-term B-lineage liquid culture conditions developed by Whitlock and Witte (12). Little difference in growth pattern between infected and control marrow was observed over the first 3 weeks of culture, during which time most of the hematopoietic elements die and disappear from the culture, and a stromal or feeder layer develops. This stromal layer provides the essential growth factors and cell–cell interactions necessary for the regrowth, maintenance, and expansion of the B-cell precursors and more differentiated elements of the B-cell lineage (13, 23, 24). By week 4, control cultures (uninfected or Mo-MuLV-infected bone marrow) had multiple small foci of hematopoietic cells growing on top of the stromal layer; these foci expanded and eventually covered the surface of the monolayer over the next 2 weeks. Cell densities of 2–4 $\times 10^5$ per ml in control cultures were routinely observed. Cell populations in control cultures are mixtures of immature B-lineage cells, pre-B cells, and B cells with diverse immunoglobulin gene rearrangements (25). Such cell populations are nontumorigenic and functional for reconstitution of diverse B-cell immune responses in immunodefective mouse strains like CBA/N or CB17 SCID (26, 27).

Cultures infected with the P210-expressing virus also developed foci of hematopoietic cells around weeks 3–4 but grew to higher densities (4–10 $\times 10^5$ cells per ml) between weeks 4 and 6. Populations examined by Wright–Giesma staining were composed of large lymphoblastoid cells with scant cytoplasm (data not shown). No significant difference in culture establishment or growth parameters was observed for cultures infected with P210 plus helper virus stocks (M1, M2, M3, and M4) versus P210 helper-free stocks (12B1, 8B1, and 8B2). By weeks 4–5 and thereafter, all P210-infected cultures were expressing high levels of the P210 protein tyrosine kinase activity as monitored by an immunoprecipitation–autophosphorylation assay (Fig. 2). Culture 8B1 expressed a slightly smaller form of the P210 protein, which lacked serological reactivity with an antiserum specific for the carboxyl terminus of the *ABL* gene product (anti-pEX-5; ref. 19). This was likely due to an internal deletion of the retroviral genome that truncated the P210 coding sequences and removed most of the *TK-neo* gene in this instance (see DNA and RNA blot analyses below). In all other cases examined (15 independent cultures), we observed stable expression of an unaltered P210 protein. We found no evidence of recombination of P210 with viral *gag* or cellular genes in these infections. The levels of P210 protein and

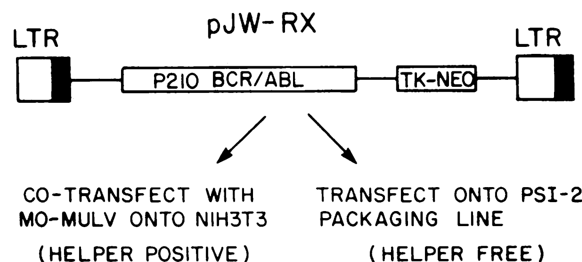

FIG. 1. P210 expression vector and rescue strategies. Structure of retroviral expression vector for P210 BCR/ABL (pJW-RX; ref. 11) is shown in proviral form. LTR is the viral long terminal repeat, and *TK-neo* is the bacterial neomycin gene driven by the herpesvirus thymidine kinase promoter. Virus is rescued by cotransfection of vector in plasmid form with Mo-MuLV DNA into NIH 3T3 cells or alone into the packaging line ψ-2 (15). Clones selected for resistance to G418 (0.5 mg/ml) were expanded and screened for high-titer transmission of P210-expressing virus (11).

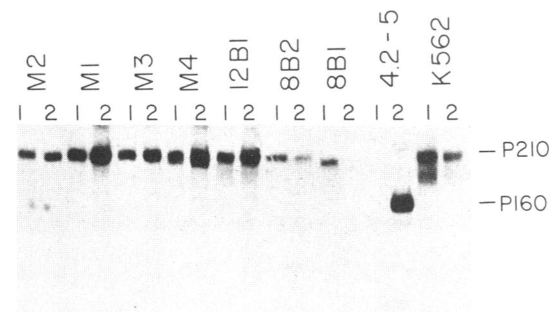

FIG. 2. Expression of P210 tyrosine kinase. Approximately 1.5 $\times 10^6$ nonadherent cells from independent cultures M2, M1, M3, and M4 (derived from P210 stocks rescued with Moloney helper virus) and 12B1, 8B2, and 8B1 (derived from ψ-2-packaged P210 stocks) at 4–5 weeks postinfection were extracted and immunoprecipitated for tyrosine autophosphorylation assay (7) using site-directed antisera specific for ABL exon 2 (lanes 1) or the carboxyl-terminal 15 kDa of the c-*abl* protein (lanes 2). Samples were analyzed by $NaDodSO_4$/7% polyacrylamide gel electrophoresis and visualized by autoradiography for 30 min. Control cell lines include the Ab-MuLV transformed B-cell line 4.2-5, which expresses P160, the human CML-derived line K562, which expresses P210.

