from other AIDS patients and identifying LAV-specific antibodies in the serum of AIDS patients, including the donor-recipient pair reported here, but not in serum from persons in groups with low AIDS incidence (*4*, *8*), provide further evidence for such an etiologic association.

An etiologic association between HTLV-III and AIDS was reported recently (*4*). The most likely explanation for the parallel evidence for HTLV-III and LAV being the cause of AIDS is that the two viruses are the same.

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References and Notes

1. M. Essex *et al.*, *Science* **220**, 859 (1983).
2. F. Barré-Sinoussi *et al.*, *ibid.*, p. 868.
3. H. W. Jaffe *et al.*, *ibid.* **223**, 1309 (1984).
4. P. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; J. Schüpbach *et al.*, *ibid.*, p. 224; M. G. Sarngadharan *et al.*, *ibid.*, p. 506.
5. V. S. Kalyanaraman *et al.*, *Nature (London)* **294**, 271 (1981).
6. L. Montagnier *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984).
7. F. C. Greenwood, W. M. Hunter, J. S. Glover, *Biochem. J.* **89**, 114 (1963).
8. V. S. Kalyanaraman *et al.*, *Science*, in press.
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Altered Transcription of the c-*abl* Oncogene in K-562 and Other Chronic Myelogenous Leukemia Cells

Abstract. *Expression of the cellular* abl *(c-*abl*) oncogene was studied in K-562 and other chronic myelogenous leukemia (CML) cells and cell lines by means of Northern blot hybridization. In contrast to non-CML cells, which contained 7.4- and 6.8-kilobase* abl*-related transcripts, the CML cells contained a predominant and novel 8.2-kilobase* abl*-related RNA. In addition, the levels of* abl*-related message were up to eight times higher in CML cell lines from patients at the blast crisis stage of the disease compared with CML cells obtained during the chronic phase and with non-CML cells.*

Chronic myelogenous leukemia (CML) is a pluripotent stem cell disease characterized in over 90 percent of cases by the presence of the Philadelphia chromosome (Ph^1) (*1*). A number of recent findings suggest that the human cellular homolog (c-*abl*) of the tranforming sequences of the Abelson murine leukemia virus (v-*abl*) may be involved in the pathogenesis of CML: the c-*abl* gene, normally located on chromosome 9, is translocated to the Philadelphia chromosome in cases of CML (*2*); moreover, in at least one case of CML the chromosomal break point on chromosome 9 is in close proximity to the c-*abl* locus, mapping within 14 kb 5′ of the c-*abl* gene (*3*); in addition, c-*abl* is amplified some four to eight times in the K-562 CML cell line, and this cell line exhibits increased expression of *abl*-related RNA (*4*). Here we demonstrate in three Ph^1 positive CML cell lines and in a fresh CML cell sample the presence of an 8.2-kb c-*abl*–related messenger RNA that is absent from cells of non-CML origin. We also show that the expression of *abl*-related messenger RNA is increased in CML cell lines obtained from patients during blast crisis compared to fresh CML cells obtained during the chronic phase and to non-CML cell lines.

Northern blot analysis of polyadenylated RNA derived from various human cell lines of non-CML origin and hybridized to a mouse v-*abl*–specific probe

