and other long-lived isotopes in the lunar surface at depths related to the earth's atmosphere and beneath the penetration of the primary particles. Most of the long-lived radioisotopes in the earth's crust were incorporated at the time of the birth of the solar system. However, continuous creation of ^{40}K and of other isotopes occurs by the breakdown of cosmic-ray nuclei in the calcium to iron groups. The deposition of carrier-free ^{11}C fragments at the end of the range may be useful for medical research: external measurement of the location of the autoradioactive deposit might provide a convenient means for finding out (for example, in patients in vivo) where the beam has stopped, and the fate and transport of beam-deposited isotopes can be further studied with in vivo counting techniques.

Most of the studies described were performed in parallel with physical improvements on the bevatron for nitrogen beam acceleration. Although the beam intensity was relatively small, it was possible to carry out a few bioexperiments demonstrating the effects of small groups of particles. In working with T. Budinger, it was shown that the individual particles can produce a sensation of light flashes and streaks when they are aimed at the human retina, whereas nitrogen particles directed to the occipital lobes of the brain, where visual sensations are elaborated, failed to elicit light sensation (*16*). The effects observed are similar to those reported by astronauts in lunar flights. Measurements of the fluorescence of various fluids placed in the nitrogen beam—including rabbit vitreous fluid and retina—have demonstrated that the observed light flashes are probably due to direct excitation and ionization.

Preliminary measurements were made by members of our biomedical group (*17*) of survival curves, relative biological effectiveness (RBE), and "oxygen effect ratio" (OER) for mammalian kidney cells in culture (T1 cells). At the 10 percent survival level the OER is between 1.0 and 1.4 and the RBE is about 3. For 250-Mev nitrogen particles, at the 10 percent survival level the OER is between 1.9 and 2.4 and the RBE is about 1.5. These data are in general agreement with theoretical prediction for the oxygen effect of fast heavy ions (*5*); it would appear that nitrogen beams and still heavier ions, when used in radiation therapy, will reduce the oxygen effect more effectively than pi-mesons (*9*) or neutrons (*11*). The group has also exposed the skin of black mice (C57-BL) to small bursts of nitrogen particles, which were allowed to stop in skin. The development of bleached hair as a consequence is an indication of the profound effects of individual nitrogen particles on the pigment cells of the hair follicles (*17*).

The effects of single accelerated nitrogen ions have also been demonstrated by exposing maize to low doses (for example, 1 rad). About 5 percent of the developing plants from irradiated seeds exhibited gross malformations as compared to unirradiated controls, which did not exhibit these effects (*17*). The morphology of these effects seems to be peculiar to heavy ion irradiation.

C. A. TOBIAS, J. T. LYMAN
A. CHATTERJEE, J. HOWARD
H. D. MACCABEE, M. R. RAJU
A. R. SMITH, J. M. SPERINDE
G. P. WELCH

Donner Laboratory and *Lawrence Berkeley Laboratory, University of California, Berkeley 94720*

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Human Leukemic Cells: In vitro Growth of Colonies Containing the Philadelphia (Ph^1) Chromosome

Abstract. *Human leukemic cells with a marker (Philadelphia; Ph^1) chromosome gave rise to granulocytic and mononuclear cell colonies when grown in vitro. All metaphases from a single colony were either Ph^1 positive or Ph^1 negative. No colonies contained a mixed cell population. This suggests that leukemic and normal cells exist simultaneously and that in vitro colonies are clonal in origin.*

Colonies of granulocytes and mononuclear cells can be grown in vitro from bone marrow and blood of animals (*1, 2*) and man (*3–7*). Colonies can be grown from patients with various hematologic and nonhematologic diseases as well as from normal individuals (*3–7*). Colonies arising from human blood and marrow contain eosinophils, neutrophils, monocytes, or macrophages (*3*). In a number of instances, cells from patients with leukemia have been observed to give rise to similar colonies (*4, 6–8*). However, it has not been definitely established that these colonies arise from the leukemic cell population.

