MOLECULAR AND CELLULAR BIOLOGY, Oct. 1988, p. 4079–4087
0270-7306/88/104079-09$02.00/0

Vol. 8, No. 10

Selective Transformation of Primitive Lymphoid Cells by the *BCR/ABL* Oncogene Expressed in Long-Term Lymphoid or Myeloid Cultures

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The *BCR/ABL* gene, formed by the Philadelphia chromosome translocation (Ph1) of human chronic myelogenous leukemia, encodes an altered *ABL* gene product, P210. P210 is strongly implicated in the malignant process of chronic myelogenous leukemia, but its precise role is unknown. Infection of long-term bone marrow cultures enriched for B-lymphoid cell types with a Moloney murine leukemia virus retroviral vector containing the *BCR/ABL* cDNA resulted in clonal outgrowths of immature B-lymphoid cells which expressed abundant P210 kinase activity. Surprisingly, infection of long-term myeloid lineage-enriched cultures also resulted in clonal outgrowths of immature B-lymphoid cells. The P210-expressing lymphoid cell lines resulting from either type of culture were resistant to the lethal effects of corticosteroids. These findings indicate that high levels of P210 expressed from a Moloney murine leukemia virus long terminal repeat preferentially stimulate the growth of immature B-lineage cells, and this effect is apparent even in myeloid lineage-enriched cultures, in which few if any lymphoid cells can be detected prior to infection.

In more than 95% of cases of chronic myelogenous leukemia (CML), the chromosome translocation Ph;t9:22q34:q11 (the Philadelphia chromosome or Ph1) is detectable in the leukemic population. As a result, a subset of the exons of the *ABL* gene is translocated into the coding region of a second gene, *BCR*, creating a new transcription unit, *BCR/ABL*, which encodes a chimeric protein, P210 *BCR/ABL* (for a review, see reference 57). P210 is a protein tyrosine kinase with properties very similar to those of the chimeric transforming protein, P160$^{gag\text{-}v\text{-}abl}$, encoded by the Abelson murine leukemia virus (A-MuLV) (9, 35). P160 transforms fibroblasts, B-lymphoid cells, and, under certain conditions, a range of other hematopoietic cell types (for a review, see N. Rosenberg and O. N. Witte, Adv. Virus Res, in press).

CML is characterized by the clonal expansion of a hematopoietic stem cell carrying Ph1 (19, 29). The disease generally has two phases. In the chronic phase, members of the leukemic clone are greatly increased in number, but still have the capacity to mature. Progression into the acute phase occurs after several months or years and is marked by the accumulation of leukemic blasts which fail to mature. Involvement of each of the various hematopoietic lineages has been found during the acute phase in individual cases of CML; however, in the majority of cases the leukemic blasts are either myeloid (75%) or B lymphoid (25%). Although P210 is expressed during both chronic and acute phases, it is possible that changes in expression are involved in progression. Konopka et al. (23) observed that clonal populations of CML cells from acute-phase patients expressed higher levels of P210 than those from chronic-phase patients. Collins et al. (4) reported an increase in *BCR/ABL* transcripts in blood samples taken from acute-phase versus chronic-phase patients, whereas Shtivelman et al. (47) reported no consistent differences.

The biological effects of the *BCR/ABL* gene have been tested in several in vitro systems. The complete *BCR/ABL* coding region isolated from a cDNA library of the CML-derived cell line, K562 (32), was inserted into the murine retroviral expression vector, pMV6(TKneo) (28). The resulting construct, pJW-RX, when introduced into rodent fibroblasts via transfection or infection, produced high levels of expression of P210 tyrosine kinase activity, but, unlike A-MuLV, did not produce morphological transformation. Rare transformants were observed, but these clones of cells expressed recombinant proteins in which a *gag* domain from the Moloney murine leukemia virus (MoMuLV) helper was fused to the N terminus of P210 (6). In contrast, if fresh murine bone marrow was infected with pJW-RX and then maintained under long-term B-lymphoid culture conditions, clonal cultures of immature B-lymphoid cells with limited transformed characteristics resulted. These findings demonstrated that P210 expression could alter the growth of some target cells present in fresh bone marrow which differentiate into immature B cells (31).

Since multiple hematopoietic lineages can be involved in CML, we investigated the ability of P210 expression to alter the growth of a variety of hematopoietic target cell populations. Two established long-term culture systems were used: B lymphoid and myeloid. Long-term B-lymphoid cultures contain a range of B cells in various stages of development; the majority represent a pre-pre B level of differentiation with heterogenous immunoglobulin heavy-chain gene rearrangements (52, 53, 55). Long-term myeloid cultures contain myeloblasts, immature granulocytes, neutrophils, and macrophages (11). In addition, by shifting cells from myeloid cultures to B-lymphoid culture conditions or by injecting into lethally irradiated animals, it was shown that myeloid cultures contain progenitors that can differentiate into B-lymphoid cells, suggesting the presence of a multipotential cell in these cultures (10, 14, 15). Use of long-term culture systems can address whether P210 expression is preferentially growth stimulatory or toxic to hematopoietic cells of various types and stages of differentiation.

We report here that high-level expression of P210 from a

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MoMuLV long term repeat (LTR) in either lymphoid or myeloid long-term cultures resulted in the clonal outgrowth of early B-lymphoid cells which are steroid resistant and display a limited transformed phenotype.

MATERIALS AND METHODS

Viral stocks and cell culture conditions. The construction of pJW-RX, a retroviral construct containing the complete *BCR/ABL* coding region, has been described previously (31). Briefly, recombined cDNA clones isolated from a library generated from the CML-derived cell line K562 were inserted into the *Eco*RI site of the retroviral vector pMV6(TKneo) provided by B. Weinstein, Columbia University. This places the control of transcription of *BCR/ABL* under the MoMuLV-derived LTR. An internal bacterial neomycin resistance gene is driven off the herpes-virus-derived thymidine kinase gene promotor segment.