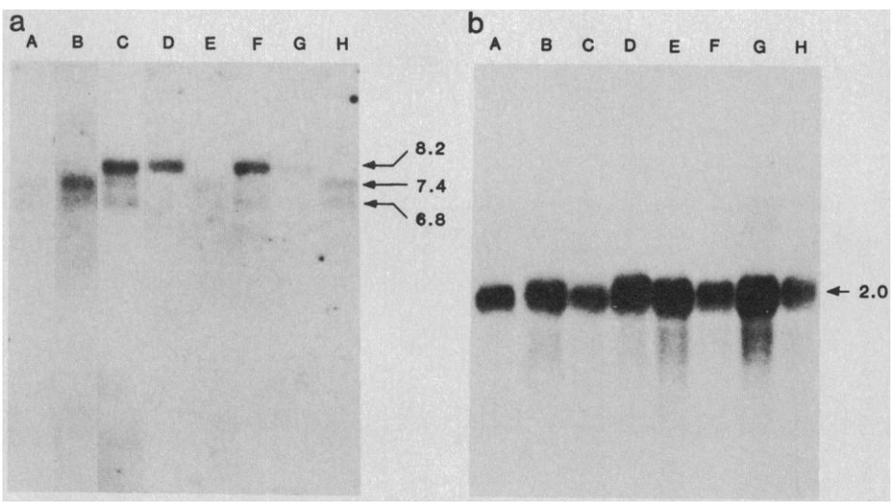

Fig. 1. (a) C-*abl*–related RNA transcripts in CML and non-CML cell lines. Cells were homogenized in guanidine thiocyanate (Eastman-Kodak) and subjected to ultracentrifugation through a cesium chloride cushion as described by Chirgwin *et al.* (*17*). The RNA pellet was suspended in 0.3 ml of water and precipitated twice with ethanol. The pellet was resuspended in 0.3 ml of water. Total RNA was enriched for polyadenylate-containing RNA by one cycle of oligo dT cellulose affinity chromatography (*18*). Portions (5 μg) of polyadenylated RNA were subjected to electrophoresis in formaldehyde gels and transferred to nitrocellulose filters as described (*19*). The filter was baked and then hybridized to the v-*abl*–specific pABlsub9 probe (*6*) that was radioactively labeled with ^{32}P by nick translation (*20*). Prehybridization, hybridization, wash, and exposure conditions were as described (*4*). Exposure time was 6 days. Sizes in kilobases were determined by comparison with 28*S* (5.0 kb) and 18*S* (2.0 kb) ribosomal RNA bands visualized by ethidium bromide staining of a marker lane excised from each gel before nitrocellulose transfer. Cells and cell lines are as follows: A, HL-60 promyelocytic leukemia (*21*); B, normal peripheral blood cells transformed with EBV; C, K-562; D, EM-2; E, Raji (Burkitt lymphoma); F, KCL-22; G, peripheral blood cells from a patient with Ph^1-positive CML; H, KG-1 myelogenous leukemia (*22*). (b) Transcripts of β-actin–related RNA in CML and non-CML cell lines. The same samples as in (a), after being allowed to undergo several half-lives of ^{32}P decay of the initial v-*abl* probe, were hybridized to the chick pAl β-actin complementary DNA probe (*23*) under the same conditions as for (a). Exposure time was 5 hours.

revealed the presence in these cells of 7.4- and 6.8-kb c-*abl*–related transcripts (Fig. 1a, lanes A, B, E, and H). Findings of similar *abl*-related RNA in human cells have been reported by others (*5, 6*), and these transcripts probably represent the normal c-*abl* transcription pattern. In contrast, the Ph[1]-positive CML blast crisis cell lines K-562 (*7*), KCL-22 (*8*), and EM-2 (*9*) displayed a predominant 8.2-kb *abl*-related messenger RNA (Fig. 1a, lanes C, D, and F). In K-562 and KCL-22 the 7.4- and 6.8-kb *abl* transcripts were also present while in EM-2 only the 8.2-kb *abl*-related transcript was demonstrated (Fig. 1a, lane D). Peripheral blood cells from a patient with chronic phase Ph[1]-positive CML display a single faint but distinct 8.2-kb *abl*-related message (Fig. 1a, lane G).

The variable intensity of hybridization of the v-*abl* probe to the different RNA samples (Fig. 1a) suggested that, like the K-562 cells (*4*), the other CML cell lines, KCL-22 (lane F) and EM-2 (lane D), might also contain increased levels of *abl*-related message. The variable intensity of hybridization was not a result of differences in the amount of RNA loaded on the gel since the same samples hybridized to a β-actin probe gave bands of essentially the same intensity (Fig. 1b). To better quantitate the levels of *abl*-related message in these various cell lines, we performed a dot blot analysis of polyadenylated RNA from these cells. As shown in Fig. 2, there was a four- to eightfold increase in the amount of *abl*-related message in the CML blast crisis cell lines KCL-22, K-562, and EM-2 compared to the amount of *abl* RNA in chronic phase CML cells and non-CML cell lines. The Northern blots (Fig. 1a) indicate that most of the increased *abl*-related message in these cell lines is accounted for by the predominant 8.2-kb transcript.

