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# Chronic myeloproliferative disease induced by site-specific integration of Abelson murine leukemia virus-infected hemopoietic stem cells

(retrovirus/v-*abl* oncogene/chronic myelogenous leukemia/hemopoietic stem cells/site-specific integration)

XIAODONG HAN, PETER M. C. WONG, AND SIU-WAH CHUNG

Morse Institute of Molecular Genetics, Department of Microbiology and Immunology, Box 44, 450 Clarkson Avenue, State University of New York Health Science Center, Brooklyn, NY 11203

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ABSTRACT **We recently showed that hemopoietic stem cells expressing the v-*abl* oncogene can cause leukemia when injected into lethally irradiated recipient mice. Progenitor cells expressing v-*abl* did not significantly contribute to disease development, and the leukemia was monoclonal in origin. By serially transplanting v-*abl*-transduced hemopoietic stem cells into normal, nonirradiated syngeneic recipients, we showed that multiple stem-cell clones do exist in some recipients. These cells fluctuated as normal stem cells do and could home to normal bone marrow. Based on the time course of disease, the recipients developed either an acute or a chronic phase of disorder. All recipients with the acute disease had stem-cell clones with random Abelson murine leukemia virus integration sites. All recipients with the chronic disorder had a specific Abelson murine leukemia virus integration site. We believe this *abl*-specific integration site, termed ASI, is important in *abl*-mediated stem-cell leukemogenesis.**

The existence of primitive hemopoietic stem cells with lymphoid and myeloid potentiality has been demonstrated by various techniques that include the use of chromosome markers (1, 2) and *W* mutant mice (3). However, the biological properties of these cells are not well understood. They represent a minute population, even in the blood cell-forming hemopoietic organs such as the fetal liver and the adult bone marrow. By a series of physical separations with immunological markers, 30 Thy-$1^{lo}$ Sca-$1^{+}$ $Lin^{-}$ cells from mouse fetal liver were reported to rescue 50% of lethally irradiated mice (4). Thus Thy-1, Sca-1, and perhaps AA4.1 (5) markers are most likely present in pluripotent hemopoietic stem cells. Recently, by marking stem cells with retroviral vectors usually carrying a neutral gene as a genetic marker, several groups have shown that these cells fluctuate, and one or a few particular stem-cell clones appear to predominate at one time (5–9).

Abelson murine leukemia virus (A-MuLV) is a naturally occurring retrovirus carrying the v-*abl* oncogene. The virus was originally isolated from a lymphoma of B-cell origin (10). Subsequently, A-MuLV-transformed macrophages (11), erythroid precursors (12), T cells (13), mast cells (14), plasmacytoma (15), and fibroblasts (16) have been reported. The ways these transformed cells were generated appear to be a function of specific routes of virus inoculation. Therefore, accessibility of virus to the appropriate target cells is probably an important determining factor.

Using the method of retroviral-mediated gene transfer and an Abelson virus stock free of helper virus, we have successfully delivered the v-*abl* oncogene into lymphoid-myeloid hemopoietic stem cells (17). When these cells are introduced into lethally irradiated recipients, a monoclonal disease develops. We conclude that A-MuLV-infected hemopoietic progenitor cells apparently do not contribute significantly to disease development. In addition, we hypothesize that, in the irradiated recipients, competition between A-MuLV-infected lymphoid-myeloid stem cells exists, so that only a single clone predominates at one time. To test this hypothesis, we changed the experimental protocol in an important way. Instead of using lethally irradiated recipients, we serially transplanted A-MuLV-infected hemopoietic stem-cell clones into syngeneic, unirradiated recipients. Under this condition, one might predict the appearance of multiple infected stem-cell clones participating in disease development in a single recipient, thus allowing the relative contribution by a particular clone to be examined. Because in these unirradiated animals the infected stem-cell clones represent only a small fraction compared with the uninfected clones, it is important to use the A-MuLV virus stock free of helper virus, so that no virus spreads from the infected clones transduced with the v-*abl* gene.

In this study, we observed the existence of several v-*abl*-transduced stem-cell clones, which fluctuated as normal stem cells do. In addition, the recipients developed either chronic or acute disease. All recipients analyzed that developed chronic disorder had the same distinct A-MuLV integration site, whereas those that died of acute disease had different A-MuLV integration sites.

