Prolonged Survival Time of Human Leukemic Lymphocytes *in vitro*. (29231)

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Blood lymphocytes from patients with chronic lymphocytic leukemia have been shown to differ from normal lymphocytes in morphology(1) and motility(2) and with respect to sensitivity to X-rays(3), guinea pig sera(1), glucocorticoids(4) and PHA(5). These studies demonstrated that blood lymphocytes from patients with chronic lymphocytic leukemia are distinctively abnormal and therefore may be characterized by the term "leukemic lymphocytes." The objective of the present study was to seek further differences between the *in vitro* reactions of normal and leukemic lymphocytes.

Methods. Heparinized blood of normal individuals and from patients diagnosed chronic lymphocytic leukemia or lymphosarcoma in leukemic phase was allowed to sediment for 30 to 60 minutes. The plasma with nonsedimented cells was removed and centrifuged. The precipitated cells were washed and resuspended in equal parts of normal human serum and tissue culture medium 199. To eliminate granulocytes and monocytes, the suspension was incubated in a T-flask for 30 minutes and the supernatant removed. In recent experiments, contaminating red blood cells were also largely eliminated. For this purpose, the blood cells precipitated from the plasma were washed and resuspended in 50% normal human serum incompatible with the cells. The suspension was incubated in a T-flask. This procedure resulted both in adherence of the granulocytes and monocytes to the glass and in phagocytosis of the red cells coated with A or B antibody. The resulting supernatant was found to be a pure suspension of lymphocytes with less than 10% of erythrocytes and other cells.

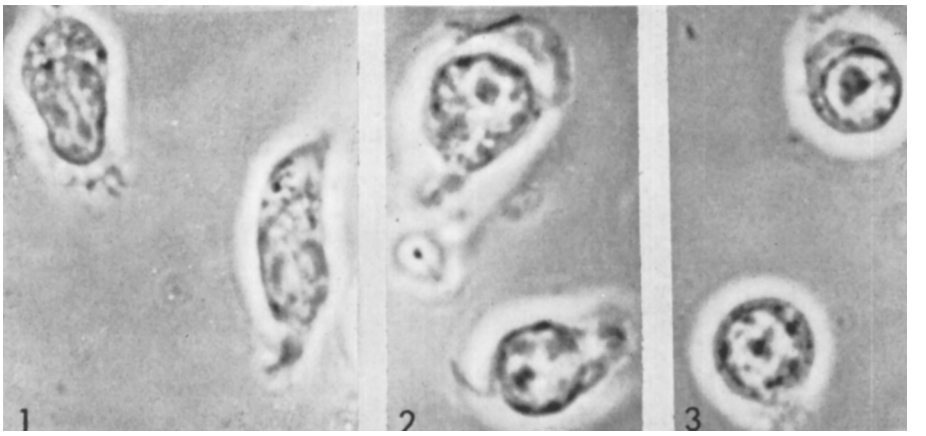
The cells in 50% normal human serum were incubated in small test tubes without shaking and without change of media. The number of viable lymphocytes before and after incubation were counted in a special slide chamber with a phase contrast microscope. The methods and the morphologic criteria for differentiating viable and dead lymphocytes have been described(5,6). The number of surviving lymphocytes after incubation were expressed as a percentage of the original number.

Results. In Table I normal and leukemic lymphocytes are compared with respect to percentage of cells surviving incubation in 50% normal human serum. In purified suspensions of normal lymphocytes, 4.8% of the lymphocytes survived 21 days. In a few experiments, some normal lymphocytes survived 28 days. On the other hand, in suspensions of blood cells from patients with chronic lymphocytic leukemia, 29.2% of the leukemic lymphocytes survived 21 days, and lymphocytes from 3 of 13 patients, survived 7 weeks. Fig. 1 presents normal lymphocytes that survived 20 days and Fig. 2 and 3 show leukemic lymphocytes that survived 49 and 56 days. The surviving lymphocytes represented in these figures had the same cytologic

TABLE I. *In vitro* Survival of Blood Lymphocytes from 7 Normal Individuals and from 13 Patients with Chronic Lymphocytic Leukemia. Cells were incubated with 50% normal human serum in small test tubes without shaking and without change of media.

