JOURNAL OF VIROLOGY, July 1987, p. 2192–2197
0022-538X/87/072192-06$02.00/0

Vol. 61, No. 7

Clonal Dominance and Progression in Abelson Murine Leukemia Virus Lymphomagenesis

PATRICK L. GREEN, DONAL A. KAEHLER, AND REX RISSER*

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

Received 22 December 1986/Accepted 25 March 1987

We examined the clonality of tumors induced by an acutely transforming retrovirus which carries a single oncogene. Contrary to our expectation, tumors induced by the Abelson murine leukemia virus (A-MuLV) showed one to four major proviral integration events. To further investigate the process by which clonality was established, we analyzed the number of cells infected and transformed by A-MuLV at various times after in vivo infection. At the midpoint of tumor latency (14 days postinfection), we found that infection of total bone marrow cells by A-MuLV was efficient and polyclonal. However, only a minority of these infected cells were transformed as assayed in cell culture, and clonal dominance had already been established in this transformed cell population. Examination of the in vitro growth properties of transformed cells recovered from preleukemic and leukemic mice indicated that preleukemic cells had lower cloning efficiencies than primary tumor cells. Our results suggest that the rate-limiting step in this system of lymphomagenesis is the initial transformation of bone marrow target cells and that these cells undergo subsequent changes in cloning ability during the course of the disease that lead to an autonomous neoplastic state.

The induction of tumors by rapidly oncogenic retroviruses has provided the means to identify oncogenes and to assess their individual contributions to the development of malignancy. Because these viruses establish viremia in the host and the disease develops within weeks of virus inoculation, most investigators have reasoned that infection of susceptible cells and expression of the viral oncogene are sufficient for tumor formation. Studies of tumors that arise in mice transgenic for an activated *myc* oncogene and expressing high levels of its product indicate that these *myc*-induced tumors are of monoclonal origin. This result suggests that steps in addition to the expression of a single activated oncogene are necessary for tumor formation (1). We examined the clonality of tumors induced by the Abelson murine leukemia virus (A-MuLV) and found that, like tumors induced by the *myc* transgene and the majority of naturally occurring tumors, these viral tumors originate from one or a few transformed cells and thus are monoclonal or oligoclonal in origin.

A-MuLV, a highly oncogenic retrovirus, encodes the v-*abl* oncogene, which is composed of both Moloney murine leukemia virus (M-MuLV) *gag* sequences and c-*abl* sequences (3, 5, 12, 19, 21, 23). This *gag-abl* fusion protein is a tyrosine-specific kinase, and its expression is necessary for transformation of cells and tumor induction (16). A-MuLV transforms primary lymphoid cells in vitro, and the number of lymphoid colonies produced depends linearly on the concentration of A-MuLV (14, 15). A-MuLV also directly transforms NIH 3T3 cells in vitro (18) and induces predominantly lymphosarcomas of the B-cell lineage in vivo (11, 13).

MATERIALS AND METHODS

Viruses and cells. Biologically cloned A-MuLV (p160 strain) was prepared by harvest of supernatant fluids from nonproducer N54 cells (16) superinfected with M-MuLV Mov3 (9). An A-MuLV (p160 strain) proviral clone (pAB_4) was recovered in lambda Charon 4A from an A-MuLV-induced tumor cell line, E102 (6), DNA library and characterized in this laboratory. The pAB_4 and M-MuLV pMov3 proviral clones were cotransfected into NIH 3T3 cells, and supernatant fluids were harvested. The titer of replication-competent M-MuLV was determined by UV-XC plaque assay on NIH 3T3 cells (17), and the titer of transforming A-MuLV was determined by focus formation on NIH 3T3 cells (18). The titer of XC PFU was 10- to 100-fold greater than focus-forming units (FFU) in the A-MuLV pools used in these studies.

Human foreskin fibroblast strain SL68 feeder layers were grown in Ham F-10 medium containing 15% fetal bovine serum and antibiotics (4). Mouse bone marrow feeder layers were cultured as described by Whitlock et al. (24, 25).

Mice. Adult (>6-week-old) BALB/cByJ or C57BL/6ByJ mice were obtained from the Jackson Laboratories (Bar Harbor, Maine), and newborn (<2-day-old) mice were obtained by breeding in our mouse colony. Adult mice were inoculated intravenously or intrathymically with 0.1 to 0.2 ml of undiluted virus stock containing 10^3 to 10^5 FFU of virus per mouse. Tumors developed in approximately 3 to 5 weeks. Newborn mice were inoculated intraperitoneally with the same virus dose.