## MATERIALS AND METHODS

**Preparation of A-MuLV Virus Stock.** High-titer virus stock of $1 \times 10^7$ focus-forming units per ml was prepared as described (17). When tested on XC cell plaque assay (18), this stock of viral supernatant did not contain any helper virus. In order to use our study protocol, the A-MuLV stock has to be free of the replication-competent helper virus to prevent further spread of A-MuLV by infected cells. Under this condition, the infected cells will therefore be marked by the proviral genome, as defined by a specific integration site. This marking enables us to determine the number of A-MuLV-infected stem cells and to follow the fate and development of these retrovirally marked stem-cell clones. For example, HA2 in Fig. 3 contained three individually marked stem-cell clones a, b, and c, which later segregated into different recipient mice.

**Animals and Cells.** Eight- to 10-week-old BALB/c inbred mice were obtained from the National Cancer Institute. For some transplantation experiments, recipient animals received 9 Gy of total body irradiation. The preparation, dissection, and collection of 12-day fetal liver cells used for retrovirus infection was done as described (19, 20).

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Abbreviation: A-MuLV, Abelson murine leukemia virus.

**Retrovirus Infection.** Fetal liver cells from 12-day-old mouse embryos from BALB/c inbred mice were incubated with A-MuLV virus supernatant stock at two million cells per ml, together with Polybrene at 4 μg/ml and 2% pokeweed mitogen-stimulated spleen cell-conditioned medium overnight at 37°C. Sixteen hours later, the cells were washed twice in R2 medium (RPMI 1640 medium/2% fetal calf serum), and one to two million cells were injected into each unirradiated or lethally irradiated recipient.

**Serial Transplantation.** Unirradiated recipient mice were maintained in regular cages in our animal quarters. Irradiated recipients were maintained in a sterile laminar-flow hood and fed with autoclaved food and acidified water. Peripheral blood was obtained by drawing blood through the retroorbital plexus. When individual mice were sacrificed, various organs and tissues were dissected out and prepared for DNA or RNA extraction or for biological assays as described (21). Bone-marrow cytoprep smears were stained with Wright–Giemsa stain. To detect factor-independent colonies formed by A-MuLV-infected progenitor cells, bone-marrow or spleen cells from recipient mice were plated in methylcellulose cultures as described (19).

**Southern Blot Analysis.** DNA was extracted from frozen tissues pulverized in liquid nitrogen. Extraction of high-$M_r$ DNA from various tissues was done by treatment with SDS followed by proteinase K digestion as described (17). DNA samples were usually digested with *Bam*HI and *Hin*dIII, enzymes that generate junctional fragments with respect to the integrated A-MuLV proviral genome. To extract DNA from the peripheral blood samples, usually three capillary tubes with a volume of 70 μl each were obtained. After spinning in a hematocrit centrifuge for 5 min, the buffy coat (containing the white blood cells) was used for DNA extraction. The total yield was up to one million nucleated cells. The cells were then lysed in extraction buffer, and the sample was processed normally for obtaining high-$M_r$ DNA.

## RESULTS

**Different Time Course and Pathological Disease Development.** One to two million A-MuLV-infected fetal liver cells were injected i.v. into each normal, syngeneic, unirradiated mouse. By 4 weeks after transplantation, recipient mice began to develop disease (Table 1). Time course for disease development differed significantly from animal to animal; this difference has been confirmed by analysis with 17 additional recipients (HA9–HA26). Among these 17 mice, 4 mice were moribund at week 4, 6 mice survived to 21 weeks, and the rest of the animals developed disease at various times. All animals analyzed carried one to three copies of A-MuLV proviral DNA. Factor-independent growth of hemopoietic colonies has been an excellent index of v-*abl* expression (19, 22, 23).