Viral stocks either containing MoMuLV or no helper virus (helper free) were generated by transfection of pJW-RX into NIH 3T3 cells along with MoMuLV DNA or into psi-2 packaging cells alone, respectively. G418-resistant colonies were selected and screened for high virus production as described previously (31).

The establishment and maintenance of long-term B-lymphoid (52, 55) and myeloid cultures (10, 11) has been described. Lymphoid cultures were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol; myeloid cultures were grown in Iscove modified Eagles medium (Irvine Scientific Sales Co., Inc.) supplemented with 5% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, and 10^{-7} M hydrocortisone (Sigma Chemical Co.). Nonadherent cells were collected from these cultures and infected for 3 h at a density of 2×10^6 cells per ml in Dulbecco modified Eagle medium containing 10% fetal calf serum, 8 μg of Polybrene per ml, and approximately 5×10^5 G418-resistant CFU of virus stock per ml. They were then suspended in culture medium and returned to their original dishes. Cultures L1 to L3 were infected with helper-free stock, and L4 to L6 were infected with helper-containing stock. Cultures D1 to D7 were infected by incubating hematopoietic cells directly over JW-RX(MoMuLV)-producing NIH 3T3 cells.

Protein and nucleic acid analysis. Extraction, immunoprecipitation, autophosphorylation with [γ-^{32}P]ATP, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography of P210 were as previously described (25) with site-directed rabbit anti-*abl* serum pEX-5 (24). Cells were labeled with [^{35}S]methionine, extracted, and immunoprecipitated with rabbit anti-mouse immunoglobulin for analysis of μ heavy-chain production (54) or rabbit anti-terminal deoxynucleotidyltransferase (48) and analyzed as previously described.

Probes for DNA and RNA analysis were labeled with [α-^{32}P]ATP by Random Primed DNA Labeling (Boehringer Mannheim Biochemicals) for glucocorticoid receptor RNA analysis or by nick translation (41) for all other probes. Preparation and analysis of cytoplasmic RNA and high-molecular-weight DNA were as previously described (43). Descriptions of probes and restriction enzyme digests are given in the figure legends.

Growth in soft agar and tumor challenges. Cell suspensions were seeded in soft agar medium at 10^3 cells per dish either with or without a stromal feeder underlayer as described (43, 56) and evaluated 12 to 14 days later for macroscopic colonies. For tumor challenges, 2.5×10^6 cells were injected intraperitoneally into 4- to 6-week-old BALB/c mice and the animals were observed for 3 months for tumors at the injection site or in the lymphoid organs.

RESULTS

Infection of long-term hematopoietic cultures with a *BCR/ABL* retroviral construct. To determine the effect of P210 expression on a spectrum of hematopoietic cell lineages, we infected two different types of established long-term cultures with the recombinant retrovirus JW-RX. JW-RX contains the complete *BCR/ABL* cDNA expressed from a MoMuLV LTR. By using retroviral infection, we expected to introduce *BCR/ABL* into each of the various cell types present in these cultures. MoMuLV-based vectors carrying other genes have been shown to efficiently enter the cells of a mixed hematopoietic population, provided a virus titer of at least 10^5 is used (13, 27). JW-RX gives high-level expression of P210 in lymphoid cultures established from fresh bone marrow (31). In addition, a granulocyte-macrophage-colony-stimulating factor-dependent myeloid cell line, DA3.15 (40), expresses high P210 kinase activity when infected with JW-RX (A. Muller, E. Chianese, and O. Witte, unpublished observations).

The structure of the virus and the scheme for growth and infection of the hematopoietic cell cultures are diagramed in Fig. 1. Murine femoral bone marrow was plated under two different conditions to obtain continuous lymphoid or myeloid cultures as described in Materials and Methods. Initially, both types of cultures form an adherent feeder layer consisting of fibroblasts, endothelial cells, macrophages, and, for the myeloid cultures, large fat cells. After 4 to 6 weeks, nonadherent cells appear in clusters over the adherent layer. These are lymphoid cells in various stages of the B lineage (B-lymphoid cultures) or myeloblasts, mature granulocytes, and macrophages (myeloid cultures). At the appearance of abundant nonadherent cells, these cultures are considered established and were infected with the JW-RX virus. Between 3 and 6 weeks postinfection, a portion of the cultures (see below) attained an increased growth rate and maintained a cell density about 10-fold higher than that of uninfected cultures. These rapidly growing cultures were designated L (lymphoid derived) or D (myeloid derived).

The rapidly growing cultures consist of clonal outgrowths of cells expressing high P210 kinase activity. To determine whether these rapidly growing cultures contained the integrated *BCR/ABL* provirus, we performed Southern blotting on high-molecular-weight DNA cleaved with *Eco*RI. Blots were probed with a ^{32}P-labeled *TK-neo* gene fragment. This strategy detects a fragment containing 3′ retroviral sequences plus downstream flanking DNA, identifying unique integration sites (Fig. 1A). The six lymphoid-derived (L) and four myeloid-derived (D) cultures examined each contain one to four unique proviruses (Fig. 2A). These results indicate that among the population of cells initially infected in each culture, a few clones had developed a growth advantage by 6 weeks postinfection.