Bone marrow transformation assay. The bone marrow transformation assay was performed essentially as described by Rosenberg and Baltimore (14). Bone marrow cells (1×10^5, 3×10^5, or 1×10^6) in 2 ml of 0.35% agarose (SeaPlaque low-gelling agarose; FMC Co., Rockland, Maine) in RPMI 1640 medium supplemented with 15% fetal calf serum and 50 μM mercaptoethanol were plated on top of 2 ml of 0.7% agarose medium prechilled (4°C) in a 60-mm tissue culture plate. Cells were incubated for 7 days at 37°C in a 5% CO_2 atmosphere and then fed 2 ml of 0.35% agarose medium. At 10 to 14 days, macroscopic colonies were scored.

To determine plating efficiencies of transformed cells, individual colonies were picked, and cells were dispersed and counted (yield, 1×10^4 to 2×10^5, 50 to 90% viability). Cells were plated in 2 ml of 0.35% agarose medium on top of a layer of 2 ml of 0.7% agarose medium covering the feeder layers. Feeder layers consisted of semiconfluent human

* Corresponding author.

foreskin fibroblasts (approximately 3×10^5/60-mm tissue culture plate) or 4- to 5-week-old semiconfluent mouse bone marrow feeder cells. Colony-forming efficiency was scored 7 to 8 days after plating.

Infectious center assay. A single-cell suspension of A-MuLV lymphoma cells was obtained by perfusing primary tumor tissue. The cells were exposed to 4,000 rads in a ^{137}Cs irradiator. The concentration of nucleated cells was determined, and 5×10^7 cells were added in duplicate to plates of NIH 3T3 cells seeded the previous day (10^5 cells per 60-mm plate). At confluence the UV-XC plaque assay was performed on one of these plates, and the other plate was cultured for 14 days and scored for focus formation.

DNA transfer and hybridizations. DNA was extracted from cells, digested to completion with restriction endonucleases (New England Biolabs), and electrophoresed in a 0.7% horizontal agarose gel as described previously (7, 8). The DNA was denatured and transferred to nitrocellulose by the procedure of Southern (20) and hybridized with ^{32}P-nick-translated probe DNA. Hybridized filters were washed and autoradiographed as described (8). Probe pSA-17 consists of a 1.9-kilobase-pair (kbp) *Sac*I-*Hin*dIII fragment isolated from linear DNA molecules of the p120-encoding A-MuLV (3). The probe pAB3 consists of a 2.3-kbp *Bgl*II-*Hin*dIII fragment of the 5.5-kbp p120-encoding A-MuLV genome (5). The probe pRI-J_H consists of a 6.2-kbp fragment spanning the mouse immunoglobulin heavy-chain joining region (2).

RESULTS

Clonal dominance in primary A-MuLV tumors. The clonality of a population of A-MuLV-infected cells can be determined by examining *abl*-specific hybridization patterns of infected-cell DNA following cleavage with restriction enzymes that do not cut within the viral genome. Tumor tissue DNA was prepared from primary lymphomas induced by A-MuLV and digested with *Eco*RI, which does not cleave the A-MuLV provirus, and the patterns of viral integration were examined by Southern blot analysis (Fig. 1). In each tumor one to four prominent proviral bands were detected, in addition to the 27-kbp c-*abl*-specific band present in all cells. All 43 A-MuLV tumors examined showed one to four major proviral integration sites, a result which suggests that each tumor cell population is dominated by one or a few clones. Examination of several DNA samples containing comigrating bands (Fig. 1, lanes 2 and 4) with other restriction enzymes indicated that these tumors had distinct proviral integration sites (data not shown). Some tumors were dominated by cells from a single clone, as judged by the comparison of the proviral band with the internal c-*abl* band (Fig. 1, lanes 2, 3, 10, 12, and 15). Twenty-six of the 43 A-MuLV tumor DNAs showed more than one proviral band, with an average of 1.8 bands per tumor. These additional proviral bands within a tumor DNA sample suggest the presence of more than one dominant clone within the tumor cell population or the presence of a clone(s) bearing more than one integrated provirus. The proviral bands of subgenomic intensity (Fig. 1, lanes 5, 6, and 7, B or T) are probably due to the emergence of additional transformed clones during tumor cell proliferation or subsequent integration events within the original clone. Examination by Southern blot analysis of the immunoglobulin heavy-chain joining (J) regions in A-MuLV tumor DNAs showed a few tumor-specific bands in each case (data not shown). This result further indicates that a major portion of the cells in A-MuLV primary tumors are descendants of a few infected cells.