Table 1. Pathological data of v-*abl*-transduced recipients

| Mouse | Time analyzed, week | State | HLP | Enlargement | | | WBC* (week) | Hct, % | Plt† | v-*abl* clone | FI col, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LN | Spl | Thy | | | | | |
| N2-9 | 6 | N | – | – | – | – | 6 (6) | 41 | ND | ND | ND |
| N2-10 | 6 | N | – | – | – | – | 7 (6) | 45 | ND | ND | ND |
| HA1 | 4 | D | + | + | + | – | 44 (3) | 56 | ND | ND | ND |
| HA2 | 4 | M | + | + | 0.3 | – | 50 (3) | 50 | ND | 3 | 19 |
| HA3 | 9 | N | – | – | – | + | 5 (9) | 50 | ND | 1 | 32 |
| HA4 | 9 | N | – | – | – | + | 5 (9) | 50 | ND | 1 | 20 |
| FA5 | 12 | D | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| FA6 | 12 | M | – | + | – | – | 100 (12) | 38 | 21 | 1 | 18 |
| FA7 | 12 | M | – | + | 0.2 | – | 53 (12) | 39 | 43 | 1 | 29 |
| FA8 | 11 | M | + | – | + | – | 12 (3) | 37 | ND | 1 | 13 |
| HA9 | 4 | M | + | + | 0.4 | + | 48 (4) | 49 | 11 | 1 | 314 |
| HA10 | 4 | M | + | + | 0.3 | + | 23 (4) | 43 | 19 | 1 | ND |
| HA14 | 4 | M | + | + | 0.3 | + | 100 (4) | 49 | 9 | 1 | 112 |
| HA2-1 | 2 | M | + | – | 0.3 | + | 15 (2) | 46 | ND | 2 | 12 |
| HA2-2 | 2 | M | + | – | 0.4 | + | 16 (2) | 52 | ND | 2 | 0 |
| HA2-3 | 2 | M | + | – | 0.4 | + | 36 (2) | 47 | ND | 2 | 5 |
| HA2-4 | 2 | M | + | – | 0.5 | + | 26 (2) | 48 | ND | 2 | 8 |
| HA2-1-1 | 3 | M | + | + | – | + | 84 (3) | 60 | ND | 2 | 63 |
| HA2-1-2 | 3 | M | + | + | – | + | 75 (3) | 53 | ND | 2 | 49 |
| HA2-1-3 | 18 | N | – | – | 0.09 | + | 20 (18) | 43 | 3 | 1 | <1 |
| HA2-1-4 | 21 | Alive | | | | | 15 (21) | 49 | 6 | | |
| HA2-4-1 | 5 | N | – | + | 0.3 | + | 3 (5) | 51 | ND | 2 | 14 |
| HA2-4-2 | 7 | M | – | + | 0.3 | – | 19 (7) | 40 | ND | 1 | ND |
| HA2-4-3 | 1 | D | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| HA2-4-4 | 16 | N | – | – | 0.1 | + | 40 (16) | 50 | 23 | 1 | 8 |

Hematopathological data of v-*abl*-transduced recipient mice. A-MuLV-infected cells were injected i.v. into normal syngeneic adult BALB/c mice at $2 \times 10^6$ cells per mouse. Eight mice (HA1–FA8) were injected together with mice that received N2-infected cells as described (17). HA3 and HA4 were sacrificed and analyzed on week 9 to determine whether there was any v-*abl* transduction. At week 10, FA5–FA8 were injected with 5-fluorouracil at a dose sufficient to kill off most cycling hemopoietic cells. In this way, dormant stem cells are spared and recruited later into cycling. On week 12, all mice were sacrificed. HA2-1–HA2-4 were recipients of HA2 bone-marrow cells (one million each). HA2-1-1–HA2-1-4 and HA2-4-1–HA2-4-4 were recipients of one million bone-marrow cells from HA2-1 and HA2-4, respectively. D, dead; M, moribund; N, normal; HLP, hind limb paralysis; LN, lymph nodes; Spl, spleen; Thy, thymus; FI col, factor-independent colonies, mainly composed of macrophage type CFU-M (colony-forming unit-macrophage) and small undifferentiated type; Hct, hematocrit. Organ enlargement of lymph nodes is defined by an obvious massive cell proliferation occurring in lymph nodes in most anatomical locations. Thymus enlargement was two to four times the normal thymus size of N2 recipients. Actual weight of spleen for N2-9 and -10 was not determined; however, the weight of two other N2 recipient spleens was 0.1 and 0.09 g. Some recipient spleens were obviously enlarged, but their actual weights were not recorded and thus are indicated by a plus sign. Average percentage of platelet counts from two other N2 recipients was $4 \times 10^5$ per $mm^3$. ND, not done.
*WBC, white blood cell count (number $\times 10^{-3}$ cells per μl).
†Plt, platelet counts (number $\times 10^{-5}$ platelets per $mm^3$).

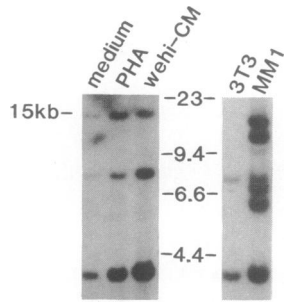


FIG. 1. Southern blot analysis of DNA from myeloid and lymphoid cells of the recipient HA15-2R. Two million spleen cells were cultured in liquid suspension with or without 1% phytohemagglutinin (PHA)/10% WEHI-3-conditioned medium (wehi-CM) for 6 days. Extracted DNAs from cells of these cultures were digested with *Hin*dIII and blotted with v-*abl*-specific sequences. Note the 15-kilobase (kb) junction fragment band in all three samples. 3T3, NIH 3T3 fibroblasts; MM1, v-*abl*-transformed mast cells.

