cally derivatized with an imidazole at Lys^{52H} by a flexible tether seven atoms in length (*5*). Under similar conditions, the imidazole-derivatized Fab hydrolyzes **1** approximately one-sixteenth as rapidly as $Fv(Y34H_L)$ (k_{cat} = 0.011 min^{-1}, *p*H 7.0). The greater reactivity of $Fv(Y34H_L)$ is likely due to the fewer degrees of freedom of the His^{34} side chain allowed by the protein backbone and surrounding side chains. In addition, the His imidazole may be better aligned for attack on the ester carbonyl of **1**, or for activation of a water molecule.

We have demonstrated the feasibility of using site-directed mutagenesis to introduce a catalytically active residue into an antibody combining site. In the absence of high-resolution structural information, chemical cross-linking data have proven useful in identifying sites for the introduction of catalytic groups. Additional site-directed or random mutagenesis combined with in situ screens or selections could be used to generate more efficient catalytic antibodies. Alternatively, Cys residues could be introduced for the subsequent attachment of synthetic catalysts to produce semisynthetic catalytic antibodies (*5*).

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21. The second-order rate constant for hydrolysis of **1** by 4-methylimidazole ($k_{4\text{-MeIm}}$) was calculated from the pseudo–first-order rate constant, obtained by plotting the initial rate of coumarin release versus [4MeIm], in 400-μl reactions containing 10 to 40 m*M* 4-methylimidazole, *p*H 6.8, and 10 μ*M* **1**. The value for $k_{4\text{-MeIm}}$ was 0.90 ± 0.06 M^{-1} min^{-1}.
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Lineage-Specific Requirement of c-*abl* Function in Normal Hematopoiesis

DANIELE CARACCIOLO, MAURO VALTIERI, DONATELLA VENTURELLI, CESARE PESCHLE, ALAN M. GEWIRTZ,* BRUNO CALABRETTA*

Structural abnormalities of the c-*abl* proto-oncogene are found in hematopoietic cells of more than 90 percent of individuals with chronic myelogenous leukemia. Therefore c-*abl* may be important in normal as well as malignant hematopoiesis. Normal human hematopoietic progenitor cells were exposed to three different c-*abl* sense or antisense oligodeoxynucleotides, and the effects on myeloid and erythroid colony formation were examined. The c-*abl* antisense oligodeoxynucleotides inhibited myeloid, but not erythroid, colony formation. The c-*abl* sense oligodeoxynucleotides and *bcr* sense and antisense oligodeoxynucleotides were not inhibitory in this assay. These data show that c-*abl* is critical in normal myelopoiesis and may explain the relatively selective expansion of leukocytes in patients with chronic myelogenous leukemia.

THE c-*abl* PROTO-ONCOGENE encodes a protein with tyrosine kinase activity. Although the functional significance of the protein is unknown (*1, 2*), it is known that more than 90% of chronic myelogenous leukemia (CML) patients have c-*abl* structural alterations in their leukocyte DNA (*2*). These structural alterations are caused by the translocation of c-*abl* from chromosome 9 to the breakpoint cluster region (*bcr*) on chromosome 22, resulting in the formation of *bcr-abl* hybrid genes. The most characteristic clinical feature of the chronic phase of CML is an increase in mature and immature myeloid elements in bone marrow and peripheral blood (*3*). Kinetic studies indicate that these abnormal cells do not proliferate or mature faster than the normal counterpart (*4*). Rather, the basic defect underlying the exuberant granulopoiesis in CML appears to be an expansion of the myeloid progenitor cell pool in bone marrow and peripheral blood (*5*). Although hematopoiesis in the chronic phase of CML is altered, it retains some normal features. These observations raise the possibility that c-*abl* function is required during normal human hematopoiesis.

Inhibition of specific gene functions by exposing target cells to synthetic antisense oligomers (*6*) was used to investigate the role of c-*myb* in normal human hematopoiesis (*7, 8*). We have now used the same technique to show that exposure of early and late hematopoietic progenitors to c-*abl* antisense oligomers inhibits in vitro myelopoiesis and spares erythropoiesis. This suggests a lineage-specific requirement of c-*abl* function in normal hematopoiesis.