We also examined DNAs isolated from different leukemic tissues of the same mouse (Fig. 1, lanes 5B and 5T, 6B and 6T, 7B and 7T, and 8T and 8L). In six of eight mice analyzed, the pattern of proviral integration events was the same in different lymphoid tissues of the same mouse. Two mice showed distinct provirus-hybridizing bands in tumor tissue DNA, and a smear of hybridization was seen in the bone marrow DNA of each mouse (data not shown). We conclude from the data presented thus far that a major portion of each tumor cell population consists of cells with one to four unique A-MuLV integration events, or the tumor is dominated by one or a few clonal cell populations. This clonal dominance was detected irrespective of mouse age, mode of virus inoculation, mouse strain, or virus dose (10^3 to 10^5 FFU).

Based on a reconstruction experiment (Fig. 2), we estimate that a proviral band would be detected if the percentage of cells containing it constituted as little as 5 to 10% of the total cell population. Comparison of the intensity of the signal found in the genomic c-*abl* *Eco*RI fragment with the signals found in the novel A-MuLV integration fragments suggests that in most tumors the dominant clone(s) composes greater than 10% of the cell population (Fig. 1).

Efficiency of infection and clonality prior to tumor emergence. One possible explanation for the clonality of A-MuLV-induced tumors is that infection or transformation in vivo is an inefficient process. To determine whether A-MuLV tumors were clonal due to inefficient infection in vivo, adult BALB/c mice were infected with A-MuLV. Total bone marrow DNA was isolated 14 days postinfection and examined to quantify the number of proviruses per cell. These DNA samples were compared in reconstruction hybridization experiments with a cloned A-MuLV cell line

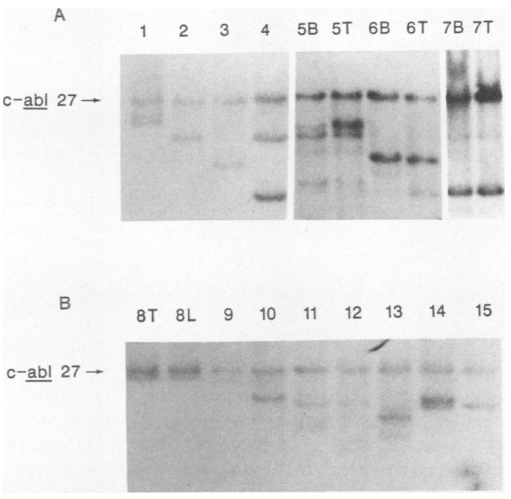

FIG. 1. Southern hybridization analysis of primary A-MuLV-induced tumor tissue or leukemic bone marrow DNA. High-molecular-weight DNA (10 μg) was digested with *Eco*RI, electrophoresed through 0.7% agarose gels, blotted to nitrocellulose paper, and hybridized to the ^{32}P-labeled *abl*-specific probe pAB3. Lanes: 1 to 4, thymomas induced by intrathymic inoculation of 10-day-old C57BL/6 mice; 5 to 7, bone marrow (B) and tumor (T) tissue of 6-week-old BALB/c mice inoculated intravenously; 8, tumor tissue (T) or lymph node (L) tissue of 6-week-old BALB/c mice inoculated intravenously; 9 to 15, tumor tissue induced by intraperitoneal inoculation of newborn (<2-day-old) BALB/c mice. Molecular weights were determined by comparison with a *Hin*dIII digest of lambda DNA (23.1, 9.42, 6.68, 4.36, 2.32, 2.03, and 0.564 kb).

shown to contain two A-MuLV proviruses. Digestion of the DNA with *Xba*I, which cleaves only in the long terminal repeat (LTR), will resolve all A-MuLV proviruses into a 5.8-kbp hybridizing band. A-MuLV proviruses were clearly detected in two total bone marrow DNA samples taken from mice infected 14 days earlier with A-MuLV (Fig. 2A, lanes A#2 and A#1), but not in DNA of total bone marrow of M-MuLV-infected mice (Fig. 2A, lane M). By comparison of the intensities of the bands found in A-MuLV infected bone marrow cells with the intensities of signals in this and subsequent reconstruction experiments, we estimate that samples A#2 and A#1 contain 0.02 and 0.4 proviruses per cell, respectively. Eight additional bone marrow samples from A-MuLV-infected mice were analyzed, and each fell within this range (data not shown). The average value from the 10 infected bone marrow samples was 0.2 proviruses per cell. Total bone marrow cells from A-MuLV-infected mice also were plated in infectious center assays. From 3 to 30% of the cells scored as A-MuLV producers, when corrected for the plating efficiency of clonal A-MuLV producer lines. Both reconstruction hybridization experiments and infectious center assays indicated that in vivo infection by A-MuLV is efficient, resulting in viral integration and virus production by a large portion of total bone marrow cells.