In approximately 90 percent of patients with typical chronic myelocytic leukemia (CML), a characteristic chromosome abnormality is present in cells arising in the marrow (*9, 10*). This abnormal marker, the Philadelphia or Ph^1 chromosome, has recently been identified as a G-22 autosome (*11*) with deletion of a substantial portion of its long arms. It is the only chromosomal marker consistently found in a human neoplasm and does not occur in any other disease, with the excep-

tion that it has been found in a few instances of acute myelocytic leukemia and related disorders. It persists throughout the course of CML both in relapse and during remission (*9*). The growth of in vitro colonies containing cells with the Ph¹ chromosome would provide evidence that such colonies arise from the leukemic cell population. The finding of Ph¹ positive cells in colonies arising from the marrow and blood of patients with CML forms the basis of this report.

Seven patients with typical features of CML and known to have the Ph¹ chromosome in marrow cells were studied. In four patients the disease was in relapse, and in two others it had undergone acute blastic transformation. One patient was recovering from busulfan therapy and was pancytopenic.

Approximately 5 ml of marrow was aspirated from the sternum or iliac crest into a syringe rinsed with heparin (1 : 1000) and allowed to sediment at room temperature. Blood leukocytes were obtained by venipuncture and treated in the same manner as marrow cells. After sedimentation, the cell-rich plasma was removed, and cells were cultured by a modification of the method described by Pike and Robinson (*4*). A known number of cells was suspended in methylcellulose (Dow) containing McCoy's 5A tissue culture medium, 15 percent fetal calf serum (FCS), and a supplement of Eagle's amino acids and vitamins. Stimulation of colony growth was achieved by either plating 1 ml of methylcellulose, FCS, and cell mixtures on top of a feeder layer of human peripheral leukocytes (*4*) or by the addition of 0.15 ml of conditioned media [prepared by incubation of human leukocytes (*2*)] to each 1 ml of the mixture. Cells were incubated in plastic petri dishes (35 by 10 mm; Falcon) at 37°C (7.5 percent CO_2 atmosphere). After 10 to 14 days of incubation, colonies (containing more than 20 cells) were overlaid with 0.2 ml of colchicine (10 μg/ml). Individual colonies were removed 18 hours later by a Pasteur pipette and prepared for chromosomal analyses by a modification of the method described by Moorhead *et al.* (*12*). An individual colony was placed on a cover glass and a drop of 0.25 percent sodium citrate was added. The cells were fixed with a mixture of methanol and acetic acid, stained with 2 percent aceto-orcein, and mounted on a microscope slide. Metaphase figures were then scored for the presence or absence of the Ph¹ chromosome. Only those metaphases that were well spread and showed detailed morphology of the smaller chromosomes were scored.

Table 1. Distribution of Ph¹ positive cells in colonies arising in vitro from CML marrow.

Patient	Disease status	Analyzable colonies/ No. examined	Ph¹ chromosomes/ analyzable metaphases
A.M.	Relapse	8/21	1/1; 5/5; 6/6; 0/4; 1/1; 3/3; 3/3; 3/3
A.P.	Relapse	6/26	0/1; 0/2; 0/1; 2/2; 3/3; 1/1
S.P.	Relapse	2/12	6/6; 3/3
D.G.	Blast crisis	2/22	1/1; 2/2

Colonies grew from the blood or bone marrow from five of seven patients studied (Table 1). Of the two patients who did not give rise to colony growth, one was in relapse and one was in the acute blastic phase of the disease at the time of study. There was nothing that distinguished these patients from those in relapse or in blastic crisis whose blood or marrow gave rise to colonies. The number of colonies observed in the five patients ranged from 30 to 90 per 2×10^5 marrow cells plated, which is within the expected range for normal or nonleukemic marrow in our laboratory. However, colony size was abnormally small and, although in two patients a few colonies contained 500 to 700 cells, most contained 20 to 200 cells. This result is in contrast to colonies arising from nonleukemic blood and marrow, which contain approximately 200 to 1500 cells (*3, 4, 6*). Colonies of eosinophils, neutrophils, monocytes, and macrophages were observed.