D. Caracciolo, Istituto di Medicina ed Oncologia Sperimentale, Sezione di Ematologia, Torino, Italy, and Department of Pathology, Temple University School of Medicine, Philadelphia, PA 19140.
M. Valtieri, Istituto Superiore di Sanita, Laboratorio di Ematologia ed Oncologia, Roma, Italy, and Department of Pathology, Temple University School of Medicine, Philadelphia, PA 19140.
D. Venturelli, Department of Pathology, Temple University School of Medicine, Philadelphia, PA 19140.
C. Peschle, Istituto Superiore di Sanita, Laboratorio di Ematologia ed Oncologia, Roma, Italy.
A. M. Gewirtz, Departments of Medicine, Pathology, and Thrombosis Research, Temple University School of Medicine, Philadelphia, PA 19140.
B. Calabretta, Department of Pathology, Medicine, and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140.

*To whom correspondence should be addressed.

We exposed partially purified and more highly purified bone marrow and peripheral blood progenitor cells to c-*abl* sense and antisense oligomers. The colony-forming ability of treated cells was then assayed under lineage-selective growth conditions (*9*). Two distinct c-*abl* mRNAs that differ in their 5′ regions have been identified (*10, 11*). We synthesized 18-base oligodeoxynucleotides complementary to either the 7.0-kb (*abl* 2) or to the 6.0-kb (*abl* 4) c-*abl* transcripts beginning from the second codon of each mRNA, and a third c-*abl* antisense oligomer (*abl* 6) complementary to 18 nucleotides of the second exon of c-*abl* (codons 2 to 7), which is common to both c-*abl* mRNAs. An 18-base c-*abl* sense sequence corresponding to each antisense sequence served as a control for each experiment. Sense and antisense oligomers corresponding to a region from the second to the seventh codon of a *bcr* transcript were also prepared (*12*). Human bone marrow cells, depleted of adherent monocyte-macrophages and T lymphocytes, were exposed to oligomer preparations (final concentration 14 μ*M*) for 15 to 18 hours (*13*) and were then cultured in semisolid medium in the presence of optimal concentrations of growth factors specific for different subsets of progenitors (*9*).

Exposure of bone marrow mononuclear cells to c-*abl* antisense oligomers did not affect erythroid colony formation deriving from burst-forming unit–erythroid (BFU-E) and colony-forming unit–erythroid (CFU-E) progenitors, but markedly inhibited (10% to 20% of residual growth in comparison to controls) myeloid colony formation deriving from colony-forming unit–granulocytic (CFU-G) and colony-forming unit–granulocytic macrophage (CFU-GM) progenitors (Table 1). In addition, the residual myeloid colonies were much smaller than those formed in the presence of c-*abl* sense oligomers.

We next analyzed the effect of c-*abl* antisense oligomers on the growth of bone marrow progenitors selected on the basis of the expression of the My10 antigen ($CD34^+$ cells) (*14*). This population is rich in BFU-E and CFU-GM progenitors (*15*) but does not contain CFU-E or CFU-G progenitors. On the basis of the model that hematopoiesis is a developmental continuum, $My10^+$ progenitors correspond to a more homogeneous, less mature population of colony-forming units than the one assayed from partially purified bone marrow cells. The c-*abl* antisense oligomers inhibited the formation of granulocyte-macrophage colonies (CFU-GM growth) but did not affect the growth of primitive erythroid colonies (BFU-E growth) (Table 2). A *bcr* antisense oligomer did not have any effect on colony number or colony size (Tables 1 and 2).

We then analyzed the effect of c-*abl* antisense oligomers on normal peripheral blood progenitors since they are antigenically distinct and less differentiated than those found in the bone marrow (*16*). CFU-GM colonies formed from peripheral blood progenitors in the presence of the c-*abl* antisense oligomer were undistinguishable from those derived from similarly treated bone marrow progenitors and were much smaller than those arising in the presence of the c-*abl* sense oligomer or the *bcr* antisense oligomer (Fig. 1). In addition, the number of colonies formed was inhibited essentially to the same degree (75% to 85%) as that observed for bone marrow progenitors (*17*). The growth of erythroid progenitors was inhibited slightly more (20% to 25%) than we have observed for bone marrow erythroid progenitors (10% to 20%).