To determine whether infection of total bone marrow was oligoclonal or polyclonal at 14 days postinfection, DNAs were digested with *Eco*RI, which resolves proviruses on the basis of their integration sites. Our limit of detection was approximately 0.05 proviruses per cell resolved into two bands. The average number of proviruses in clonally dominant tumors was 1.8 proviruses per cell. Sample A#1 (0.4 proviruses per cell) clearly would yield a resolvable band if a distinct integration site were present in a major portion of the infected bone marrow cell population, and A#3 (0.05 proviruses per cell) most likely would yield a detectable band. We did not detect a dominant proviral pattern in either of these two samples (Fig. 2B, A#1 from above and A#3) or in the other eight samples tested (data not shown). Thus, many sites of integration are occupied in infected bone marrow cells. We conclude that at the midpoint of tumor latency (14 days postinfection) A-MuLV infection is efficient and polyclonal.

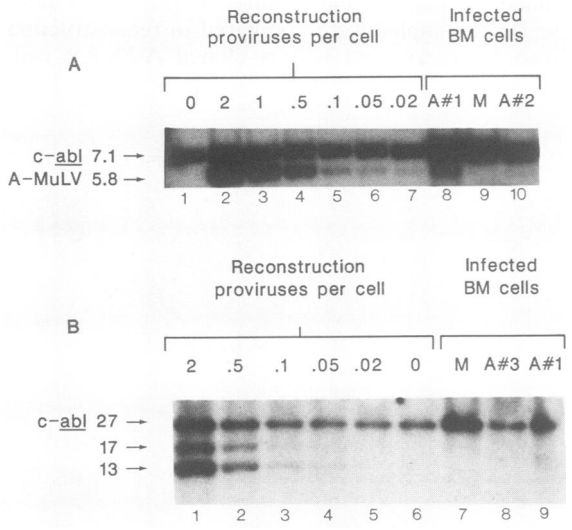

FIG. 2. Determination of the number of A-MuLV proviruses per cell and clonality of total bone marrow (BM) cell DNAs isolated 14 days postinfection. High-molecular-weight DNAs were digested with *Xba*I (A) or *Eco*RI (B), electrophoresed, blotted, and hybridized with PSA-17 as described in the legend to Fig. 1. (A) Lanes: 1, 10 μg of normal BALB/c liver DNA; 2, 10 μg of E102 cloned-cell DNA, shown to contain two proviruses per cell; 3, 5 μg of BALB/c liver DNA and 5 μg of E102 DNA; 4, 7.5 μg of BALB/c liver DNA and 2.5 μg of E102 DNA; 5, 9.5 μg of BALB/c liver DNA and 0.5 μg of E102 DNA; 6, 9.75 μg of BALB/c liver DNA and 0.25 μg of E102 DNA; 7, 9.9 μg of BALB/c liver DNA and 0.1 μg of E102 DNA; 8, 20 μg of A-MuLV (A#1)-infected bone marrow DNA; 9, 10 μg of M-MuLV-infected bone marrow DNA; 10, 10 μg of A-MuLV (A#2)-infected bone marrow DNA. (B) Lanes: 1 to 6, reconstruction similar to that described for panel A; 7, 10 μg of M-MuLV-infected bone marrow DNA; 8 and 9, 10 μg of A-MuLV-infected bone marrow DNA. Sizes are indicated to the left (in kilobase pairs).