Bone-marrow cultures of 15 of 16 primary recipient mouse developed factor-independent hemopoietic colonies, indicating expression of the transduced v-*abl* gene in these animals.

By serially transplanting bone-marrow cells from moribund donor animals into normal recipients, the disease was shown to be transplantable (Table 1). However, pathological development was significantly different among the recipients and the donor parent. Most prominent was the variation in hind limb paralysis and enlargement of lymph nodes, spleens, or thymus (Table 1).

**Evidence of Stem-Cell Infection by A-MuLV.** In all primary recipients analyzed, stem cells of lymphoid-myeloid origin were transduced with v-*abl*. Recipients HA2, -9, -10, and -14 developed acute disease within 4 weeks, displaying lymphadenopathy and spinal tumors. The acute syndromes seen in these mice are similar to those of Abelson disease (10), wherein pre-B cells have been the target cells for A-MuLV (24, 25). However, in marked contrast to features seen with the Abelson disease, these animals had elevated white blood cell count with a clear presence of circulating blast cells (15–58%); enlargement of the thymus and spleen was often seen (Table 1). Histological examination of spleens indicated diffused infiltration of lymphoblastoid and monocytic cells. Also, unreported for the Abelson disease, bone-marrow cells of these animals could develop many factor-independent colonies composed mainly of macrophage type CFU-M (Table 1).

DNAs from the enlarged spleen, thymus, and/or lymph node revealed rearrangements of T-cell receptor and immunoglobulin genes in appropriate sites (data not shown); yet in recipients HA9, HA10, and HA14, only a single copy of integrated A-MuLV proviral DNA could be detected (Table 1). The same corresponding A-MuLV-integrated proviral DNA was detected in DNA from pooled factor-independent macrophage colonies and from mast cells stimulated with WEHI-3-conditioned medium. Fig. 1 is one example in which spleen cells from HA15-2-R, one of the lethally irradiated recipients of HA15, were stimulated with either phytohemagglutinin (mitogen for lymphoid cells) or WEHI-3-conditioned medium (mitogen for myeloid cells) for 6 days. The same A-MuLV proviral DNA could be observed in stimulated and unstimulated cultures. These data indicate the involvement of lymphoid-myeloid hemopoietic stem cells, even in these recipients that developed acute disease. Similar data were obtained in HA2-4-4, FA7-1, and FA7-2 (two lethally irradiated recipients of FA7, analyzed 15 weeks later) recipients that developed chronic disorder.

**Clonal Fluctuation of the Transduced Stem Cells and an Association of a Particular v-*abl* Clone with Chronic Disorder.** In the serial transplantation experiments, one striking observation was noted by comparing recipients of two animals, HA2 and HA16 (Fig. 2 and Table 1). While serial bone-marrow recipients of HA16 manifested uniformly short latent periods each time after transplantation, tertiary recipients of HA2 developed the disease at various times (Fig. 2).

In attempting to explain this variation, we first determined the number of v-*abl*-transduced stem-cell clones in HA2. Digestion of genomic DNA from HA2 with two restriction enzymes, *Bam*HI and *Hin*dIII, yielded three distinct clones, a, b, and c (Fig. 3). Clone a (9.5-kb *Bam*HI or 16-kb *Hin*dIII fragment) was the dominant population in the spleen, lymph nodes, and lung of HA2. After one million bone-marrow cells of HA2 were transplanted into each of the four secondary recipients (HA2-1–HA2-4), clonal segregation was seen among various organs. This segregation was most obvious