To determine whether the *BCR/ABL* gene of the proviruses present in these cultures was expressed, we performed Northern (RNA) blot analysis for the genomic-length transcript and assayed for P210 kinase activity. All of the six L and four D cultures synthesize the full-length 9.5-kilobase (kb) mRNA which encodes the P210 protein (Fig. 2B). Cell lysates of 10^7 cells from each culture were immunoprecipitated with an antiserum that recognizes the extreme C terminus of Abelson proteins (anti-pEX-5), and immunopre-

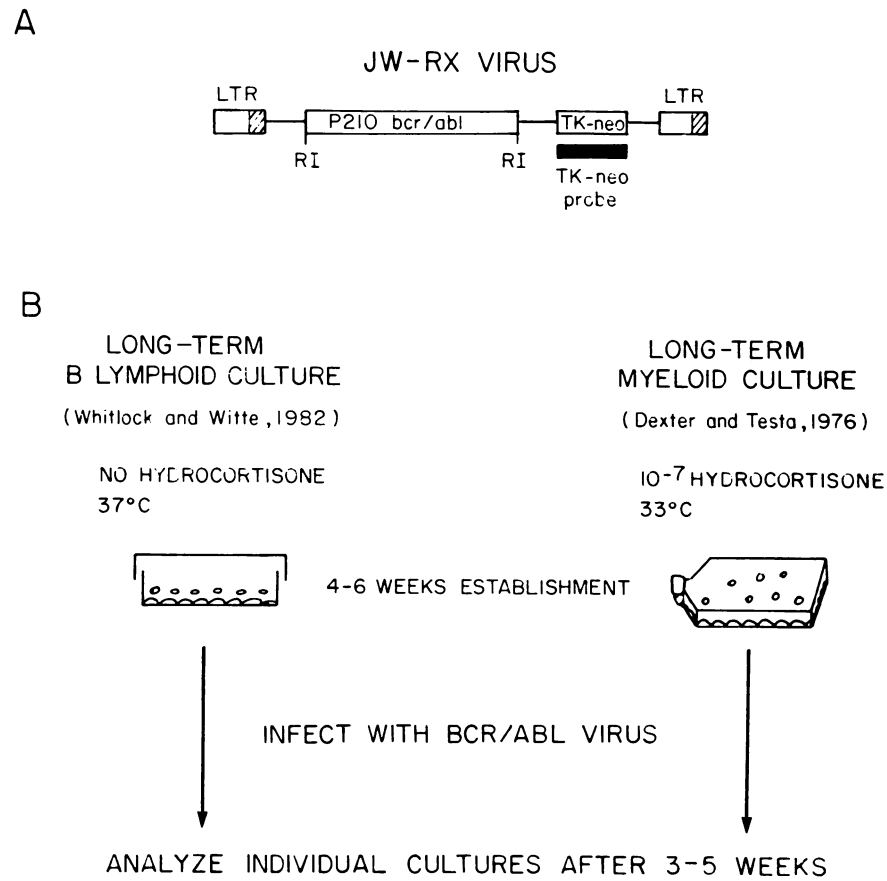

FIG. 1. Scheme for infection of hematopoietic cultures with BCR/ABL virus and analysis of the provirus. (A) Structure of the retroviral expression vector for P210 *BCR/ABL* is shown in the viral form. The box marked TK-neo shows the bacterial neomycin gene driven by the herpesviral thymidine kinase promotor. *Eco*RI restriction enzyme sites (RI) are indicated. Symbol: ▬, region recognized by the TK-neo probe. (B) Long-term B-lymphoid and myeloid cultures initiated from BALB/c bone marrow were established by culturing for 4 to 6 weeks. They were then infected with JW-RX virus and analyzed 3 to 5 weeks later.

cipitates were subjected to the immune complex kinase assay. P210 kinase activity was detected in all of these cultures (Fig. 2C). Over the course of this work, approximately 50 lymphoid and 150 myeloid cultures were infected and evaluated. Rapid growth, proviral DNA, and P210 kinase activity were observed in about 80% and 10 to 20% of the lymphoid and myeloid cultures infected, respectively. We believe that this difference is due to differences in the target cell populations in the two culture types. This point is further discussed below.

The P210-expressing cultures exhibit an early B-lymphoid phenotype. Figure 3 shows a Wright-Giemsa staining to compare the morphology of the cells in the P210-expressing cultures (lower panels) with those in uninfected cultures (upper panels). The cells of uninfected B-lymphoid cultures (top left) have a single large nucleus and scant cytoplasm. The uninfected myeloid cultures (top right) contain various recognizable elements of the myeloid lineage such as macrophages and metamyelocytes. Surprisingly, the P210-expressing cultures derived from either type of culture (bottom panels) consist of cells with a very similar large blastlike appearance. The D cultures have no recognizable myeloid elements. Furthermore, in uninfected myeloid cultures, 25 to 50% of the cells stained positive for the myeloid markers Sudan Black and Mac-1 antigen, whereas the P210-expressing D cultures contained no cells which stained for these markers (Table 1).

To determine the lineage of these blastlike cells, we analyzed immunoglobulin gene rearrangement, which is a characteristic of B lineage and some early T cells. High-molecular-weight DNA isolated from the cultures and cleaved with *Eco*RI was analyzed with a J_H region fragment probe which detects D-J joining. This is the first rearrangement of the Ig_H loci to occur in developing B cells. Figure 4 shows the hybridizing fragments detected in each of the cultures as compared with the 6.2-kb germ line configuration fragment detected in mouse liver (Fig. 4, lane LIVER). Each culture lacks the germ line fragment but contains two or more new fragments, indicating that the cells of both the L and D cultures have Ig_H rearrangements on both alleles.