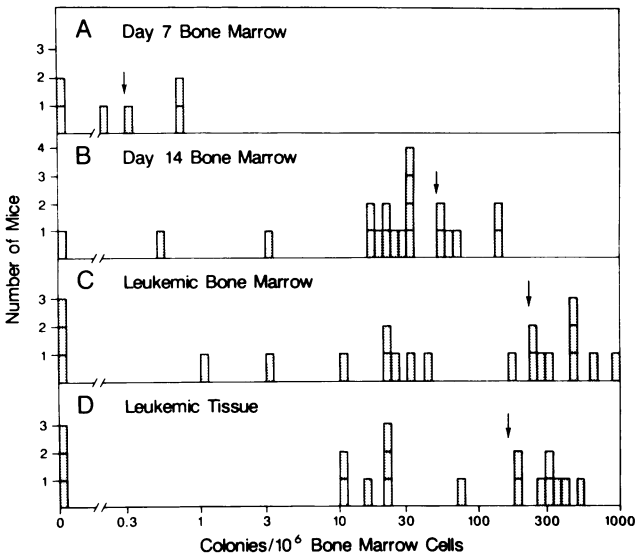

FIG. 3. Histogram summary of the number of A-MuLV-transformed cells throughout tumor latency. Six-week-old BALB/c mice were infected with A-MuLV; bone marrow cells were removed from individual mice at days 7 and 14 postinfection and bone marrow and tumor cells were removed at 25 to 30 days postinfection and plated in an agarose bone marrow transformation assay. Each box represents the number of transformed colonies found in one mouse. The arrows indicate the mean in each category. Macroscopic transformed colonies were scored 10 to 14 days postplating. Bone marrow cells removed from six M-MuLV-infected mice 7 to 50 days postinfection gave no transformed colonies.

Appearance of transformed cells prior to tumor emergence. To determine whether A-MuLV-induced tumors were clonal due to inefficient transformation of cells by A-MuLV in vivo, we quantified the number of transformed cells present in total bone marrow prior to tumor emergence. Mice were infected with A-MuLV, and at various times throughout the tumor latent period, total bone marrow cells were removed and plated in the in vitro agarose colony assay. As early as 7 days after infection with A-MuLV, transformed cells were detected. The number of transformed colonies increased throughout tumor latency (Fig. 3). However, it is also worth noting that 6 of the 21 individual tumors tested in this assay contained very few colony-forming cells (Fig. 3C).

Growth properties of transformed cells from preleukemic and leukemic mice. To determine whether the growth potentials of transformed cells isolated from preleukemic bone marrow or leukemic bone marrow were equivalent, we tested cells from individual colonies for their ability to give rise to permanent cell lines and to form colonies when replated in the absence or presence of feeder layers.

TABLE 1. Plating efficiencies of A-MuLV-transformed cells[a]

Colony source	No. of colonies tested	Efficiency of plating (%)		
		None	HF	MBM
Established lines	4	74	66	55
Leukemic bone marrow	15	0.4	48	68
Preleukemic bone marrow	19	0.02	0.07	9

[a] Colonies containing 1×10^4 to 2×10^5 cells were replated on human fibroblasts (HF), mouse bone marrow (MBM), or no feeder layer (none).

We attempted to establish permanent cell lines by picking transformed colonies from agarose 7 to 10 days after plating and subculturing them in microtiter wells. Cultures were subdivided every 7 days, or 3 to 4 days if substantial growth was observed, and were continued for 4 to 6 weeks. Of 52 colonies picked from agarose assays of preleukemic bone marrows (day 10 or 14 postinfection), none gave rise to estalished cell lines. Of 23 colonies picked from agarose assays of leukemic bone marrow, 14 gave rise to permanent cell lines. These frequencies were not altered by supplementing the medium with 20% medium conditioned by WEHI-3B cells, a source of interleukin 3 (10) or 50% medium conditioned by ANN-1 A-MuLV nonproducer cells. From these results we conclude that the frequency of cells that can give rise to permanent cell lines is much higher in leukemic cell populations than in preleukemic cell populations.

To test the growth requirements of cells in transformed colonies, individual colonies were picked and cells were replated over three different feeder layers. One recipient plate contained no feeder cells; one recipient plate contained human foreskin fibroblast feeder layers (SL68), previously shown to support the growth of Epstein-Barr virus-transformed lymphocytes (22); and one recipient plate contained mouse bone marrow feeder layers, previously shown to support the growth of some in vitro A-MuLV-transformed lymphocytes (26). Preleukemic cells had much lower cloning efficiencies than leukemic cells in both the absence and presence of feeder layers (Table 1). From these results we conclude that A-MuLV preleukemic and leukemic cells differ in their requirement for continued proliferation in vitro. The increase in the number of transformed cells detected in the bone marrow of A-MuLV-infected mice during the course of leukemia development (Fig. 3) presumably reflects both the increase in the absolute number of transformed cells and the increase in their cloning efficiency.