tyrosine kinase activity were comparable in most cases to those expressed in Ab-MuLV-infected lymphoblastoid cells lines like 4.2-5 (Fig. 2; ref. 28).

Although clearly growth-stimulated compared to the uninfected control cultures, P210-expressing populations grew less vigorously than cultures infected with wild-type Ab-MuLV strains like P160 or P120 (12) or cultures doubly infected with *ras*- and *myc*-expressing retroviruses (18), which reach cell densities of 3–5 × 10^6 per ml over the first 2–4 weeks after infection. P210-infected lines required the presence of the bone marrow stromal layer for continuous growth *in vitro* even when assayed 12 weeks after infection. Almost all Ab-MuLV—infected cultures can be weaned from the feeder layer within 1–2 months (12).

Infection with P210-Expressing Virus Results in Clonal or Pauciclonal Outgrowths of Immature B-Lymphoid Cells. High molecular weight DNA was prepared from seven independent P210-infected cultures between weeks 4 and 6 after infection, digested with an appropriate restriction endonuclease, and analyzed by blot hybridization to determine clonality and stage of lymphoid differentiation on the basis of viral integration sites and immunoglobulin and T-cell antigen-receptor gene rearrangements. Most of the lines showed a single viral integration site, indicating clonal dominance even at this early stage of culture (Fig. 3*A*), when analyzed using a *TK-neo* probe and a restriction enzyme that cuts uniquely in flanking cellular DNA and internally in the viral genome. Lines 8B1 and 8B2 showed three and two integrated genomes, respectively, which could represent multiple infections per cell or a mixture of a small number of independent clones. The weak hybridization in line 8B1 is probably due to partial deletion of the *TK-neo* segment, since there is abundant *ABL* tyrosine kinase expressed (Fig. 2) and viral RNA detected with an *ABL* probe (see below).

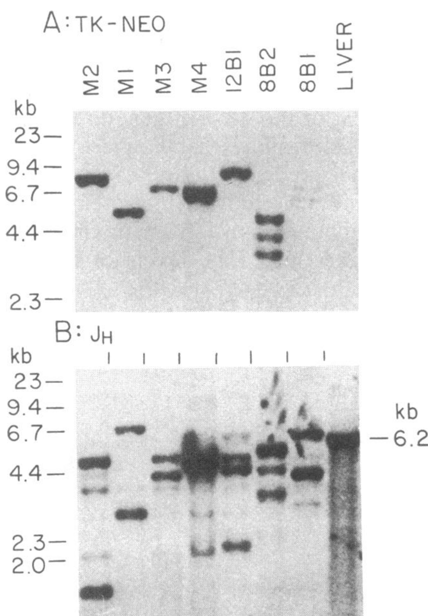

FIG. 3. Proviral integration and immunoglobulin gene rearrangements. (*A*) High molecular weight DNA from P210-infected lines and normal BALB/c mouse liver was digested with *Hin*dIII, fractionated in a 0.8% agarose gel, transferred to nitrocellulose, and probed with a 2-kb fragment of the *TK-neo* insert from vector pMV-6(TKneo) (14) to detect viral integration sites. (*B*) DNA digested with *Eco*RI was fractionated as above and probed with a mouse J_H probe (29) to detect DJ and VDJ rearrangements of the μ immunoglobulin heavy chain loci. (D, J, and V represent diversity, joining, and variable gene segments.) The germ-line 6.2-kb fragment seen in liver DNA is noted. Size markers at left are from bacteriophage λ DNA digested with *Hin*dIII.