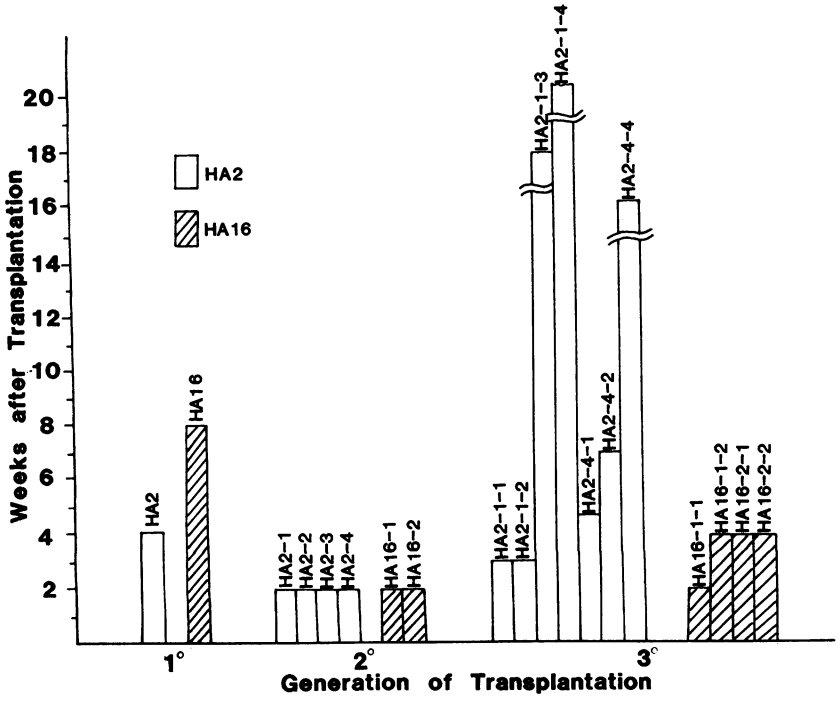


FIG. 2. Time course for disease development. Time was defined by the number of weeks required for recipient mice to become moribund, except for recipients HA2-1-3, 2-1-4, and 2-4-4, shown with broken bars. HA2-1-3 and HA2-4-4 were still healthy when sacrificed. Recipient HA2-1-4 was still alive at week 21, indicated by an uneven line at the top of the bar. Recipients HA2-1–HA2-4, and HA16-1–HA16-2 each received one million bone-marrow cells from HA2 and HA16, respectively. Recipients HA2-1-1–2-1-4, HA2-4-1–2-4-4, HA16-1-1–16-1-2, and HA16-2-1–HA16-2-2 each received one million bone-marrow cells from HA2-1, HA2-4, HA16-1, and HA16-2, respectively. All recipient mice were not irradiated.

