In vitro Sensitivity of Human Leukemic Cells to X-Rays.* (22912)

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This study explores the feasibility and utility of testing radiosensitivity of blood cells of leukemic patients by time-lapse cinemicrography and phase microscopy. These methods have been used to determine the effects of x-rays on rabbit(1) and human† lymphocytes and other cells. It was found that lymphocytes treated with small doses of x-rays (50 to 1000r) died with intranuclear vacuolization, lobulation and finally pyknosis of the nucleus. Granulocytes obtained from human blood and from peritoneal exudates of the rabbit, however, were resistant to large doses of x-rays. A few investigators have studied the *in vitro* effects of x-rays on blood cells from leukemic patients. Osgood(2) irradiated fluid tissue cultures of blood cells of one patient with subacute myelocytic leukemia. Irradiation with 300r caused a relative decrease of progranulocytes in 48 and 96 hours presumably due to inhibition of mitosis. Gunz(3) cultivated blood cells from 8 patients with chronic myeloid leukemia and found that 100 and 1000r caused inhibition of mitosis of cells in the cultures but had no effect on resting cells. Schrek(4) tested the effect of 1000r on human leukemic cells by the method of unstained cell counts. Irradiation decreased survival time of blood cells from 4 out of 5 patients with chronic lymphocytic leukemia but had no effect on survival of cells from 7 patients with chronic myelocytic leukemia. According to these 3 studies, irradiation with 1000r or less 1) inhibits mitosis of leukocytes of chronic and subacute myelocytic leukemia, 2) accelerates death of blood cells from most patients with chronic lymphatic leukemia and 3) has no effect on non-dividing leukocytes of myelocytic leukemia.

The methods used in the previous work on normal cells have been extended to a study of blood cells of 12 patients with chronic lymphocytic leukemia and 6 patients with other types of leukemia.

Methods. To 10 ml of blood from a patient with leukemia, heparin (0.2 ml of 1% solution) was added and the erythrocytes allowed to settle at 37°C for 15 to 60 minutes. The plasma with its suspended leukocytes was then removed. Leukocytes were precipitated by low speed centrifugation, washed twice with Earle's solution and resuspended in a saline medium. The suspension diluted to about 6 leukocytes/μml was mixed with an equal amount of serum, either autologous or of the same blood group. A 0.2 ml drop of the mixture was placed between 2 cover slips separated from each other by a metal ring which formed a chamber 30 mm in diameter and 0.8 mm in depth. The slide preparations were irradiated with an x-ray machine, 100 kv, 5 ma. Doses above 200r were administered at 670 rpm at 10.5 cm from source, and a half layer value of 1.3 mm aluminum. For 50 to 200r, the distance was increased to 31.5 cm to give a rate of 67 rpm. For less than 50r, a ¼ mm copper filter was added, the distance was 31.5 cm, and the rate 7.6 rpm. The preparations were examined daily by means of an inverted phase microscope with a fluorite objective, 45x, N.A. 1.0, to observe the number and morphology of surviving cells. In some experiments differential counts were made of viable and dead cells. The number of surviving cells was represented by symbols 5+ to 1+ with 5+ representing survival of all cells and 1+ the survival of less than 5% of the original cells. The differentiation of viable and dead cells was based on direct phase microscopy supplemented by time-lapse cinemicrography. The viability of leukemic lymphocytes was indicated by their large nuclei, thin nuclear walls, and coarse dark chromatin masses. Some viable cells

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TABLE I. Radiosensitivity of Blood Cells from Patients with Chronic Lymphocytic Leukemia.

Patient	Date of test	Survival of lymphocytes, 3 days after irradiation — Dosage of x-rays in roentgens									
		0	4000	1000	500	200	100	50	20	10	5
AM*	8-22	4+	0	0	0	0	1+	2+	2+	2+	3+
WD	-20	3+	0	0	0	0	0	1+	1+	2+	2+
AWC	-13	5+	0	0	0	0	0	0	1+	2+	3+
FJS	- 6	4+	1+	2+	2+	2+	2+	2+	2+		
AW	7-11	4+	0	0	0	2+	2+	3+	3+		
JSB	6-15	5+	0	1+	1+	3+	3+	4+			
MK	-14	5+	0	0	0	1+	2+	3+			
GS	5-31	4+	0	0	0	1+					
JS	-29	5+	0	0	0	2+					
FS	-16	5+	2+	4+	5+	5+					
MKD	3-23	3+	0	0	0						
AO	-23	4+	0	0	1+						

* Observations made 2 days after irradiation.