The number of colonies containing cells in metaphase was small, and not all colonies yielded analyzable chromosome preparations (Table 1). In colonies which contained metaphases that could be scored, cells were either Ph¹ positive or Ph¹ negative. Both Ph¹ positive and Ph¹ negative cells were observed in colonies grown on feeder layers of leukocytes and when stimulated with conditioned media. No colonies were found to contain a mixed population of Ph¹ positive and Ph¹ negative cells.

One patient, L.S., was recovering from busulfan therapy and was pancytopenic at the time of study. In this patient chromosome analyses were made from a combined colony preparation (20 colonies) rather than from individual colonies, and, therefore, these results were not included in Table 1. In the combined colony preparation, 21 metaphases were observed, ten of which could be scored. The Ph¹ chromosome was not identified in any of the metaphases scored in this patient.

Blood leukocytes from three patients gave rise to in vitro colonies. In the one patient (S.P.) with analyzable metaphases, these cells also contained the Ph¹ chromosome.

The results of our study indicate that cells from patients with CML containing the Ph¹ chromosome can be grown in vitro in a soft gel medium.

The finding that colonies contained either Ph¹ positive or Ph¹ negative cells suggests that a mixed population of normal and leukemic cells exists in the marrow of patients with CML throughout the course of the disease. Not all cells in direct marrow preparations from patients with CML have been found to contain the Ph¹ chromosome; but whether the negative population was lymphoid or myeloid in origin remains in question (*8, 9*). Persistence of normal myeloid stem cells (if we assume that the Ph¹ chromosome identifies "leukemic cells") has important therapeutic implications (*13*). Unless normal stem cells do persist, recovery from "total leukemia cell kill" would seem impossible.

Colonies arising from leukemic cells were considerably smaller than those arising from normal individuals or from patients with nonhematologic diseases. The reason for the abnormally small colonies is unknown. A longer-than-normal generation time would result in smaller colonies, but whether the generation time is prolonged in CML is not entirely clear (*14*). Morphologically colonies consisted of either eosinophils, neutrophils, monocytes, or macrophages, similar to those observed to arise from normal individuals (*3*). In view of their apparent clonal origin, these data provide further evidence that monocytes and neutrophils share a common stem cell.

PAUL A. CHERVENICK
LAWRENCE D. ELLIS, SYLVIA F. PAN
ALTON L. LAWSON
University of Pittsburgh School of Medicine and *Graduate School of Public Health, Department of Medicine and Radiation Health, Pittsburgh, Pennsylvania 15213*

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Detection of an Antigen Associated with Acute Leukemia

Abstract. *Antiserums to a purified cell membrane component from a Burkitt's lymphoma tissue culture cell line were produced in rabbits. These antiserums were cytotoxic to peripheral white blood cells from 8 of 15 patients with acute leukemia and 5 of 41 relatives, but not to peripheral white blood cells from leukemia patients in clinical remission or from normal individuals. These antiserums appear to be detecting an acute leukemia associated antigen or antigens.*

Serums obtained from rabbits immunized with purified cell membrane components (*1*) from several tissue culture cell lines were cytotoxic to all of a large panel of peripheral blood lymphocytes (*2*). However, when a cell membrane fraction from the tissue culture cell line Raji (derived from the patient with Burkitt lymphoma) was used to immunize rabbits, a more limited pattern of cytotoxic reactivity was observed. Serums from two of three animals immunized with the same cell membrane component were found to be cytotoxic only to the peripheral white blood cells from some patients who had acute lymphocyte or acute myelocytic leukemia (*3*). These serums were not absorbed before they were tested.

The cytotoxic reactivity of these antiserums to the peripheral white blood cells of 15 patients with acute leukemia, 41 of their relatives, and 527 random donors was determined by a microdroplet cytotoxicity test (*4*).