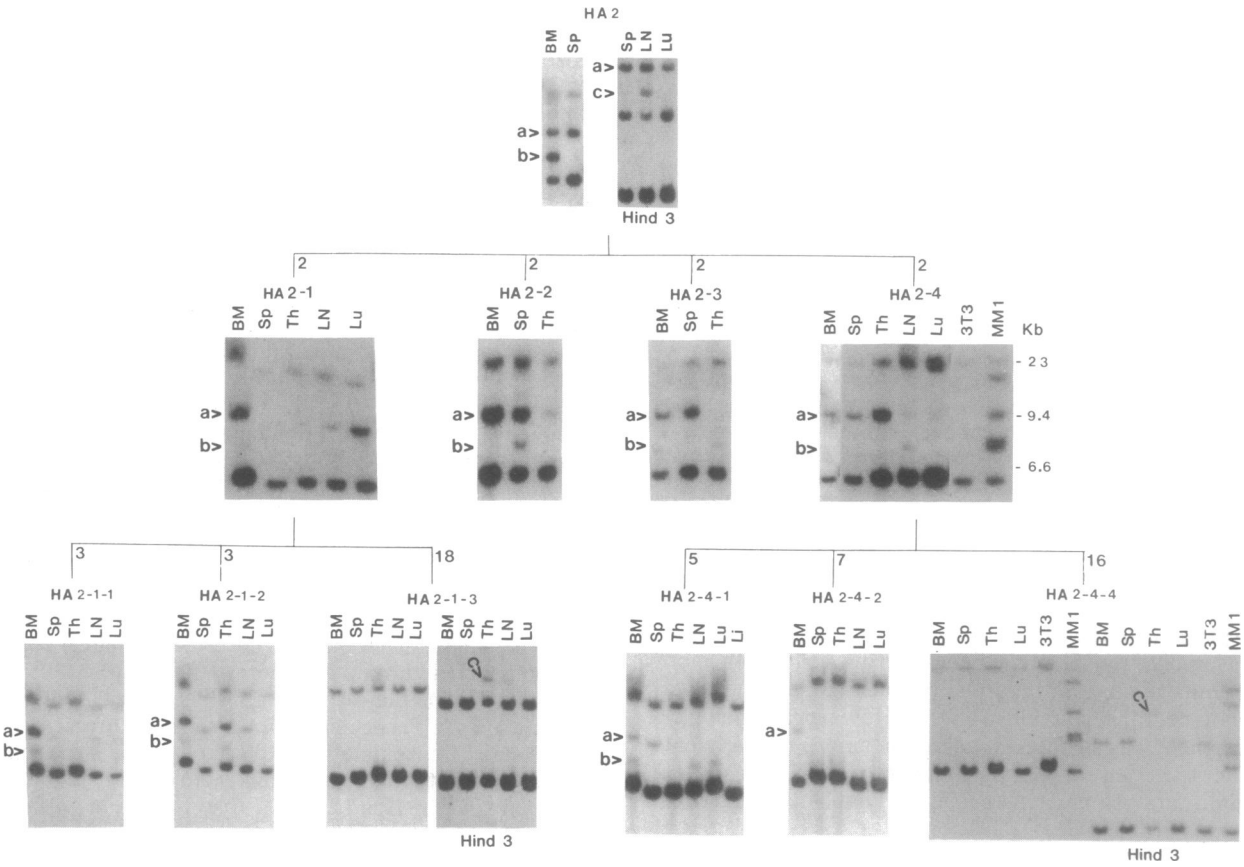


FIG. 3. Clonal segregation in recipient mice of HA2 revealed by Southern blot analysis. HA2 was the primary recipient of A-MuLV-infected fetal liver cells. HA2-1–2-4, HA2-1-1–2-1-3, and HA2-4-1–2-4-4 were secondary and tertiary recipients, as described in the legend of Table 1. Unless otherwise stated, all samples were digested with *Bam*HI. Numbers on top of each recipient were the number of weeks after transplantation. BM, bone marrow; Sp, spleen; Th, thymus; LN, lymph nodes; Lu, lung, 3T3, NIH 3T3 fibroblasts; MM1, v-*abl*-transformed mast cells.

when tertiary recipients were examined. Clone b (7.3-kb *Bam*HI) was absent in HA2-4 bone marrow. Although it reappeared in tertiary recipient HA2-4-1, clone b was not detectable in either HA2-4-2 or HA2-4-4. Most interestingly, all mice containing predominant clone a, with or without minor clone b, became moribund within 2 months after transplantation, whereas only clone c (9.9-kb *Hin*dIII fragment) was present in the two long-term recipients HA2-1-3 and HA2-4-4, which were free of symptoms at 4–5 months when they were sacrificed for analysis. Southern blot analysis of the peripheral blood DNA of the third animal, HA2-1-4, still alive at 27 weeks with elevated white blood cell count, contained clone c as well (Fig. 4).

**Recipients with the Chronic Disorder Had a Distinct A-MuLV Integration Site.** We then examined whether clone c was present in all recipients with a long latency. Table 2 indicates that the 9.9-kb *Hin*dIII fragment (clone c in Fig. 3) occurred in all recipients that developed chronic disorder, as defined by a latency >8 weeks.

Most recipients with the chronic disorder had elevated white cell counts. To determine whether the peripheral blood leukocytes are the progeny of A-MuLV-infected stem cells, Southern blot analysis was done on DNA of peripheral blood of three of these recipients (Fig. 4). FA6-1 and FA7-3 were lethally irradiated (15 weeks after transplantation) recipients of FA6 and FA7 bone-marrow cells. HA2-1-4 was a recipient of serially transplanted bone-marrow cells that originated from HA2, at 23 weeks after transplantation. Fig. 4 indicates

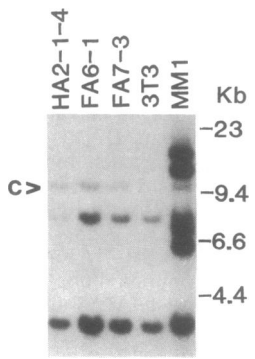


FIG. 4. Presence of clone c in the peripheral blood of recipients with chronic leukemia. Southern blot analysis was done on *Hin*dIII-digested DNA extracted from recipient leukocytes of 200 μl of peripheral blood. Note the 9.9-kb band (clone c) in all three leukocyte samples. 3T3, NIH 3T3 fibroblasts; MM1, *abl*-transformed mast cells.