5+ to 1+: % of lymphocytes surviving treatment; 5+ represents survival of all or nearly all lymphocytes and 1+ survival of only an occasional cell.

0: No surviving lymphocytes.

had anterior pseudopods and a posterior tail and showed ameboid motion which was accentuated in the cinemicrographic films. Lymphocytes in early stages of degeneration (spontaneous or after 50 to 1000r) were usually lobulated and had one or more large intranuclear vacuoles surrounded by a dark chromatin ring. In latter stages the chromatin ring contracted to a crescent and finally to a round dark pyknotic nucleus. These degenerating and dead cells could be readily differentiated from the morphologically intact, viable lymphocytes. Other types of degeneration and death required recognition and diagnosis by phase microscopy. On addition of fixatives, cells are known to die with only minimal changes in morphology, *i.e.* death by fixation. In this study lymphocytes and other cells irradiated with 4000r were killed in about 3 hours with only minor morphologic changes and this process was termed death by delayed fixation. These changes have been described previously for normal rabbit lymphocytes(5). Here it is only necessary to describe the morphologic features used to diagnose the cells killed by 4000r. In comparison to viable cells, fixed lymphocytes after 4000r had darker chromatin masses and nuclear walls and the nucleoplasm was more profuse and brighter. A few cells had bi- or tri-lobed nuclei. In a few hours the fixed cells underwent autolysis and the autolyzed cells had shrunken dark, blurred nuclei and profuse transparent cytoplasm. There was some difficulty in recognizing the fixed cells as dead but there was no problem in identifying the autolyzed cell. Further information on the appearance of dead and living cells under phase microscopy is given by Bessis(6).

Results. Chronic lymphocytic leukemia. Blood specimens were obtained from 12 patients with chronic lymphocytic leukemia. In most experiments, nearly all non-irradiated leukemic lymphocytes maintained their morphologic characteristics in slide preparations for 3 to 6 days incubation at 37°C and many cells showed normal ameboid motion during this time. In a few experiments, the leukemic cells survived only 2 or 3 days. The poor survival seemed to be correlated with prior recent x-ray treatment of the patients, but the number of such cases was too small to give conclusive results. In comparison with other studies†, lymphocytes from leukemic patients with chronic lymphocytic leukemia usually survived as well as lymphocytes from normal blood.

Table I shows that *in vitro* irradiation with 200r killed in 3 days, all or almost all lymphocytes of 11 out of 12 leukemic patients. Even 20r had a considerable cytotoxic effect on cells of 5 patients and 5r caused an appreciable decrease in percentage survival of cells of 3 patients. As can be seen in the Table, 50r killed all the AWC cells in 3 days

and allowed 2+ survival for FJS cells and 4+ survival for cells of JSB. Although further work is indicated, the present data suggest that there is some variability in radiosensitivity of blood cells in these 3 patients. Nearly all FJS cells were killed by irradiation with 20r although a small percentage of cells (2+) resisted even 1000r of x-rays. These findings suggest that the FJS preparations contained lymphocytes of different sensitivities.

We may now turn to a discussion of the unique results obtained with cells of FS. In slide preparations, the FS cells did not differ appreciably from cells of other patients with chronic lymphatic leukemia in morphology, in *in vitro* survival time and in type of spontaneous death. Irradiation of FS cells with 200r or even with 1000r had little or no effect on survival of the cells during 3 days of observation (4+ survival). Irradiated cells continued to die by vacuolization and pyknosis at the same rate as non-irradiated cells. Evidently the degenerative process in these cells was not accelerated by irradiation with 1000r. By time-lapse cinemicrography, the FS cells treated with 4000r were seen to die by the method of delayed fixation in 3 hours.

The findings on the cells of FS were so different that the tests were repeated on 2 additional blood samples with substantially the same results. In addition, the stained smears of peripheral blood and bone marrow were reviewed and the diagnosis of chronic lymphocytic leukemia was confirmed. It may be concluded that FS cells unlike the cells of other patients with chronic lymphocytic leukemia were not affected by irradiation with less than 1000r but gave the same reactions to 4000r as the other leukemic lymphocytes.