Since c-*abl* antisense oligomers inhibited colony formation arising from different subsets of progenitors, we hypothesized that c-*abl* mRNA levels would be constant during the in vitro differentiation of early progenitors. $My10^+$ elements were therefore isolated from normal bone marrow and cultured for 0, 4, 8, and 12 days in the presence of interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Total RNA was extracted, and c-*abl* mRNA levels were assessed by reverse transcriptase–polymerase chain reaction (RT-PCR) analysis (*18*). Concentrations of c-*abl* mRNA appeared essentially unchanged during in vitro culture of bone marrow cells enriched in early progenitors. These findings further suggest that the functional requirements of c-*abl* are independent of the stage of differentiation of myeloid progenitors.

To determine whether the effect on myeloid colony formation was due to a reduced synthesis of the c-*abl*–encoded protein, we examined the ability of a c-*abl* antisense oligomer complementary to the common second exon to inhibit the expression of the hybrid *bcr-abl* product in K562 cells, which have multiple copies of the hybrid *bcr-abl* gene (*19*). In these leukemic cells, the *abl* second exon is spliced to *bcr* exons 2 and 3 (*10*), and therefore treatment of K562 with the c-*abl* antisense oligomer (*abl* 6) complementary to *abl* second exon should effect a decrement of Bcr-Abl protein concentrations. We assumed that the observed effects in K562 cells could be extrapolated to normal progenitors as already shown in experi-

Table 1. Effect of sense and antisense c-*abl* oligomers on in vitro growth of partially purified bone marrow progenitors. Values represent mean ± SD of quadruplicate control cultures (no oligodeoxynucleotide added) and duplicate experimental cultures from three separate experiments for each colony type. Adherent and T cell–depleted low-density bone marrow cells (2.5×10^4) were plated in 1 ml Iscove's modified Dulbecco's medium (IMDM) supplemented with 30% fetal bovine serum (FBS), 5×10^{-5} β_2-mercaptoethanol, and 0.9% methylcellulose in the presence of appropriate growth factors (*9*).

Oligodeoxy-nucleotide	Colonies or clusters			
	BFU-E	CFU-E	CFU-GM	CFU-G
Control (no oligomer added)	54 ± 2	298 ± 20	120 ± 5	327 ± 38
c-*abl* 1 (sense)	51 ± 8	276 ± 31	98 ± 10	298 ± 12
c-*abl* 2 (antisense)	48 ± 6	256 ± 18	24 ± 5	75 ± 10
Control	60 ± 8	280 ± 16	118 ± 4	300 ± 10
c-*abl* 3 (sense)	55 ± 10	274 ± 12	120 ± 10	285 ± 8
c-*abl* 4 (antisense)	52 ± 8	268 ± 10	16 ± 8	60 ± 10
Control	71 ± 5	294 ± 30	93 ± 5	280 ± 38
c-*abl* 5 (sense)	56 ± 8	255 ± 28	74 ± 24	270 ± 25
c-*abl* 6 (antisense)	45 ± 4	246 ± 27	8 ± 4	58 ± 10
Control	65 ± 10	290 ± 18	138 ± 10	280 ± 10
bcr 1 (sense)	68 ± 4	320 ± 12	120 ± 8	270 ± 20
bcr 2 (antisense)	66 ± 8	340 ± 20	135 ± 12	320 ± 20

Table 2. Effect of c-*abl* sense and antisense oligomers on the in vitro growth of human $My10^+$ progenitors. Values represent mean ± SD of quadruplicate control cultures (no oligodeoxynucleotide added) and duplicate experimental cultures from three separate experiments for each colony type. $My10^+$ cells (4×10^3) that were isolated by immunorosetting (*14*) were plated in each dish. CFU-GM colonies were obtained after 14 days from cultures stimulated by GM-CSF (10 ng/ml) and IL-3 (100 U/ml). BFU-E colonies were obtained after 14 days from cultures stimulated by GM-CSF (10 ng/ml), IL-3 (100 U/ml), and erythropoietin (3 U/ml).