Table 2. A-MuLV proviral integration sites in various recipients

| Time to moribundity, week | Recipient | Junction fragments, size in kb | |
|---|---|---|---|
| | | *Bam*HI | *Hin*dIII |
| 4 | HA2 | 9.5, 7.3 | 9.9, 16 |
| 4 | HA9 | 10.7 | 8.8 |
| 4 | HA10 | 9.4 | 8.8 |
| 4 | HA14 | 13 | 6.1 |
| 5 | HA12 | 13.3 | 8.8 |
| 5 | HA13 | 14.8 | 6.4 |
| >9 | HA3 | Absent | 9.9 |
| >9 | HA4 | Absent | 9.9 |
| 11 | FA8 | Absent | 9.9 |
| 12 | FA6 | Absent | 9.9 |
| 12 | FA7 | Absent | 9.9 |

Mice HA3 and HA4 were not moribund when sacrificed for analysis. Both *Bam*HI and *Hin*dIII had one recognition site within the A-MuLV proviral genome. The v-*abl* probe was derived from a 1.6-kb *Bgl* II fragment of the p160 A-MuLV proviral DNA.

the presence of clone c in the peripheral blood of all three animals.

## DISCUSSION

By serially transplanting A-MuLV-infected hemopoietic stem cells into normal, syngeneic recipients, we have shown that the v-*abl*-transduced stem cells fluctuated as normal stem cells do and could home to normal bone marrow. All recipients that developed acute disease had stem cells with random A-MuLV integration sites, whereas those that developed the chronic disorder had stem cells with a specific integration site.

There was no infectious helper virus present either in our A-MuLV virus stock or in the conditioned medium of the spleen cells from serially transplanted HA2 recipients. Were infectious particles present in the recipient animals, one would predict effective virus spread in these nonirradiated, normal recipients, and one should see the appearance of a relatively large number of newly integrated A-MuLV proviral genome: this large-scale integration was not observed; all animals had only one to three copies of A-MuLV proviral DNA.

Disappearance of clones a and b in HA2-1-3 and HA2-4-4 most likely was not from an immunological response against the v-*abl*-transduced cells. No helper virus was present in the animals analyzed and, therefore, no synthesis of viral antigen occurred. Within the A-MuLV p160 genome, the gag–v-abl protein is not known to be transmembrane (26, 27) and, therefore, should not be available on the cell surface for immune attack. Lastly, if there were an overall immune reaction against the v-*abl*-transduced cells, cells of clone c should disappear as well, and they did not.

Transplantation of normal marrow cells into normal syngeneic mice has been reported to be unsuccessful (28, 29). In our studies, v-*abl*-transduced stem cells readily home to the bone-marrow microenvironment. One interpretation is that donor stem cells do home into normal bone marrow but are usually not detectable, as reported (30). Expression of v-*abl* in these cells expands the clones to a detectable level. Alternatively, the expression of v-*abl* in the transduced hemopoietic stem cells induced a modification at the cell surface, enhancing their adhesive ability for the marrow stroma. Such altered adhesive interactions with marrow stroma by hemopoietic progenitor cells in chronic myeloid leukemia, in which the activated *bcr–abl* gene is a hallmark of this disease, has been demonstrated by Gordon *et al.* (31). However, the abnormal progenitor cells become less adherent to the marrow stroma (31).

True leukemic clones should proliferate autonomously and continuously. Finding clonal fluctuation of v-abl-transduced stem cells within the initial 3-month period and only one remaining clone 5 months later was, thus, unexpected. These data closely resemble those of normal, uninfected stem cells (5–9). Whether v-*abl* was expressed at the beginning of clonal expansion of the transduced lymphoid-myeloid stem cells is impossible to definitely determine. However, progeny progenitors did express v-*abl*, as indicated by their ability to develop factor-independent colonies.

HA2-1-3 and HA2-4-4 remained apparently normal 5 months after transplantation. Thus clone c is not frankly neoplastic, and yet clonal expansion was consistently seen, as indicated by elevation of white blood cell counts and occasional increase of platelet counts. For the surviving recipient, HA2-1-4, chronic elevation of white cells derived from clone c has been seen (Fig. 4). Although blast cells were clearly present in the peripheral blood smear from all three recipients, a normal spectrum of differentiated neutrophils, monocytes, and large and small lymphocytes could be identified easily. This condition resembles a state of chronic leukemia.

One might argue that as a result of small changes such as point mutations within the A-MuLV genome, v-*abl* expression is low in animals with chronic disorder compared with those that had the acute disease. This explanation is unlikely because all animals were derived from independently A-MuLV-infected stem-cell clones, and in two additional independent infection experiments, clone c was consistently observed.