To define more precisely the stage of differentiation in the B lineage represented by these P210-expressing cultures, we hybridized Northern blots of total cellular RNA with ^{32}P-labeled probes which detect μ heavy-chain and κ light-chain transcripts. Each culture synthesized μ heavy-chain transcripts (Fig. 5A), and a portion of the cultures synthesized immunoprecipitable μ protein (Table 1). However, all cultures had unrearranged κ light-chain genes (data not shown), and, as expected, no κ light-chain transcripts were detected (Fig. 5B). λ light-chain genes were also in germ line config-

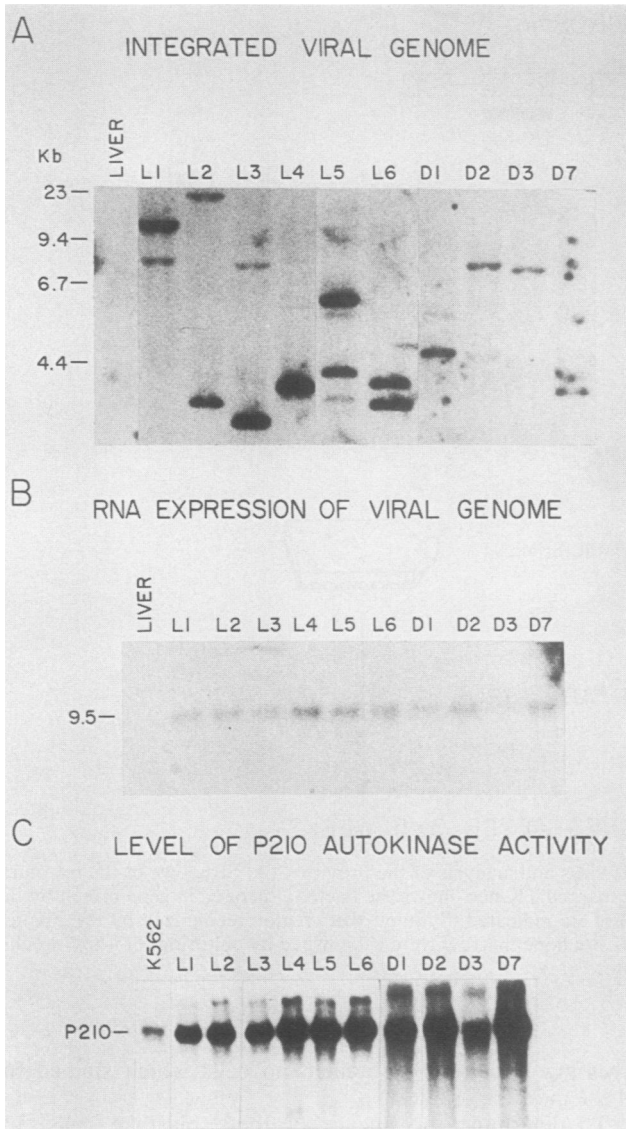

FIG. 2. Clonality of the *BCR/ABL* virus-infected cultures and expression of the *BCR/ABL* gene. (A) Southern blot analysis of high-molecular-weight DNA isolated from normal BALB/c mouse liver, the nonadherent cells from six *BCR/ABL*-infected lymphoid-derived cultures (L1 to L6), and four myeloid-derived cultures (D1 to D7). The DNA was cleaved with *Eco*RI, fractionated on a 0.8% agarose gel, transferred to nitrocellulose, and probed with a 2-kb fragment containing the TK-neo insert from vector pMV6(TKneo). Size markers are from bacteriophage lambda DNA digested with *Hin*dIII. The exposure time was 30 h at −70°C. (B) Northern blot analysis of total cytoplasmic RNA isolated from the same cell types as for panel A. A 20-μg portion of RNA was denatured, separated on a formaldehyde–1% agarose gel, transferred to nitrocellulose, and probed with the TK-neo probe. The full-length retroviral transcript of 9.5 kb, which codes for P210, is noted. The exposure time was 4 h. (C) Detergent lysates prepared from 5×10^6 to 10×10^6 nonadherent cells were immunoprecipitated with pEX-5 antiserum, which recognizes the C terminus of ABL proteins, and subjected to the immune complex kinase assay. Reaction mixtures were separated on a 7% polyacrylamide gel. The CML-derived cell line, K562, was used as a positive control. The exposure time was 1.5 h for K562 and L cultures and 4 h for D cultures.

uration (data not shown). These data indicate that both L and D P210-expressing cultures have a phenotype similar to the pre-B stage of differentiation, at which immunoglobulin heavy-chain genes are expressed but no complete cytoplasmic or surface immunoglobulin molecules are assembled as a result of the absence of light-chain gene expression.

Figure 5C shows a Northern blot hybridized with a probe which recognizes the B220/T200 surface antigen transcripts. Each culture tested synthesized immunoprecipitable terminal deoxynucleotidyltransferase (TdT) (Table 1). These two antigens are found in early lymphoid cells, and their presence is further evidence that these cultures consist of immature lymphoid cells.

It was surprising that the clonal P210-expressing lines derived from myeloid cultures all had a pre-B lymphoid phenotype that was indistinguishable from that of the lymphoid culture-derived lines. No pre-B cells exist in long-term myeloid cultures. These lines may have arisen from multipotential or lymphoid-committed stem cells present in these cultures (15, 16).

The P210-expressing cultures exhibit limited transformed characteristics. As described above, our initial observation was that some of the cultures infected with JW-RX showed an increase in growth rate and acidification of the medium within 5 weeks of infection. It was these cultures that were found to be expressing P210. These cultures maintain a cell density of 1×10^6 to 3×10^6 cells per ml instead of 2×10^5 to 4×10^5 cells per ml, maintained by uninfected cultures.

We examined the ability of cells from the L and D cultures to form colonies when plated in semisolid medium at 2 and 5 months postinfection. All were able to form colonies (Table 1); however, they displayed a range of colony-forming frequencies of 10% or less, which is considerably lower than those reported previously (50 to 80%) for A-MuLV-infected clonal lymphoid lines (56). There was no significant change between the values obtained at 2 or 5 months, and the values listed are averages of the two time points.