Establishment of clonal dominance prior to tumor emergence. To determine whether clonal dominance had been established in the transformed cells prior to overt tumor formation, mice were infected with A-MuLV, and at 10 to 14 days postinfection total bone marrow cells from several individual mice were isolated. Half the cells from each mouse were used to make DNA, and the other half were plated in the agarose transformation assay. As was found in the samples analyzed previously (Fig. 2), total bone marrow cell DNA from individual mice showed efficient polyclonal infection.

To determine whether the transformed colonies were composed of a few cell clones, 100 colonies from each individual mouse were pooled, and DNAs were isolated. These DNAs were digested with *Hin*dIII, which cleaves once in the A-MuLV provirus 3′ to the v-*abl* oncogene, and analyzed by Southern blot hybridization. In all, eight of eight DNA samples showed distinct proviral integration patterns, one of which is shown in Fig. 4, lane 7. From these results we conclude that at 10 to 14 days postinfection with A-MuLV, the population of bone marrow cells transformed by in vitro criteria is dominated by a few cell clones.

Although these results indicate the presence of clonally dominant populations of transformed cells in preleukemic mouse bone marrow, they do not establish the relationship of this population of cells transformed by in vitro criteria to the clones of cells that will dominate the tumor in vivo. To investigate that question, a BALB/c mouse was infected with A-MuLV, and 14 days later the bone marrow was removed. Most of the cells were plated in agarose suspension and allowed to form colonies. DNA was isolated from 100 colonies (approximately 10^6 to 10^7 cells) and analyzed by Southern blot hybridization following digestion with *Hin*dIII (Fig. 4, lane 7).

The remainder of the bone marrow cells from the A-MuLV-infected mouse were injected subcutaneously into five weanling BALB/c mice at a dose of 3×10^5 cells per mouse (40 in vitro colony-forming cells per mouse). Tumors developed at the site of inoculation 17 to 21 days later in all of the mice. Tumors were excised, and DNA was isolated from each individual tumor. Following digestion of the DNAs with *Hin*dIII, the pattern of A-MuLV proviral integration events present in the in vitro-transformed colony DNA (Fig. 4, lane 7) was compared with the patterns of A-MuLV integration in the five individual tumors induced by the same inoculum of cells (Fig. 4, lanes 2 to 6). Each of the four proviral bands present in the in vitro-transformed colony DNA (Fig. 4, lane 7) was also present in at least one of the tumor DNA samples (Fig. 4, lanes 2 to 6). Two additional proviral bands were present in the tumor DNA samples but were not found in the colony DNA. A similar result was found after cleavage of the DNA with *Eco*RI (data not shown).

To compare the frequency of comigration found in the tumor DNA samples in Fig. 4 with that which would be found in unrelated tumors, we determined the frequency of comigrating bands in one set of 10 individual A-MuLV primary tumors run on the same gel. The frequency of comigrating bands in this set of unrelated samples was 1 of 14 (data not shown). The frequency of comigrating bands found in tumors induced by bone marrow cells of the same preleukemic mouse (4 of 6) was significantly higher than random tumor samples ($P < 0.014$). Therefore, the bone marrow of preleukemic mice contains clones of cells that are or will become a clonally dominant tumor.

Comparison of each individual tumor DNA sample (Fig. 4, lanes 2 to 6) with the colony DNA sample (lane 7) indicated that the chance that a proviral band observed in the tumor

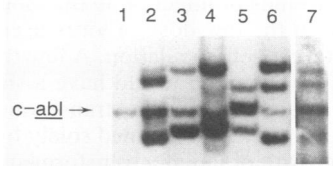

FIG. 4. Southern blot analysis of tumors induced by day 14 preleukemic bone marrow and transformed colonies arising from this bone marrow. DNAs were isolated from transformed colonies which grew out from 14-day preleukemic bone marrow cells and from tumors induced by subcutaneous inoculation of weanling BALB/c mice with the same 14-day preleukemic bone marrow cells. These DNAs were digested with *Hin*dIII, electrophoresed, blotted, and hybridized with the pSA-17 probe. Lanes: 1, 10 μg of BALB/c liver DNA; 2 to 6, 10 μg of primary tumor tissue DNA; 7, 10 μg of preleukemic day 14 transformed-colony DNA.