All of the lines showed rearrangement at both alleles of the immunoglobulin heavy chain joining (J_H) region (Fig. 3*B*), characteristic of B-lineage cells. Generally, two dominant rearrangements were seen, but additional rearranged fragments, perhaps representing secondary rearrangements from a single original clone or a mixture of two or more independent clones, could be detected. We have not determined precisely which of these fragments represent DJ rearrangements and which represent VDJ rearrangements. In two of the seven lines tested (M4 and 12B1) synthesis of μ heavy chain was barely detectable by metabolic labeling with [^{35}S]methionine and immunoprecipitation (data not shown), and none of the lines expressed high levels of μ mRNA (see Fig. 4). None of the lines showed rearrangements at the κ light chain J loci or T-cell-receptor β-chain loci, but two of the clones showed rearranged fragments of the T-cell γ-chain family (data not shown). This rearrangement pattern is typical of very immature B-lineage cells obtained by transformation of susceptible target cells in fetal liver or bone marrow with Ab-MuLV or with *ras* or *src* oncogenes expressed from murine retroviral vectors (9, 18, 30, 31).

All of the P210-stimulated lines expressed high levels of *ABL* mRNA of retroviral genome length (≈9.5 kb) except line 8B1, which expressed a 7.5-kb form (Fig. 4*A*). The level of P210 retroviral message was equal to or greater than that from the Ab-MuLV-transformed B-cell line 4.2-5 and greater than the level of the 8.5-kb *BCR/ABL* mRNA from the CML-derived cell line K562. Some variation was due to differences in amount of RNA per lane, as evidenced by hybridization with a probe for a "housekeeping" gene (Fig. 4*F*). The normal murine and human cellular *abl* mRNAs (37) were not detected at this level of exposure of the autoradiogram due to their low copy number. A *TK-neo* probe detected the same P210-retrovirus-derived genome-length RNAs and a small amount of a *TK-neo* RNA in all P210-infected lines except for 8B1. *TK-neo* subgenomic mRNA expression is low, probably because these lines were not selected in medium containing G418. No hybridization was seen for the control cell lines or spleen RNA, as expected (Fig. 4*B*). These data and Fig. 2 demonstrate that high levels of the P210 retroviral mRNA and gene product are stably expressed in these cells.

Each of the P210-infected lines expressed mRNA sequences reactive with a probe for the T200/B220 family of lymphocyte-specific glycoproteins (ref. 33; Fig. 4*C*). Many of the lines expressed the B-cell-specific epitope of the B220 molecule detected with monoclonal antibody 14.8 (38) (data not shown). Authentic μ mRNA species were easily detected from the surface-IgM-positive B-cell line 4.2-5 and spleen (Fig. 4*D*), and all of the P210 lines showed low levels of two μ-reactive RNAs of similar size, neither of which appeared to correlate with variation in level of μ protein expression as described above. None of the p210 lines showed detectable κ mRNA species (Fig. 4*E*). All of the P210-infected lines showed high levels of the lymphoid-specific marker, terminal deoxynucleotidyltransferase, by immunoprecipitation analysis compared to a thymus control (Fig. 5). These results support the conclusion that the phenotype of the P210-infected lines is that of immature lymphoid cells of the B lineage.

Cell Lines Infected with P210-Expressing Retrovirus Show Variation in Oncogenic Behavior. P210-infected lines were tested after 15 weeks of continuous culture for cloning efficiency in agar media plated with or without stromal feeder layers present (Table 1). In general, the cloning efficiencies were quite low. They ranged from 0% to 15.5%. This is similar to that observed with wild-type Ab-MuLV-infected cultures very early after infection but should be contrasted with the cloning efficiency of established Ab-MuLV-transformed lines, which can be as high as 70–90% in the same assay (12, 13). Low agar-cloning efficiency was reflected in