The clinical histories of the leukemic patients were reviewed with reference to reaction to x-ray therapy. Five patients received spray x-ray treatments over the anterior and posterior trunk. Table II compares the clinical response of patients to x-ray therapy and the *in vitro* findings on radiosensitivity of the blood cells. As seen previously, the blood cells of 4 patients showed poor survival 3 days after irradiation *in vitro* with 200r. X-ray treatment of these 4 patients resulted in a rapid decrease of white cell counts, associated with clinical improvement and remission of symptoms. In these 4 patients the leukemic lymphocytes seemed to be radiosensitive both *in vitro* and *in vivo*.

TABLE II. Comparison of *In Vitro* Radiosensitivity of Blood Cells of 5 Patients with Chronic Lymphocytic Leukemia and Clinical Reaction of Patients to Spray X-ray Treatment.

Patient	Survival of cells, 3 days after 200 r *in vitro*	X-ray treatment, r	White blood cell count (cells/μml) Before treatment	White blood cell count (cells/μml) After treatment
GS	1+	75	210	8.4
FJS	2+	60	137	20
JS	2+	180	182	48
AW	2+	120	350	60
FS	5+	180	580	300

Symbols as in Table I.

The fifth patient FS had blood cells which have been described as resistant even to 1000r of x-rays. FS was given 6 spray treatments of 30r each or a total of 180r. Following x-ray therapy, the white cell count showed only a minor decrease, the clinical condition deteriorated, and the patient died 2 months after admission to the hospital. The findings suggest a correlation in this patient between the *in vitro* radioresistance of blood cells and failure of patient to respond to x-ray therapy.

The observed radioresistance of leukemic lymphocytes of patient FS suggests that *in vitro* tests may be useful in treating patients with chronic lymphocytic leukemia. Additional tests are being made to determine whether the cells of other patients with chronic lymphocytic leukemia will prove to be radioresistant and whether this information will be useful in outlining treatment and prognosis.

Other leukemias. Blood specimens were obtained from 3 patients with chronic myelogenous leukemia and 3 with acute leukemia. The slide preparations of blood cells showed various types of cells including myelocytes, myeloblasts and lymphoblasts. In the present preparations, none of the immature cells were seen to undergo mitotic division although some cells were observed for long

TABLE III. Radiosensitivity of Blood Cells from Patients with Chronic Myelogenous Leukemia or Acute Leukemia.

Patient	Diagnosis	Date of test	Survival of cells, 2 days after irradiation: Dosage of x-rays in roentgens 0	4000	1000	500	200
WES	CML	6- 6	4+	0	3+	3+	
AM	"	6-13	4+	2+	3+	3+	
AD	"	7-30	3+	1+	3+	3+	
WF	ALL	8-27	3+	0	2+		3+
EB	AML	9-13	5+	2+	4+		4+
ED	"	9-24	5+	2+	4+		4+

CML: Chronic myelocytic leukemia. AML: Acute myelocytic leukemia. ALL: Acute lymphoblastic leukemia.
Other symbols as in Table I.

periods of time by time-lapse cinemicrography. The cells of 3 patients with chronic myelocytic leukemia survived only 3 to 5 days while the cells of 2 patients with acute myelocytic leukemia showed good survival for 7 days.

The effects of x-rays on survival of leukemic cells of this group are shown in Table III which presents observations made 2 days after irradiation. According to previous experiments, most cells of chronic lymphocytic leukemia were killed by 100r of x-rays in 2 days. In contrast, 1000r produced a slight or no decrease in the number of viable cells of patients with chronic myelocytic leukemia or acute leukemia. Irradiation with 4000r killed all or most blood cells of the 6 patients. It may be concluded that the immature nondividing cells in these preparations, namely myelocytes, myeloblasts and lymphoblasts, were killed by large doses of x-rays but not by small doses. These findings on the effect of x-rays on survival of cells should not be confused with effects of irradiation on other properties of the cells such as mitotic activity.

Summary. 1. This study determined the *in vitro* sensitivity to x-rays of the blood cells of 12 patients with chronic lymphocytic leukemia. The methods used were time-lapse cinemicrography and direct observations with phase microscopy. Non-irradiated leukemic lymphocytes survived 4 to 7 days under the *in vitro* conditions. Dosages of 5 to 1000r reduced survival time of blood cells of 11 out of 12 patients with chronic lymphocytic leukemia. 2. Blood cells of one patient with chronic lymphocytic leukemia were resistant to dosages of 1000r but were killed by irradiation with 4000r. This patient was given clinically 180r spray x-ray treatment but the white cell count showed only a slight decline and the patient failed to respond to the therapy. 3. Irradiation with 1000r produced little or no effect on survival of blood cells from 3 patients with chronic myelogenous leukemia and 3 with acute leukemia.

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