To test the ability of the P210-expressing cells to form tumors in syngeneic animals, 2.5×10^6 cells taken at 2 or 6 months postinfection were injected intraperitoneally into 4- to 6-week-old BALB/c mice. The total number of animals with tumors as a function of the number of animals injected is reported in Table 1. None of the D cultures produced tumors. Four of the six L cultures produced tumors in some of the animals injected. Most tumors appeared within 9 to 17 days (two tumors produced by L4 appeared after 40 days). Only one culture, L3, showed an increased frequency of tumor formation at 6 months postinfection over 2 months postinfection (from no mice [four injected] to two mice [two injected]). A-MuLV-infected clonal lymphoid lines carried for more than 5 months have been shown to cause tumors in 100% of the animals injected (56). These data suggest that except for L3, the P210-expressing cultures did not show progression of the transformed phenotype with time by these criteria.

The P210-expressing early B lymphocytes are steroid resistant. Immature cells of the B-lymphocyte lineage are killed when grown in the presence of corticosteroids as a result of triggering of an intracellular lysis pathway (20). When 10^{-7} M hydrocortisone is added to long-term lymphoid cultures, lysis of nearly all of the nonadherent cells occurs within 24 to 48 h (data not shown). We were therefore surprised to find that the P210-expressing D cultures had a lymphoid phenotype, since they were grown chronically in the presence of 10^{-7} M hydrocortisone. However, unlike long-term myeloid cultures which have not been infected with *BCR/ABL* virus,

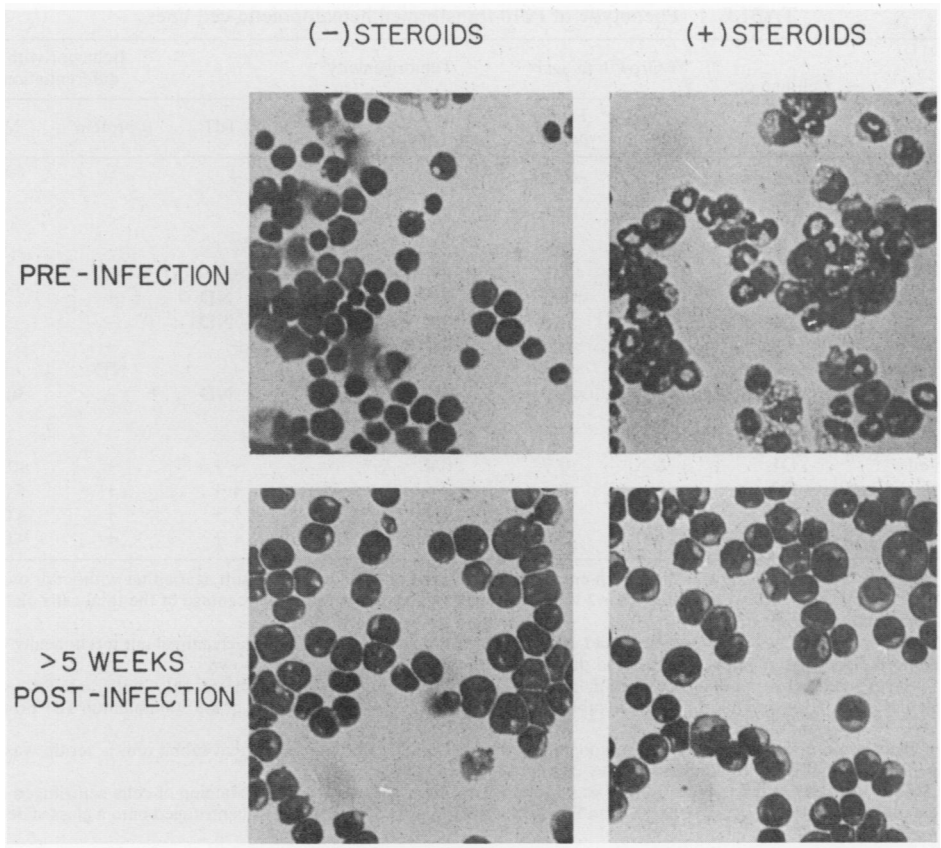

FIG. 3. Wright-Giemsa staining of long-term cultures. Top: Nonadherent cells of uninfected established B-lymphoid (left) and myeloid (right) cultures. Bottom: Nonadherent cells of P210-expressing B-lymphoid-derived (left) and myeloid-derived (right) cultures.

the D culture transformants also grew well when hydrocortisone was removed from the medium. Furthermore, when 10^{-7} M hydrocortisone was added to the medium of the P210-expressing L cultures, these cells remained viable and maintained the same doubling time as they had prior to hydrocortisone addition. They could be grown indefinitely in the presence of hydrocortisone.

Steroid-sensitive cells can develop resistance by several different mechanisms. Some mechanisms involve alterations in the glucocorticoid receptors (GR), such as lowered expression or mutations which produce receptors defective in hormone binding, nuclear transit, or DNA binding (34, 38). Cells which are resistant despite functional GRs have also been described (8, 20). The precise alterations in such cells has not been determined; however, they may involve components of the lysis pathway. To investigate the mechanism of steroid resistance in our P210-expressing pre-B cells, we first examined levels of GR RNA. Northern blotting was performed on total cytoplasmic RNA isolated from L and D cultures grown in the absence or presence of hydrocortisone. The opposite condition from usual growth was maintained for 2 weeks before RNA collection. We probed blots with a GR cDNA fragment (7) and a glyceraldehyde 3-phosphate dehydrogenase gene fragment (36) to monitor the amount of RNA in each lane. The L cultures synthesized levels of the 6- and 7-kb GR transcripts comparable with those of the steroid-sensitive lymphoblast cell line, clone K, regardless of whether they were challenged with hydrocortisone (Fig. 6). The D cultures displayed several different responses. D1 expressed a small amount and D6 expressed no detectable transcripts in the absence or presence of hydrocortisone. D2 showed an increase in GR transcripts when grown in the absence of hormone. Four additional D cultures expressed no detectable transcripts when grown in the absence or presence of hormone (not shown). Therefore, turning GR transcription down or off may be a mechanism whereby the D cultures attained steroid resistance. P210-infected progenitors in the myeloid cultures may fail to initiate GR transcription as they differentiate into pre-B cells owing to the presence of hydrocortisone. In contrast, when the L cultures are challenged with hydrocortisone, they remain viable without turning down GR transcription. This was also found to be true of $P160^{v\text{-}abl}$-expressing lymphoid bone marrow cultures (J. C. Young and O. N. Witte, unpublished observations). Gross GR gene rearrangement or deletion does not appear to be involved, since the transcripts are full length and no fragment length differences were detected in Southern blots (data not shown). It is possible that Abelson proteins can affect the way cells respond to steroids by regulating GR-induced transcriptional activation of genes of the lysis pathway.