Oligodeoxynucleotide	BFU-E	CFU-GM
Control (no oligomer)	56 ± 3	75 ± 5
c-*abl* 1 (sense)	58 ± 4	70 ± 4
c-*abl* 2 (antisense)	48 ± 2	20 ± 2
Control	58 ± 6	70 ± 3
c-*abl* 3 (sense)	60 ± 8	62 ± 2
c-*abl* 4 (antisense)	52 ± 2	10 ± 3
Control	84 ± 7	51 ± 3
c-*abl* 5 (sense)	68 ± 3	46 ± 3
c-*abl* 6 (antisense)	56 ± 4	10 ± 2
Control	64 ± 6	68 ± 5
bcr 1 (sense)	84 ± 6	88 ± 8
bcr 2 (antisense)	76 ± 6	86 ± 10

ments in which myeloperoxidase and c-*myb* expression were inhibited by the respective antisense oligomers (*7, 8*). The expression of the hybrid Bcr-Abl protein in K562 cells was detected by immunofluorescence (*7*); the intensity of fluorescence staining was analyzed by computer-assisted microspectrophotometry and expressed as the mean ± SEM of 100 individual measurements on an arbitrary intensity scale of 0 to 100% (*7*). As expected, exponentially growing K562 cells expressed high levels of Bcr-Abl protein and had a fluorescence intensity of 70 ± 12%. Further, Bcr-Abl protein levels were unaffected by exposure to a c-*abl* sense oligomer (*abl* 5) (fluorescence intensity 81 ± 17%) but were significantly reduced in the presence of c-*abl* antisense oligomer (*abl* 6) (fluorescence intensity 29 ± 9%).

The decrease in Bcr-Abl protein synthesis in antisense-treated cells was likely due to greatly decreased Bcr-Abl RNA transcripts, as established by use of the highly sensitive RT-PCR technique. This method allowed detection of *bcr-abl* mRNAs in untreated K562 cells and in cells exposed to a c-*abl* sense oligomer (Fig. 2, lanes a and b). In contrast, *bcr-abl* mRNA levels were significantly reduced (80% to 90% decrease) in K562 cells treated with the antisense oligomer (Fig. 2, lane c). These findings suggest that the formation of specific DNA-RNA duplexes provides a substrate for cellular ribonuclease H activity, which can then degrade c-*abl* mRNA (*20*).

To establish that the marked decrement of *bcr-abl* transcripts in K562 cells was a specific effect of the exposure of a c-*abl* antisense oligomer, we also measured mRNA levels of β_2 microglobulin, and they appeared to be very similar in untreated and c-*abl* oligomer–treated K562 cells (Fig. 2).

Direct evidence that the function of c-*abl* is lineage-specific is provided by the findings that c-*abl* function is required for the formation of myeloid colonies but is apparently unnecessary for the formation of erythroid colonies. In addition, c-*abl* functional requirements were found to be independent of proliferative activity and differentiation stage of myeloid progenitor cells. Thus c-*abl* gene function is important for carrying out the normal proliferation and differentiation routines of early and late myeloid progenitor cells. Whether the protein is required for commitment to the myeloid lineage remains to be determined.

The effect of c-*abl* antisense oligomers on myeloid colony formation was observed with three different oligomers: *abl* 2, *abl* 4, and *abl* 6. Since the two species of c-*abl* mRNA differ only in the region corresponding to the two distinct first exons (exon 1a and 1b) of c-*abl* gene, our findings imply the existence of two c-*abl* protein products and the need for both proteins during the process of in vitro myelopoiesis. This suggestion is based on our functional studies and appears to contradict the finding that there is only one c-*abl* gene product, p145. The small size difference between the two putative c-*abl* protein products might prevent, however, the detection of two distinct species. Alternatively, both proteins might be synthesized only in myeloid progenitors in which the function of c-*abl* seems of critical importance. The finding that a c-*abl* antisense oligomer (*abl* 6) corresponding to the common second exon inhibits myeloid colony formation leaves unresolved the issue of whether two c-*abl* gene products exist in myeloid progenitors, but demonstrates that this gene is important in the control of normal myelopoiesis. A *bcr* antisense oligomer did not appear to adversely affect normal myelopoiesis.