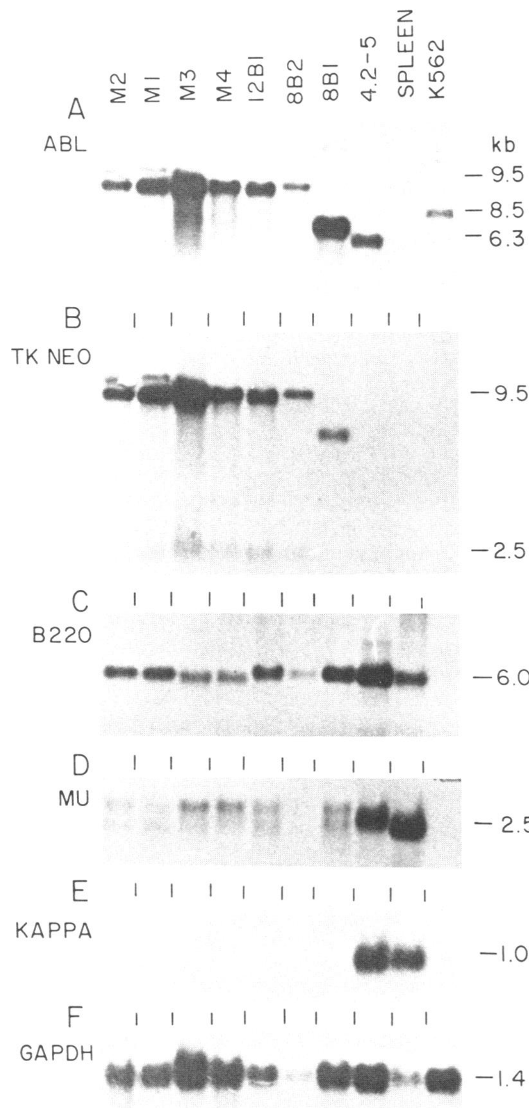

FIG. 4. Transcription of retroviral and lymphoid-specific RNAs. Total cytoplasmic RNA was isolated from P210-infected lines, Ab-MuLV-transformed B-cell line 4.2-5 (28), BALB/c mouse spleen, and K562 [a human CML-derived line of early erythroid phenotype (32)]. The RNA samples (10–20 μg per lane) were denatured and electrophoresed in a formaldehyde-containing 1% agarose gel and then transferred to nitrocellulose (19). (*A*) Probed with human *ABL* sequences from clone 215 (10); autoradiographic exposure time 24 hr. Full-length 9.5-kb P210 retroviral transcript, 6.3-kb Ab-MuLV P160 strain genomic RNA, and 8.5-kb Ph[1]-derived *BCR/ABL* RNA are noted. (*B*) Probed with *TK-neo* fragment as in Fig. 3; exposure time 24 hr. P210 retroviral genome (9.5 kb) and subgenomic *TK-neo* RNA (2.5 kb) are noted. (*C*) Probed with a 400-bp *Hgi* I-*Eco*RV fragment of a cDNA clone (T200) reactive with the T200/B220 family of lymphocyte-specific glycoproteins (33); exposure time 40 hr. Two closely migrating species of about 6.5 kb are noted. (*D*) Probed with a 400-bp fragment (PstBM12) of the constant region of the μ heavy chain gene (34) reactive with both the membrane (2.7-kb) and secreted (2.4-kb) forms of μ mRNA; exposure time 24 hr. (*E*) Probed with a 2.7-kb *Hin*dIII–*Bam*HI fragment of the κ light chain constant region gene (35) that detects the family of ≈1-kb κ RNAs; exposure time 4 hr. (*F*) Probed with the 200-bp fragment of the rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (36) expressed in all cells and used as a monitor of RNA concentration in each preparation; exposure time 5 hr. A single 1.4-kb species is seen. All probes were labeled by nick-translation with [α-^{32}P]dATP to specific activities of 2–5 × 10^8 cpm/μg of DNA. Size estimates are relative to ribosomal markers (not shown) or known sequence lengths.

a low rate and long latency of tumor formation for the P210-infected lines inoculated into syngeneic BALB/c mice (Table 1). Some lines have not induced any tumors (M2, 8B2),

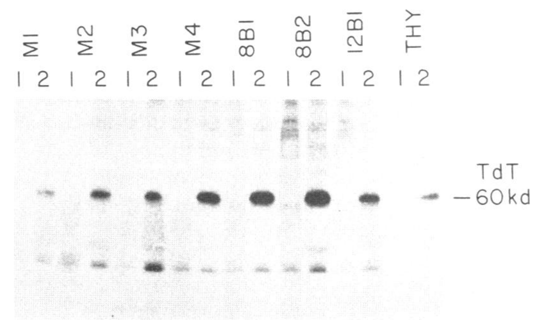

FIG. 5. Expression of immunoreactive terminal deoxynucleotidyltransferase (TdT) in P210-infected lines. Cells (10^7) of each P210-infected line and freshly explanted BALB/c thymus (THY) were washed and labeled with [^{35}S]methionine (200 μCi) for 3 hr in 2 ml of methionine-free medium at 37°C. Cells were extracted and immunoprecipitated with a rabbit anti-TdT serum (20) that precipitates a 60-kDa form from immature murine lymphoid cells. Samples were analyzed in $NaDodSO_4$/10% polyacrylamide gel developed by fluorography (exposure time 2 days).