DISCUSSION

We have shown that expression of the abnormal P210 *BCR/ABL* protein of CML in two different hematopoietic culture systems resulted in overgrowth by early B-lymphoid cells. In this study, B-lymphoid and myeloid long-term cultures were examined. The lymphoid cultures contain early B cells in various stages of differentiation, whereas the

TABLE 1. Phenotype of P210-transformed hematopoietic cell lines

Cell type	Cell line or population	% Growth in agar[a]		Tumorigenicity[b]		Behavior with following differentiation marker:			
		Feeder absent	Feeder present	No. positive/ no. tested	Latency (days)	TdT[c]	μ protein[d]	Mac-1[e]	Sudan black[f]
Uninfected lymphoid (mass population)	4 populations	<0.01	<0.01	ND[g]		+	+	ND	ND
Lymphoid culture-derived cloned lines	L1	3.5	4.5	5/6	9–17	+	–	ND	ND
	L2	3.0	6.0	0/4		+++	ND	ND	ND
	L3	3.5	6.5	2/6	9–14	ND	+	ND	ND
	L4	7.0	3.5	4/6	13–51	ND	–	ND	ND
	L5	5.0	6.5	4/4	9–11	+	–	ND	ND
	L6	8.0	5.5	0/6		+	ND	ND	ND
Uninfected myeloid (mass population)	4 populations	<0.01	<0.01	ND		ND	–	30–50%	25%
Myeloid culture-derived cloned lines	D1	1.5	0.7	0/4		++	+	<0.01%	<0.01%
	D2	0	0.5	0/4		++	+	<0.01%	<0.01%
	D3	3.0	5.0	0/4		++	+	<0.01%	<0.01%
	D7	5.0	2.0	0/4		++	+	<0.01%	<0.01%

[a] Growth in agar was determined by plating 10^3 cells from each uninfected population or P210 cell line in soft agar either without or over a preestablished bone marrow stromal feeder layer. Duplicate plates were scored after 2 weeks for macroscopic colonies as a percentage of the total cells plated. Values are averages of determinations at 2 and 5 months postinfection.

[b] P210 lines were evaluated for tumorigenesis in 4- to 6-week-old syngeneic BALB/c mice by inoculating each animal intraperitoneally with 2.5×10^6 cells. The number of mice with tumors (divided by the number tested) and the range of days of tumor latency are shown.

[c] Expression of TdT was evaluated by immunoprecipitation from lysates of 10^7 [^{35}S]methionine-labeled cells with anti-TdT serum, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and fluorography. Symbols: +++, detection of a similar amount of the 60-kilodalton TdT protein to that in 10^7 freshly explanted thymus cells; ++, +, lower but detectable amounts.

[d] Synthesis of μ heavy-chain protein was evaluated by immunoprecipitation as for TdT above, except that rabbit anti-μ serum was used. Symbols: +, the 76-kilodalton heavy chain was detectable; –, 76-kilodalton heavy chain was not detectable.

[e] The presence of the Mac-1 myeloid lineage-specific antigen was evaluated by immunoglucose oxidase staining of cells centrifuged onto a glass slide (43).

[f] The presence of the granulocytic histochemical marker, Sudan Black B, was evaluated by staining cells centrifuged onto a glass slide as described previously (43).

[g] ND, Not determined.

myeloid cultures contain some multipotential cells, myeloblasts, and mature myeloid elements. Nonetheless, on P210 expression these two culture systems evolved to contain very similar cell types by the analysis used here. This result was unexpected for several reasons. (i) In CML, *BCR/ABL* expression has been detected in cells from each of the various lineages. (ii) A variety of cell types were available in the cultures. (iii) MoMuLV expression systems have worked efficiently in both myeloid and lymphoid cell types. (iv) The myeloid cultures are grown in the presence of hydrocortisone, which is normally lethal to early B cells.

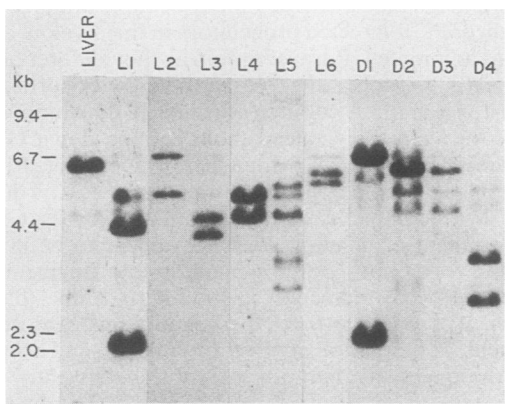

FIG. 4. Immunoglobulin gene rearrangement in cultures expressing P210. High-molecular-weight DNA from normal BALB/c mouse liver (lane LIVER) and P210-expressing lymphoid-derived (lanes L1 to L6) and myeloid-derived (lanes D1 to D7) cultures was cleaved with *Eco*RI, fractionated in a 0.8% agarose gel, transferred to nitrocellulose, and probed with a mouse J_H probe (17) to detect DJ and VDJ rearrangements of the μ immunoglobulin heavy-chain loci. The liver DNA contains the 6.2-kb germ line fragment. The exposure time was 24 h.