The lineage specificity of c-*abl* function might explain the increase in mature and immature myeloid elements in bone marrow and peripheral blood of individuals with CML. The basic function of c-*abl* likely remains unchanged in this disease, but the generation of the hybrid *bcr-abl* gene exaggerates the normal c-*abl* function. Accordingly, these studies suggest a mechanism whereby CML diverges clinically from other myeloproliferative disorders and suggest a therapeutic approach for controlling or arresting the abnormal myelopoiesis that characterizes CML.

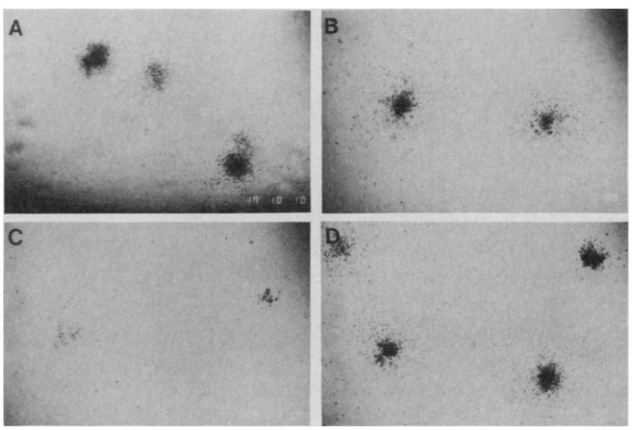

Fig. 1. Effect of c-*abl* sense and antisense oligomers on CFU-GM colony formation from peripheral blood progenitors. CFU-GM colonies grown in the presence of recombinant GM-CSF and IL-3 from adherent and T cell–depleted peripheral blood mononuclear cells after 16 days of culture. (**A**) Control; (**B**) after treatment with a c-*abl* sense oligomer (*abl* 5); (**C**) after treatment with a c-*abl* antisense oligomer (*abl* 6); and (**D**) after treatment with a *bcr* antisense oligomer (*bcr* 2).

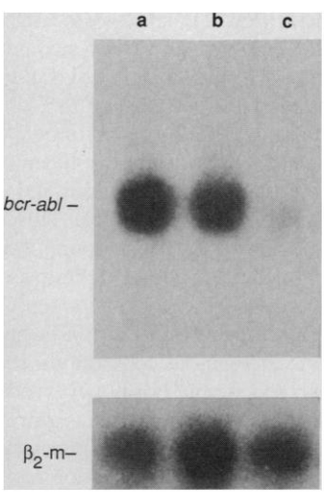

Fig. 2. Expression of *bcr-abl* and β_2 microglobulin (β_2-m) mRNAs in K562 cells treated with c-*abl* sense and antisense oligomers. K562 cells were grown for 3 days in 1 ml of RPMI with 10% fetal bovine serum in three separate wells. Cultures were supplemented daily with oligomers up to a final concentration of 14 μ*M*. At day 3, total RNA was extracted from 1.2×10^5 cells for each condition in the presence of 20 μg of *Escherichia coli* ribosomal RNA as described (*27*). RNA was reverse-transcribed with 500 U of Moloney murine leukemia virus reverse transcriptase and 0.2 μg of oligo(dT) as primer for 1 hour at 37°C. The resulting cDNA fragments were amplified with 5 U of *Thermus aquaticus* polymerase and 5′ and 3′ 22-base primers corresponding to a 200-base sequence in the 3′ region of c-*abl* mRNA (*10*), or with 5′ and 3′ β_2 microglobulin primers corresponding to a 239-base sequence in the 3′ region of β_2 microglobulin mRNA (*22*) during 60 cycles of polymerase chain reaction (PCR) (*18*). We separated 20 μl of the 100-μl PCR reaction in a 4% Nusieve agarose gel and transferred it to a nitrocellulose filter. The resulting filter was hybridized with a synthetic 40-base c-*abl* fragment or a 40-base β_2 microglobulin fragment end-labeled with [γ-^{32}P]ATP and polynucleotide kinase. The c-*abl* probe recognizes the amplified 156-bp sequence contained within 5′ and 3′ c-*abl* primers, whereas the β_2 microglobulin probe recognizes the 195-bp sequence contained within 5′ and 3′ β_2 microglobulin primers. Lane a, Exponentially growing K562 cells; lane b, c-*abl* sense–treated K562 cells; and lane c, c-*abl* antisense–treated K562 cells.