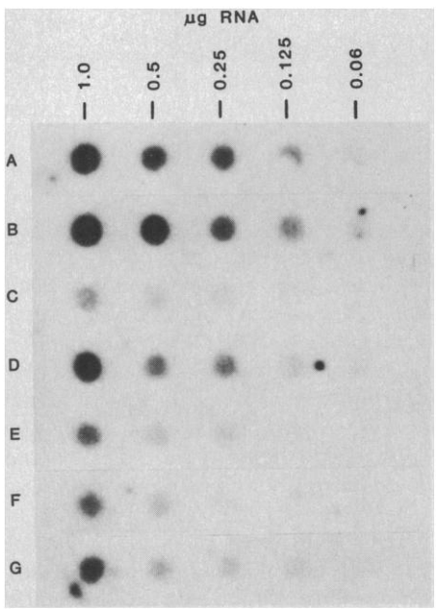

Fig. 2. Enhanced expression of *abl*-related messenger RNA in CML cell lines. Polyadenylated RNA was isolated from the indicated cells as described in Fig. 1a. The indicated quantity of RNA was diluted in 100 μl of water containing 6× SSC (standard saline citrate) and 2.2*M* formaldehyde and then heated for 15 minutes at 60°C. The samples were cooled on ice and were applied with a Hybri-Dot manifold (Bethesda Research Laboratories) to a nitrocellulose filter previously soaked in 20× SSC. The filter was immediately dried under a heat lamp, baked for 2 hours at 80°C, and hybridized to the ^{32}P-labeled v-*abl*–specific pABlsub9 probe (*6*) as described (*4*). Exposure time was 4 days. Samples are as follows: A, K-562; B, KCL-22; C, peripheral blood cells from a patient with Ph[1]-positive CML; D, EM-2; E, HL-60 promyelocytic leukemia; F, KG-1 myelogenous leukemia; G, EBV-transformed B lymphoblasts.

The enhanced expression of *abl*-related RNA in the K-562 cell line is associated with amplification and rearrangement of the c-*abl* gene in these cells (*4*). To determine if the similarly enhanced expression of c-*abl* in the KCL-22 and EM-2 blast crisis cell lines was also associated with c-*abl* gene amplification or rearrangement, we hybridized the v-*abl* probe to Southern blots of DNA from these cells, digested with restriction endonucleases. In contrast to K-562, intense hybridization of the v-*abl* probe to DNA from the KCL-22 and EM-2 cell lines was not noted, indicating that c-*abl* sequences are not amplified in these cell lines (Fig. 3). Moreover, these blots (Fig. 3) reveal identical restriction bands in KCL-22 and EM-2 cells compared with other non-CML cells, indicating that there are no gross rearrangements of the c-*abl* locus in these cell lines. Thus, the increased c-*abl*–related RNA in these three CML blast crisis cell lines may arise through different pathways: in K-562 c-*abl* gene amplification may be the major pathway, whereas in KCL-22 and EM-2 the pathways are unknown.

Both the λ immunoglobulin light chain constant region (C_λ) and c-*abl* genes are amplified to a similar extent in K-562 (*4*). Moreover, C_λ, which is normally present on the long (q) arm of chromosome 22, remains on the Ph[1] chromosome in the 9;22 translocation characterizing CML (*3*). These findings suggest that the c-*abl* gene in Ph[1]-positive CML may be translocated from chromosome 9 into or near the C_λ locus on chromosome 22, a situation that would be analogous to the translocation of the c-*myc* oncogene into the heavy chain immunoglobulin region in cases of Burkitt lymphoma (*10, 11*). To determine whether any C_λ-related messenger RNA occurs in K-562 and other CML cells and if such RNA is contained in the abnormal 8.2-kb transcript, we hybridized the Northern blots shown in Fig. 1 to a C_λ probe. This probe consisted of a 6.2-kb R_1 subclone of Huλ 5 (*12*) and was specific for λ immunoglobulin Ke^-Oz^- constant region sequences. It hybridized, as expected, to the RNA from lymphoblasts transformed by Epstein-Barr virus (EBV) but did not hybridize to RNA from any of the CML cell lines (blot not shown). In addition, a probe specific for the cellular oncogene c-*sis* also did not hybridize to RNA from any of the CML cells, indicating that the translocation of the c-*sis* gene from chromosome 22 to chromosome 9 that has been previously demonstrated in CML (*13*) is not associated with the transcriptional activation of this oncogene.