Several possible factors could favor the outgrowth of a particular cell type in these cultures. First, we cannot rule out that there is some preference for cell types which become infected in our cultures, although this seems unlikely, since receptors for MoMuLV are present on the surface of lymphoid, myeloid, and stem cell elements (see references below). A second possibility is that the MoMuLV LTR enhancer and promoter are exhibiting cell-type-specific expression. However, MoMuLV LTR-driven expression of various genes has been obtained in both lymphoid and myeloid cell types (13, 46, 49). Also, A-MuLV has been shown to express efficiently in myeloid and erythroid cell types (5, 37, 39, 51), and *BCR/ABL* expression from JW-RX has previously been demonstrated in lymphoid bone marrow cultures (31) and the GM-colony-stimulating factor-dependent myeloid cell line, DA3.15 (Muller et al., unpublished observations). In the majority of our infected myeloid cultures (80 to 90%), B-lymphoid outgrowth did not occur. These cultures remained myeloid in morphology, and P210 kinase activity was not detectable. Therefore, it was possible that the lack of myeloid transformation was due to inefficient expression of the integrated *BCR/ABL* construct in these myeloid cells. However, on Southern hybridization we detected no construct in the genomic DNA of these nonexpressing cultures from 48 h postinfection (data not shown). This finding supports the notion that the cells containing the

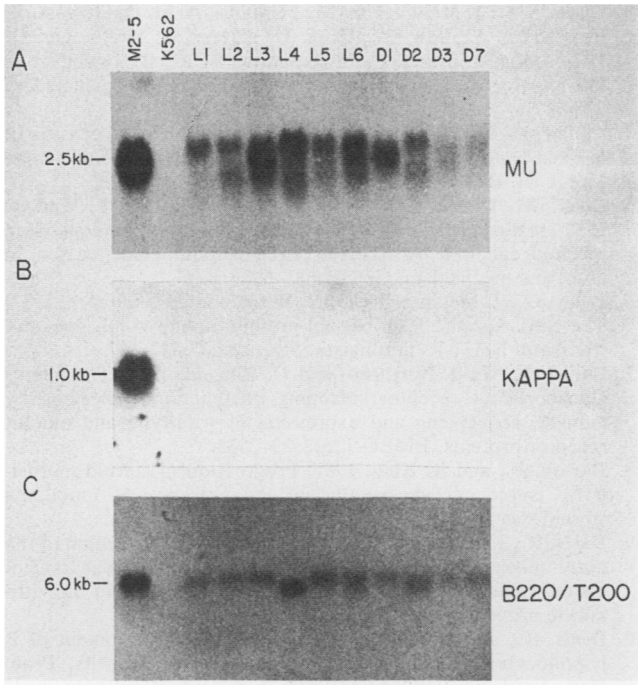

FIG. 5. Expression of lymphoid-specific RNAs. Total cytoplasmic RNA (20 μg) was denatured, fractionated on formaldehyde–1% agarose gels, transferred to nitrocellulose, and probed with (A) a 400-base-pair *Pst*I fragment of the μ heavy-chain gene constant region (42) reactive with both membrane (2.7 kb) and secreted (2.4 kb) forms of μ RNA (exposure time, 18 h); (B) a 2.7-kb fragment of the κ light-chain constant region (44), which detects the approximately 1-kb family of κ RNAs (exposure time, 2 h); (C) a 4.7-kb cDNA fragment of the mouse *Ly-5* gene (45) reactive with the B220/T200 family of lymphoid-specific glycoproteins (exposure time, 48 h). M2-5 (used as a positive control) is an A-MuLV-transformed B-lymphoid cell line which synthesizes immunoglobulin. K562 (used as a negative control) is a CML-derived cell line of early erythroid phenotype. The P210-expressing cultures are as described in the legend to Fig. 2. Size estimates are relative to rRNA markers (not shown).

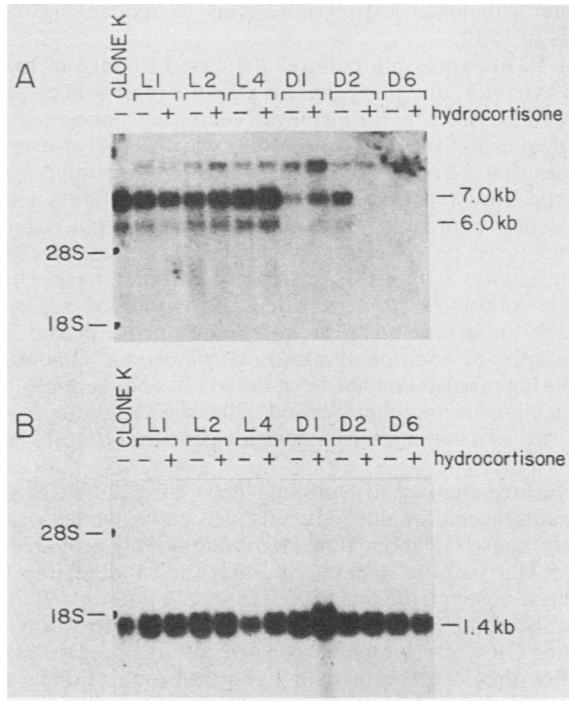

FIG. 6. Analysis of glucocorticoid receptor RNA expression. Total cytoplasmic RNA (20 μg) was analyzed by Northern blotting as described in the legend to Fig. 5. The blot was probed with (A) a 1-kb *Hin*dIII fragment from pWREC (7), which codes for the C terminus of the mouse GR; and (B) a 200-base-pair fragment of the rat glyceraldehyde 3-phosphate dehydrogenase gene (36) to monitor the amount of RNA in each lane. Exposure times were 24 and 10 h, respectively. Clone K is a clonal lymphoid cell line isolated from a long-term bone marrow culture. P210-expressing cultures are as described in the legend to Fig. 2. Cells were grown either without (−) or with (+) 10^{-7} M hydrocortisone in the growth medium. The cells were shifted to the opposite condition 2 weeks prior to RNA isolation.

construct are rapidly selected against rather than present but unable to express *BCR/ABL*. Nonetheless, *BCR/ABL* expression from a myeloid-specific LTR may favor the growth of P210-expressing myeloid cells; this is currently being tested.