while others have uniformly given tumors with latencies in the 3- to 6-week range (12B1). Tumors progressed from localized masses at the site of intraperitoneal injection to spleen and other lymphoid organs. Tumors analyzed by DNA blot analysis had the same P210 retroviral integration fragment as the cell line used as the innoculum (data not shown). No evidence for secondary host-cell-derived tumors was observed. Comparable doses of established Ab-MuLV-transformed lines kill all animals with widely disseminated tumors within 2 weeks of inoculation (12, 13). Thus, despite abundant expression of the P210 tyrosine kinase and monoclonal dominance in cultures, many of the P210-infected lines have not progressed toward a highly oncogenic phenotype. The nature of the genetic changes that determine this progression are not known.

DISCUSSION

The experiments described here provide direct evidence that the *BCR/ABL* gene of human CML can act as a growth stimulus for murine hematopoietic cells of the B lineage. Infection with a P210-expressing retrovirus leads to the

Table 1. Transformation behavior for cell lines infected with P210-expressing retrovirus

Cell line	Growth in agar,* %		Tumorigenicity†	
	−	+	No. positive/ no. tested	Latency, days
M1	3.2	4.6	0/6	
M2	4.3	15.5	0/6	
M3	0.3	8.5	ND‡	
M4	4.8	12.0	2/6	24–30
8B1	0.3	4.8	5/6	18–31
8B2	0	0.1	0/6	
12B1	12.1	12.6	8/8	14–30

*Growth in agar was determined by plating 10^3 cells of each P210-infected line 15 weeks after initial infection. The cells were plated in soft agar either without (−) or over a pre-established bone marrow stromal feeder layer (+) as described (17, 18). Duplicate plates were read at 2 weeks for evidence of macroscopic colonies. Data are expressed as a percentage of the total.

†P210 cell lines were evaluated for tumorigenesis in 4-week-old syngeneic BALB/c mice by inoculating each animal intraperitoneally with 2 × 10^6 cells. The number of mice with tumors (divided by the number of mice tested) and the range of days of tumor latency before detection are shown.

‡Not determined.

outgrowth of clonal cell lines with the phenotypic properties of immature lymphoid cells. Some of these clonal lines can progress in their transformed phenotype to a fully malignant state as monitored in syngeneic tumor challenges. The availability of an *in vitro* test system will facilitate a dissection of sequences required for transformation by the *BCR/ABL* gene. Our retroviral construct for expression of P210 was designed to qualitatively and quantitatively mimic the transcriptional apparatus of Ab-MuLV. Our bone marrow culture system was chosen because previous work showed that wild-type and even partially defective strains of Ab-MuLV could efficiently transform immature lymphoid cells under these conditions (12). The cell lines resulting from P210 infection showed evidence of dominance by a single or limited number of infected cells in the population by 6 weeks of culture. Further work will be needed to assess the precise number of infected cells and the rate at which a single clone becomes dominant among the population. It is possible that many cell types might express P210 without a dramatic change in growth rate or other properties. Recent work using pre-established populations of immature B-lineage cells from our long-term culture system (infected after formation of the stroma and regrowth of B-lineage cells) has shown that P210 infection can acutely stimulate growth of multiple clones in the same infection (J. Young, E.C., J.M., and O.N.W., unpublished observations).

Although all the cell lines we characterized had high levels of P210 expression, only some showed oncogenic behavior in syngeneic tumorigenicity experiments (Figs. 2 and 4 and Table 1). One explanation is that secondary genetic or epigenetic events occurring randomly in culture lead to the outgrowth of more transformed subclones in some of the cultures. This would be analogous to the pathway of tumor progression seen in human CML, but there is no evidence that these postulated genetic changes are similar in both cases. The availability of lines like 8B2, which express P210 and grow well in liquid culture but grow very poorly in agar and do not induce tumors in animals, should be useful in gene-transfer experiments to assay for cooperating oncogenes.

The prominent expansion of early myeloid cell types during the chronic phase of CML suggests that P210 should have pronounced effects on myeloid progenitor cells. It is not known whether the relative lineage specificity of myeloid over B-lymphoid expansion in chronic-phase CML is due to quantatitive differences in expression or to qualititative differences in the function of P210 in different cell types. Alternative P210-expressing retroviral vectors with promoters constructed to function in early stem cells and their progeny will need to be tested in various culture and animal models to answer this question.

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