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A Baculovirus Blocks Insect Molting by Producing Ecdysteroid UDP-Glucosyl Transferase

DAVID R. O'REILLY AND LOIS K. MILLER

The predicted amino acid sequence of a newly identified gene of the insect baculovirus *Autographa californica* nuclear polyhedrosis virus was similar to several uridine 5′-diphosphate (UDP)–glucuronosyl transferases and at least one UDP-glucosyl transferase. Genetic and biochemical studies confirmed that this gene encodes an ecdysteroid UDP-glucosyl transferase (egt). This enzyme catalyzes the transfer of glucose from UDP-glucose to ecdysteroids, which are insect molting hormones. Expression of the *egt* gene allowed the virus to interfere with normal insect development so that molting was blocked in infected larvae of fall armyworm (*Spodoptera frugiperda*).

BACULOVIRUSES CONSTITUTE A LARGE group of DNA-containing viruses that infect only invertebrate hosts. These viruses, many of which infect pest lepidopteran species, are of particular interest because of their potential as biological control agents (*1*). Studies of *Autographa californica* nuclear polyhedrosis virus (AcMNPV), the model system for baculovirus research, are revealing the molecular mechanisms by which the virus implements its replication strategy. Most of the genes identified to date are involved in the interaction of the virus with its host cell, but little is known of the molecular aspects of infection at the organismal level. We now report that AcMNPV has a gene that allows the virus to manipulate the hormonal regulation of development of its larval host.

The region of the AcMNPV genome containing the *egt* gene, spanning 8.4 to 9.6 map units, first came to our attention as a hypermutable region in serially propagated viruses (*2*). The sequence of this region revealed an open reading frame that could encode a 57-kD polypeptide of 506 amino acids (Fig. 1). The *egt* product shares 21 to 22% amino acid sequence identity with several mammalian UDP-glucuronosyl transferases, also shown in Fig. 1. In mammals, the UDP-glucuronosyl transferases catalyze the transfer of glucuronic acid to a wide variety of exogenous and endogenous lipophilic substrates (*3*). This conjugation reaction is of critical importance in the detoxification and safe elimination of a multitude of drugs and carcinogens. In addition, the normal metabolism and disposal of various endogenous compounds, such as bilirubin and steroid hormones, proceed through their conjugation with glucuronic acid. Available evidence on insect systems indicates that sugar conjugation reactions of this type involve glucose rather than glucuronic acid transfer (*4*). No sequences of UDP-glucosyl transferase genes were available in GenBank at the time of our search, but the sequence of a UDP-glucosyl transferase gene from *Zea mays* (maize) was subsequently reported (*5*). The COOH-terminal portion of this protein also displays homology to egt and to mammalian UDP-glucuronosyl transferases (Fig. 1).

Mammalian UDP-glucuronosyl transferases are known to be membrane-bound, and the amino acid sequences of these proteins

Table 1. Substrate specificity of *egt* gene product. Substrates were incubated in the presence of medium derived from appropriately infected cells and 0.05 μCi UDP–[U-^{14}C]glucose (312.5 mCi/mmol) for 1 hour at 37°C. All substrates were used at a concentration of 1 m*M*. Other conditions were as described in the legend to Fig. 2. Amounts of glucose transferred were calculated after scintillation counting of the appropriate regions of the chromatography plates. A hyphen indicates that less than 1% of the glucose was transferred.

Substrate	wt (pmol glucose transferred)	vEGTZ (pmol glucose transferred)
p-Aminobenzoic acid	–	–
Bilirubin	–	–
Chloramphenicol	–	–
Cholesterol	–	–
Diethylstilbestrol	–	–
Ecdysone	155.7	–
β-Estradiol	–	–
20-Hydroxyecdysone	87.8	–
8-Hydroxyquinoline	–	–
Makisterone A	89.6	–
(–) Menthol	–	–
Methylumbelliferol	–	–
α-Naphthol	–	–
p-Nitrophenol	–	–
Phenolphthalein	–	–
Testosterone	–	–
α-Tetralol	–	–

Departments of Entomology and Genetics, University of Georgia, Athens, GA 30602.