Malignancy is probably a multistage process involving a series of genetic changes rather than a single mutational

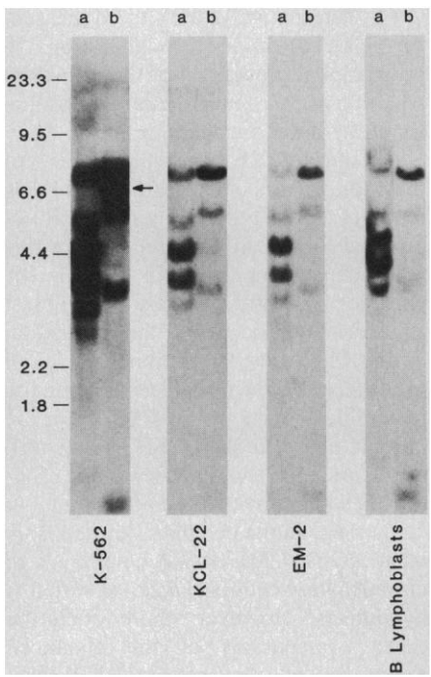

Fig. 3. Comparative restriction enzyme analysis of the c-*abl* locus of CML and non-CML cell lines. Cell lines analyzed included the CML blast crisis cell lines K-562 (*7*), KCL-22 (*8*), and EM-2 (*9*), as well as normal peripheral blood cells transformed with EBV. DNA was isolated (*24*) and incubated with various restriction endonucleases, subjected to electrophoresis overnight in a 0.8 percent agarose gel (10 μg of DNA per lane), denatured, neutralized, and transferred to nitrocellulose as described (*25*). The filter was then hybridized to the ^{32}P-labeled v-*abl*–specific pABlsub9 probe (*6*) as described (*4*). ^{32}P-Labeled Hind III digestion fragments of λ phage DNA served as the indicated molecular weight markers. Lanes a are Eco RI digests; lanes b are Pst digests. A 6.6-kb Pst band in K-562 (arrow) is not noted in the other samples.

event. One such genetic change in the pathogenesis of CML appears to be the acquisition of the Ph^1. We have demonstrated the presence of a novel 8.2-kb *abl*–related transcript in four of four Ph^1-positive CML cells and cell lines. Recently, a similar sized *abl*-related RNA transcript (8.0 kb) was found in the leukemic cells of five of six patients with Ph^1-positive CML, but was absent in Ph^1-negative CML (*14*). Together, these results suggest a close correlation between the presence of the Ph^1 and an abnormal c-*abl* transcript. Indeed, this novel transcript may serve as a leukemia-specific marker analogous to the Ph^1 marker in patients with CML. It is possible that the translocation of c-*abl* from chromosome 9 to the Ph^1 that occurs in CML results in altered transcription of the c-*abl* locus manifest by the appearance of the novel 8.2-kb message. Such an altered transcript might give rise to an abnormal c-*abl* product that could somehow be related to the pathogenesis of CML. Alternatively, the altered messenger RNA could lead to alterations in translational processing of the transcript resulting in changes in the rate of synthesis of the c-*abl* oncogene product.

Although the Ph^1 is probably critical to the pathogenesis of CML, the blast crisis stage of CML probably cannot be accounted for by the Ph^1 alone, since this marker may be present for years in the chronic phase of the disease before blast transformation occurs. If the acquisition of the Ph^1 leads to an abnormal c-*abl* product, it may be possible to explain the inevitable progression of CML from the chronic phase to blast crisis as a quantitative increase in this novel c-*abl* product. The high levels of c-*abl*–related 8.2-kb message in the cell lines derived from blast crisis CML cells compared to chronic phase cells is consistent with this hypothesis. However, there could be other explanations of the enhanced expression of c-*abl*–related RNA message in CML blast crisis cell lines. For example, the enhanced expression could be a reflection of selective pressure in vitro for cells expressing relatively high levels of c-*abl* RNA. This possibility could be addressed by comparing levels of c-*abl* RNA expression in fresh, uncultured blast crisis CML cells and chronic phase cells from the same patient. Alternatively, c-*abl*–related RNA expression may diminish as cells mature and differentiate. For example, expression of RNA related to the *myc* oncogene markedly diminishes in HL-60 promyelocytic leukemia cells as the cells are chemically induced to differentiate (*15, 16*), and a similar phenomenon may occur with respect to the *abl* oncogene during differentiation of CML cells. Since chronic phase CML cells consist predominantly of relatively mature granulocytes, they might be expected to show lower levels of c-*abl* expression than the immature and less differentiated blast phase cells.