A third possibility is that the P210 protein is tissue specific for growth stimulation. Transformation by onco-proteins of the tyrosine kinase family has been shown to display tissue specificity. For example, the sarcoma virus oncogenes v-*src* and v-*fps* can produce a fully transformed phenotype in vitro in fibroblasts, but not in hematopoietic cells, even when expressed from lymphotropic or amphotropic LTRs (1, 22). Similarly, the lymphoid transforming gene, v-*abl*, produces B-lymphomas in vivo even when expressed from the myeloid-specific myeloproliferative sarcoma virus or Friend murine leukemia virus LTRs (21), and the v-*abl* kinase region produces lymphoid transformation even when linked to N-terminal *src* sequences (30). The B-lymphoid outgrowth observed here could be produced if P210 *BCR/ABL* preferentially stimulated the proliferation of committed but resting B-lymphoid precursors in the myeloid cultures or stimulated B-lymphoid differentiation and proliferation from uncommitted stem cells. We routinely observed that the proportion of infected cultures progressing to P210-expressing cultures was higher for lymphoid (80%) than for myeloid (10 to 20%) cultures. This observation could be explained by the increased number of targets for P210 growth stimulation in the B-lymphoid cultures.

There is a precedent for P210 *BCR/ABL* selectivity in the finding that $P160^{v\text{-}abl}$ produces transformation and outgrowth of early B-lymphoid cells in murine myeloid long-term cultures (12, 50; Young and Witte, unpublished findings). As stated above, approximately 25% of CML blast crises are B lymphoid. In addition, a translocation involving the *BCR* and *ABL* genes which is similar to that of CML can be associated with human acute lymphoblastic leukemia. In this case, the *BCR/ABL* gene product (P185) is smaller than the CML protein (3). However, there are no direct data which demonstrate a difference in the potency of either protein for lymphoid or myeloid cells at this time.

A fourth possibility is that high-level P210 expression is preferentially toxic to some cell types. P210 kinase activity detected in cells carrying JW-RX is higher than that seen in the erythroid-derived CML cell line K562. We have consistently observed a toxic effect of P210 expression similar to that of $P160^{v\text{-}abl}$ in established rat and mouse fibroblasts. If myeloid precursors are more sensitive than lymphoid precursors to P210 toxicity, the result could be B-lymphoid outgrowth in cultures in which a cell line capable of B-lineage differentiation was infected. We are currently testing

vectors with lower expression levels to reduce the toxic effects of P210.

The P210-expressing cultures displayed a range of oncogenic behavior, and it is not clear why the lymphoid culture-derived lines in general displayed increased oncogenic behavior over the myeloid culture-derived lines. However, it appears that *BCR/ABL* is a less potent transforming agent than the viral tyrosine kinase oncogenes, including v-*abl*, even when expressed from a similar LTR. This reduced potency may be reflecting the disease process of CML, which initiates with a less aggressive chronic phase. Other events working in concert with P210 expression are most probably involved in the progression to acute phase. The appearance of additional specific chromosomal abnormalities during progression has been noted (2), and, recently, the *RAS* gene from peripheral blood cells of CML patients was identified as a dominant transforming gene for NIH 3T3 cells (18).

Transformation of B-lymphoid cells by P210 *BCR/ABL* abrogates steroid-induced lethality. Recently, we have used an anti-mouse GR monoclonal antibody (kindly provided by Robert Harrison) to demonstrate that the 94-kilodalton GR protein is absent from our P210-expressing lines in which no GR transcripts were detected, but is present in those expressing GR RNA (lymphoid-derived cultures), regardless of whether they were grown in hydrocortisone (Young and Witte, unpublished observations). We are currently assessing these receptors for functionality. Perhaps GR or gene products responsible for lysis of lymphoid cells are substrates in a cascade of phosphorylations, initiated by P210, which ultimately leads to regulation of lysis.

Either transient early B-lineage cells or uncommitted progenitor cells capable of differentiation along the B-lineage pathway must exist in myeloid cultures and be a target for P210 transformation. This cell type could be the same one that can give rise to continuous B-lymphoid cultures when myeloid cultures are shifted to B-lymphoid culture conditions (10, 14) or from the marrow of steroid-treated mice (26). A stem cell that is capable of producing continuous B-lymphoid cultures and that expresses no B220 antigen and a small amount of Thy-1 antigen was recently identified in mouse bone marrow (33). We are currently assessing the P210-expressing cultures for stem cell or early progenitor cell markers which may give clues of the phenotype of the target cell.

ACKNOWLEDGMENTS

We thank Erin Chianese, Eric Spoor, Carol Crookshank, and Barbara Mueller for excellent photographic and secretarial work on this manuscript. We thank Kathleen Denis, Tracy Lugo, and Ann Marie Pendergast for comments on the manuscript. We thank Jami McLaughlin for general advice and help with the biochemical analysis and Erin Chianese for derivation of cell lines and histochemical analysis. We thank Mark Danielsen and Gordon Ringold (Syntax Co.) for the gift of the glucocorticoid receptor probe.

This work was supported by research grants from the National Cancer Institute to O.N.W. J.C.Y. is a Postdoctoral Fellow of the Leukemia Society of America. O.N.W. is an Investigator of the Howard Hughes Medical Institute.

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