DNA would also be observed in the colony DNA was 0.63. Comparison of each individual tumor sample (Fig. 4, lanes 2 to 6) with three unrelated colony DNA samples indicated that the chance that a proviral band observed in the tumor DNA (lanes 2 to 6) would also be observed in the unrelated colony DNA was 0.05. Statistical analysis indicated that these frequencies were significantly different ($P < 0.001$). Therefore, some of the clones that dominate the in vitro-transformed population will also come to dominate the tumor in vivo. These results indicate that clonal dominance is established in preleukemic transformed bone marrow cells, as assayed in vitro, by 14 days after infection with A-MuLV. The results also indicate that most if not all of these transformed clones can go on to form tumors.

DISCUSSION

Contrary to our expectation, clonally dominant tumors were induced by the rapidly oncogenic retrovirus A-MuLV. Examination of the patterns of A-MuLV integration events revealed that all tumors tested contained one to four major sites of proviral integration. This clonality was not due to inefficient infection by A-MuLV, because a sizable portion of total bone marrow cells were polyclonally infected at the midpoint of tumor latency (14 days postinfection). However, the number of transformed cells in that population was much lower. Thus, the in vivo infection of bone marrow cells is not equivalent to transformation, a result consistent with the limited target cell pool for A-MuLV in in vitro transformation (14).

The plating of bone marrow cells from preleukemic mice in the in vitro agarose transformation assay provided a means to isolate cells transformed by in vitro criteria prior to overt tumor formation. Southern analysis of pools of transformed colonies from individual mice indicated that in eight of eight cases clonal dominance was established in the transformed population by 14 days postinfection. Transplantation experiments demonstrated that some of these cells transformed by in vitro criteria had the potential to form a clonally dominant tumor. The use of the in vitro transformation assay also pointed out some difficulties in equating in vitro transformation with the emergence of A-MuLV tumors. Some tumors had little if any plating ability in this assay. However, the same samples had readily detectable proviral integration events (Fig. 1, lanes 5B and 5T).

Two explanations may account for the appearance of clonally dominant tumors following infection with a rapidly transforming retrovirus such as A-MuLV. We demonstrated that efficient polyclonal infection of bone marrow was established by 10 to 14 days postinfection; however, it could be that the initial rounds of infection in the mouse were limited in number due to the low dose of virus used in comparison with the total target cell population. A few transformed cells arising soon after infection would have a significant kinetic advantage compared with cells transformed later. Thus, clonal dominance may be explained solely by the kinetics of infection and growth of initially transformed cells and not by the selection of clones with proliferative advantages.

The majority of transformed cells recovered from preleukemic A-MuLV-infected mice had low in vitro cloning efficiencies and did not yield established cell lines, unlike primary A-MuLV tumor cells. These preleukemic cells also plated with greater efficiency in the presence of mouse bone marrow feeder layers, a result which confirms the work reported by Whitlock et al. for some bone marrow cells infected in vitro with A-MuLV (26, 27). These observations could be interpreted to indicate that a second event(s) is involved in A-MuLV tumorigenesis, and cells having selective growth advantages are selected during the course of the disease. To address that question critically, it will be necessary to follow the progression of phenotypes within a single clone of A-MuLV-infected cells in the absence of viral replication, an experiment in progress with A-MuLV stocks prepared with retroviral packaging cells.

The simplest interpretation of the results presented here is that very few cells are initially transformed in vivo by A-MuLV, and this early event constitutes the rate-limiting step in the disease. Within clones of initially transformed cells, a subsequent event(s) may occur to increase the cloning efficiencies of these transformed cells. The role of the *abl* oncogene in promoting this secondary event is unknown. It is possible that secondary changes relating to cloning efficiency or growth requirements occur as a consequence of continued proliferation induced by the oncogene, and these do not reflect secondary genetic events.

ACKNOWLEDGMENTS

We thank Norman Drinkwater for advice on statistical analysis and Michael Potter, Bill Sugden, and Howard Temin for their comments on the manuscript.

This work was supported by Public Health Service grants CA-41302, CA-07175, and CA-09135 from the National Cancer Institute.