Table 1 summarizes the cytotoxic reactivity (as determined with two rabbit antiserums) of the peripheral white blood cells from the leukemia patients, together with the clinical status of the disease at the time of the testing. These two antiserums showed positive cytotoxic reactivity with the peripheral white blood cells of 8 of 15 leukemic patients tested. The percentage of blast cells in the bone marrow is an indication of active (acute) disease or remission. Six patients had from 14 to 90 percent blast cells in their bone marrow and were considered to be in an acute phase of the disease. Reactive peripheral white blood cells were found in two patients who had 5 percent blast cells in the bone marrow. In contrast, peripheral white blood cells from two of the seven patients showing no cytotoxic reaction with the two antiserums had 10 to 20 percent blast cells in their bone marrow. The remainder of the patients could be considered in clinical remission, and no cytotoxic reaction of their peripheral white blood cells was observed. A total of 41 relatives was tested; more than 60 percent cytotoxicity was observed with the peripheral white blood cells from four relatives, either siblings or one or the other parent. The two rabbit antiserums showed no cytotoxicity to peripheral white blood cells from 527 random donors.

The HL-A alloantigenic profiles of cells from the leukemia patients, their relatives, and the normal donors were simultaneously determined with 78 alloantiserums capable of detecting 15 HL-A alloantigens. The cytotoxic reactivity of the two rabbit antiserums did not correspond to the presence of a particular HL-A alloantigen or a combination of different HL-A alloantigenic determinants.

An attempt was made to determine whether the antigen present on leukemic cells might also be present on normal cells but in concentrations or density that did not allow direct cytotoxic reactions. These studies were done with a cytotoxic inhibition assay.

Two tissue culture cell lines—Raji and R-4265—were labeled with ^{51}Cr. The two rabbit antiserums were tested against these cells in the presence of complement at varying dilutions, and a dilution was selected which lysed 70 to 80 percent of 10^4 target cells. The

Table 1. Diagnosis, clinical status (percent blast cells in bone marrow) and cytotoxic reactions of peripheral white blood cells from patients with acute lymphocytic (ALL) or acute myelocytic leukemia (AML). Positive reaction (+) indicates more than 60 percent cell death with two rabbit antiserums. Negative reaction (−) indicates less than 10 percent cytolysis.

Patient	Diagnosis	Blast cells in bone marrow (%)	Cytotoxic reaction
Hm	AML	90	+
Gd	AML	71	+
Rt	ALL	68	+
Hr	ALL	15	+
Sm	ALL	15	+
Hs	ALL	14	+
Dl	AML	5	+
Ad	AML	5	+
Dh	AML	20	−
Mt	ALL	10	−
Dt	ALL	3	−
El	ALL	<5	−
Ba	ALL	<5	−
Cl	AML	<5	−
Ob	ALL	<5	−

Table 2. Inhibition of antibody activity (AA) to the acute leukemia associated antigen with peripheral white blood cells (PWBC), bone marrow (BM), PHA stimulated white blood cells (PHA-WBC), and long-term cultured lymphoblast (CLB). N.I., no inhibition of cytotoxicity.

Cell type	Number tested	Clinical status	Cell numbers needed to inhibit AA by 50 percent
PWBC	9	ALL,AML (acute)	$1,2,3,4,5 \times 10^4$; $1,2,5 \times 10^5$; 1×10^6
PWBC	3	ALL,AML (remission)	N.I.* 10^6
PWBC	17	Normal	N.I. 10^6
BM	2	Normal	N.I. 10^6
BM	2	ALL,AML	10^4; 10^4
PHA-WBC	2	Normal	N.I. 10^6
CLB	9		$2,3,5,7 \times 10^3$; $1,2,3,5 \times 10^4$
Hela, Hep.	2		N.I. 10^6

* The cytotoxicity of the antiserums detecting the leukemia associated antigen to 10^4 target cells was not inhibited by concentrations of 10^6 cells.