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References and Notes

1. J. D. Rowley, *Nature (London)* **243**, 290 (1973).
2. A. de Klein *et al.*, *ibid.* **300**, 765 (1982).
3. N. Heisterkamp *et al.*, *ibid.* **306**, 239 (1983).
4. S. J. Collins and M. T. Groudine, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4813 (1983).
5. E. Westin *et al.*, *ibid.* **79**, 2490 (1982).
6. J. Wang and D. Baltimore, *Mol. Cell. Biol.* **3**, 773 (1983).
7. C. B. Lozzio and B. B. Lozzio, *Blood* **45**, 321 (1975).
8. I. Kubonishi and I. Miyoshi, *Int. J. Cell Cloning* **1**, 105 (1983).
9. A. Keating, W. Raskind, J. W. Singer, *Proceedings of the UCLA Symposia on Molecular and Cellular Biology*, D. Golde and P. Marks, Eds. (Liss, New York, 1983), pp. 513–525.
10. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982).
11. R. Dalla-Favera *et al.*, *Science* **219**, 963 (1983).
12. P. Heiter, G. Hollis, S. Korsmeyer, T. Waldmann, P. Leder, *Nature (London)* **294**, 536 (1981).
13. J. Groffen *et al.*, *J. Exp. Med.* **158**, 9 (1983).
14. E. Canaani *et al.*, *Lancet* **1984-I**, 593 (1984).
15. E. H. Westin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2490 (1982).
16. P. H. Reitsma *et al.*, *Nature (London)* **306**, 492 (1983).
17. J. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).
18. H. Aviv and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408 (1972).
19. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), p. 202.
20. P. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
21. S. Collins, R. Gallo, R. Gallagher, *Nature (London)* **270**, 347 (1977).
22. H. Koeffler and D. Golde, *Science* **200**, 1153 (1978).
23. D. Cleveland *et al.*, *Cell* **20**, 95 (1980).
24. M. Groudine and H. Weintraub, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5351 (1980).
25. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
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The Latency of Pathways Containing the Site of Motor Learning in the Monkey Vestibulo-Ocular Reflex

Abstract. *The vestibulo-ocular reflex helps to stabilize retinal images by generating smooth eye movements that are equal to and opposite each rotatory head movement. It is well known that the reflex undergoes adaptive plasticity or "motor learning" whenever there is persistent image motion during head turns: the resulting changes in the reflex occur gradually and help to restore image stability. A new approach makes it possible to identify the pathways containing the site of motor learning according to their total latency in response to natural vestibular stimuli. The fastest pathways required 14 milliseconds to initiate a vestibulo-ocular reflex, but the site of motor learning was in pathways having latencies of at least 19 milliseconds.*

The capacity for adaptive regulation or "motor learning" plays an important role in establishing and maintaining the excellent performance of the adult vestibulo-ocular reflex (VOR). In normal individuals, the VOR helps to minimize image motion during head turns; it does so by generating smooth eye movements that are equal to and opposite each rotatory head movement. If visual or vestibular inputs are altered so that the VOR is no longer appropriate to stabilize visual images, an adaptive mechanism modifies the VOR and corrects its performance. The adaptive changes occur over a gradual and repeatable time course and, once acquired, are retained if either visual or vestibular inputs are withheld (*1, 2*).

The site of motor learning has not yet been discovered, but existing knowledge of the neural mechanisms underlying the normal VOR suggests several parallel pathways in which learning could occur. These include (i) the classical disynaptic reflex arc (*3*), (ii) a less-direct brainstem pathway that is thought to perform a mathematical integration of vestibular inputs (*4*), and (iii) an inhibitory side loop through the flocculus of the cerebellum (*5*). Because these three classes of pathways contain different numbers of synapses, they might exert their first effects on eye movement at different latencies after a transient vestibular stimulus: recordings of the VOR at short latencies after a rapid and sudden head acceleration might reveal the latency of the pathways containing the site of motor learning.

Motor learning was induced in the VOR by fitting two rhesus monkeys with optical devices that altered the normal