Frequent sites of retroviral integration associated with myeloid leukemia have been reported (32, 33). The specific association of a specific A-MuLV integration site with the development of a state of chronic disorder is particularly interesting in our studies. As a result of this specific A-MuLV integration, termed ASI, a gene appears to have been either activated or inactivated. Consequently, the signal transduction induced by v-*abl* might have been interrupted or altered, modifying leukemic development.

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1. Wu, A. M., Till, J. E., Siminovitch, L. & McCulloch, E. A. (1967) *J. Cell Physiol.* **69**, 177–183.
2. Abramson, S., Miller, R. G. & Pillips, R. A. (1977) *J. Exp. Med.* **145**, 1567–1574.
3. Mintz, B., Anthony, K. & Litwin, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7835–7839.
4. Spangrude, G. J., Heimgeld, S. & Weissman, I. L. (1988) *Science* **241**, 58–62.
5. Jordan, C. T., McKearn, J. P. & Lemishka, I. R. (1990) *Cell* **61**, 953–963.
6. Capel, B., Hawley, R., Covarrubias, L., Hawley, T. & Mintz, B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4564–4568.
7. Lemischka, I. R., Raulet, D. H. & Mulligan, R. C. (1986) *Cell* **45**, 917–927.
8. Snodgrass, R. & Gordon, K. (1987) *EMBO J.* **6**, 3955–3960.
9. Jordan, C. T. & Lemishka, I. R. (1990) *Genes Dev.* **4**, 220–232.
10. Abelson, H. T. & Babstein, L. S. (1970) *Cancer Res.* **30**, 2213–2222.
11. Raschke, W., Baird, S., Ralph, P. & Nakoinz, I. (1978) *Cell* **15**, 261–267.
12. Waneck, G. L. & Rosenberg, N. (1981) *Cell* **26**, 79–89.
13. Cook, W. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2917–2921.
14. Mendoza, G. R. & Metzger, H. (1976) *J. Immunol.* **117**, 1573–1578.
15. Potter, M., Sklar, M. D. & Rowe, W. P. (1973) *Science* **182**, 592–594.
16. Scher, C. D. & Siegler, R. (1975) *Nature (London)* **253**, 729–731.
17. Chung, S. W., Wong, P. M. C., Durkin, H., Wu, Y. S. & Petersen, J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1585–1589.
18. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1970) *Virology* **42**, 1136–1139.
19. Chung, S. W., Ruscetti, S. & Wong, P. M. C. (1988) *Blood* **71**, 973–977.
20. Wong, P. M. C., Chung, S. W. & Nienhuis, A. W. (1987) *Genes Dev.* **1**, 358–365.
21. Wong, P. M. C., Chung, S. W., Dunbar, C. E., Bodine, D. M., Ruscetti, S. & Nienhuis, A. W. (1989) *Mol. Cell. Biol.* **9**, 798–808.
22. Chung, S. W., Wong, P. M. C., Shen-Ong, G., Ruscetti, S., Ishizaka, T. & Eaves, C. J. (1986) *Blood* **68**, 1074–1081.
23. Wong, P. M. C., Chung, S. W., Raefsky, E., Eaves, C. J. & Nienhuis, A. W. (1988) *Exp. Hematol.* **16**, 5–11.
24. Rosenberg, N., Baltimore, D. & Scher, C. D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1932–1936.
25. Boss, M., Greaves, M. & Teich, N. (1979) *Nature (London)* **278**, 551–553.
26. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22**, 777–785.
27. Prywes, R., Foulkes, J. G., Rosenberg, N. & Baltimore, D. (1983) *Cell* **34**, 569–579.
28. Brecher, G., Tijo, J. H., Haley, J. E., Narla, J. & Beal, S. L. (1979) *Blood Cells* **5**, 237–246.
29. Micklem, H. S., Clarke, C. M., Evans, E. P. & Ford, C. E. (1968) *Transplantation* **6**, 299–314.
30. Sadelain, M. W. J. & Wegmann, T. G. (1989) *Blood* **74**, 2325–2329.
31. Gordon, M. Y., Dowding, C. R., Riley, G. P., Goldman, J. M. & Greaves, M. F. (1987) *Nature (London)* **328**, 342–344.
32. Gisselbrecht, S., Fichelson, S., Sola, B., Bordereaux, D., Hampe, A., Andre, C., Galibert, F. & Tambourin, P. (1987) *Nature (London)* **329**, 259–261.
33. Moreau-Gachelin, F., Tavitian, A. & Tambourin, P. (1988) *Nature (London)* **331**, 277–280.