LITERATURE CITED

1. **Adams, J. M., A. W. Harris, C. A. Pinkert, L. W. Corcoran, W. S. Alexander, S. Cory, R. D. Palmiter, and R. L. Brinster.** 1985. The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature (London) **318**:533–538.
2. **Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore.** 1981. Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. Cell **27**:381–390.
3. **Dale, B., and B. Ozanne.** 1981. Characterization of mouse cellular DNA homologous to Abelson murine leukemia virus-specific sequences. Mol. Cell. Biol. **1**:731–742.
4. **Drinkwater, N. R., R. C. Corner, J. J. McCormick, and V. M. Maher.** 1982. An in situ assay for induced diphtheria-toxin-resistant mutants of diploid human fibroblasts. Mutat. Res. **106**:277–289.
5. **Goff, S., E. Gilboa, O. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
6. **Green, P. L., W. W. Lamph, J. Dudley, A. Arfsten, R. Risser, L. L. Lanier, N. L. Warner, J. Tung, and M. P. Scheid.** 1985. Phenotypic variation in clonal Abelson virus lymphoma cells. J. Immunol. **134**:1268–1275.
7. **Grunwald, D. J., B. Dale, J. Dudley, W. W. Lamph, B. Sugden, B. Ozanne, and R. Risser.** 1982. Loss of viral gene expression and retention of tumorigenicity by Abelson lymphoma cells. J. Virol. **44**:92–103.
8. **Horowitz, J. M., and R. Risser.** 1982. A locus that enhances the induction of endogenous ecotropic murine leukemia viruses is distinct from genome-length ecotropic proviruses. J. Virol. **44**:950–957.
9. **Jahner, D., and R. Jaenisch.** 1980. Integration of Moloney leukemia virus into the germ line of mice: correlation between site of integration and virus activation. Nature (London) **287**:456–458.
10. **Lee, J. C., A. Hapel, and J. Ihle.** 1982. Constitutive production of a unique lymphokine (IL-3) by the WEHI-3 cell line. J. Immunol. **128**:2393–2398.
11. **Potter, M., M. D. Sklar, and W. P. Rowe.** 1973. Rapid viral induction of plasmacytomas in pristane-primed BALB/c mice.

Science **182**:592–594.
12. **Reddy, E. P., M. J. Smith, and A. Srinivasan.** 1983. Nucleotide sequence of Abelson murine leukemia virus genome: structural similarity of its transforming gene product to other *onc* gene products with tyrosine specific kinase. Proc. Natl. Acad. Sci. USA **80**:3623–3627.
13. **Risser, R., M. Potter, and W. P. Rowe.** 1978. Abelson virus induced lymphomagenesis in mice. J. Exp. Med. **148**:714–726.
14. **Rosenberg, N., and D. Baltimore.** 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. J. Exp. Med. **143**:1453–1463.
15. **Rosenberg, N., D. Baltimore, and C. D. Scher.** 1975. *In vitro* transformation of lymphoid cells by Abelson murine leukemia virus. Proc. Natl. Acad. Sci. USA **72**:1932–1936.
16. **Rosenberg, N. E., D. R. Clark, and O. N. Witte.** 1980. Leukemia virus mutants deficient in kinase activity and lymphoid cell transformation. J. Virol. **36**:766–774.
17. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology **42**:1136–1139.
18. **Scher, C. D., and R. Sigler.** 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. Nature (London) **253**:729–731.
19. **Shields, A., S. Goff, M. Paskind, G. Otto, and D. Baltimore.** 1979. Structure of Abelson murine leukemia virus genome. Cell **18**:955–962.
20. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
21. **Srinivasan, A., E. P. Reddy, and S. A. Aaronson.** 1981. Abelson murine leukemia virus: molecular cloning of infectious integrated proviral DNA. Proc. Natl. Acad. Sci. USA **78**:2077–2081.
22. **Sugden, B., and W. Mark.** 1977. Clonal transformation of adult human leukocytes by Epstein-Barr virus. J. Virol. **23**:503–508.
23. **Wang, J. Y.-J., F. Ledley, S. Goff, R. Lee, Y. Groner, and D. Baltimore.** 1984. The mouse c-*abl* locus: molecular cloning and characterization. Cell **36**:349–356.
24. **Whitlock, C. A., D. Robertson, and O. N. Witte.** 1984. Murine B-cell lymphopoiesis in long term culture. J. Immunol. Methods **67**:353–369.
25. **Whitlock, C. A., and O. N. Witte.** 1982. Long-term culture of B lymphocytes and their precursors from murine bone marrow. Proc. Natl. Acad. Sci. USA **79**:3608–3612.
26. **Whitlock, C. A., and O. N. Witte.** 1981. Abelson virus-infected cells can exhibit restricted in vitro growth and low oncogenic potential. J. Virol. **40**:577–584.
27. **Whitlock, C. A., S. Ziegler, and O. N. Witte.** 1983. Progression of the transformed phenotype in clonal lines of Abelson virus-infected lymphocytes. Mol. Cell. Biol. **3**:596–604.