	Mean	S.E. of mean (days)	Range
% of normal lymphocytes that survived incubation at 37°C			
14 days	23.7(17)*	4.11	.6–56.7
21 "	4.8 (7)	2.83	0 –20.6
% of leukemic lymphocytes that survived incubation at 37°C			
14 days	43.9(14)	5.71	21.3–69.8
21 "	29.2(13)	6.97	0 –76.9
49 "	11.9 (3)	6.30	2.5–24.3

* Number in parentheses indicates number of individuals used for determination of mean.



Photomicrographs were taken with a phase contrast microscope. × 2000.

FIG. 1. Two normal lymphocytes that survived incubation for 20 days.
FIG. 2. Two leukemic lymphocytes (patient CON) that survived 49 days.
FIG. 3. Two leukemic lymphocytes (patient PAU) that survived 56 days.

Note differences in morphology of normal and leukemic cells. Leukemic lymphocytes have large dark nucleoli, dark chromatin masses adherent to the nuclear walls, and anterior thin cytoplasmic projections. Normal lymphocytes have soft, gray chromatin masses and broad pseudopods.

characteristics as the cells in the original suspensions. It was concluded that, on the average, leukemic lymphocytes survived longer *in vitro* than normal lymphocytes.

The data were analyzed to study the variability in survival capacity of the lymphocytes of different patients. It was seen previously that in suspensions of normal blood lymphocytes, only 4.8% survived 21 days *in vitro*. Therefore, this period of incubation seemed of particular interest to study variability. It was found that the survival percentages of leukemic lymphocytes incubated 21 days varied from 0 to 76.9% (Table I). In fact, the tests on the blood cells of 3 of 13 patients showed survival percentages of 5 or less; *i.e.*, the lymphocytes of these patients did not show any prolongation of survival as compared to normal. It seemed that the survival capacity of the lymphocytes were characteristic for the given patient.

An attempt was made to correlate survival time *in vitro* with various clinical factors. In this analysis, the absolute lymphocyte count of the patient proved of interest. Fig. 4 shows the relationship between survival percentage of leukemic lymphocytes incubated 21 days and the absolute lymphocyte count at time of the first *in vitro* test. Each point in the graph represents the average survival percentage in 1 to 4 tests for a patient. The Figure shows a linear relationship between the logarithm of the absolute lymphocyte count of the patient and average survival time of the lymphocytes *in vitro*. The regression equation for the graph is:

$$S = 38.7 \log L - 138$$

where S is percentage of lymphocytes surviving 21 days and L is absolute lymphocyte count.

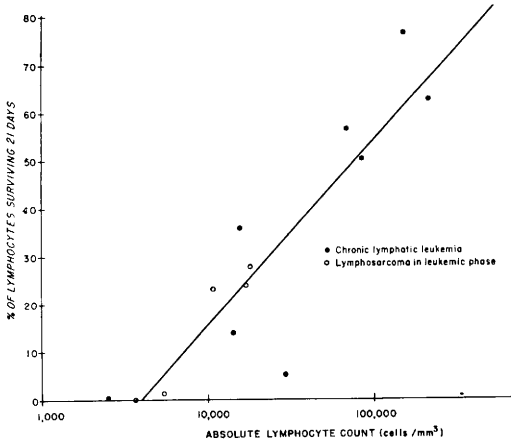


FIG. 4. Relationship between absolute blood lymphocyte count of 13 patients and percentage of lymphocytes surviving *in vitro* 21 days. Clinical diagnoses of the patients were chronic lymphocytic leukemia or lymphosarcoma in leukemic phase.

Discussion. *In vitro* experiments showed that leukemic lymphocytes survived, on the average, longer than normal lymphocytes. Does this finding indicate an intrinsic difference in the capacity of normal and leukemic lymphocytes to survive?

Technical factors were carefully controlled to obtain similar conditions for the suspensions of normal and leukemic lymphocytes. It was found possible to obtain highly purified suspensions of normal lymphocytes with less than 10% contaminating cells including erythrocytes. It is not possible to state categorically that there were no differences in technique but it is probable that they were not responsible for the finding of the prolonged survival time of leukemic lymphocytes.

The observed difference in survival may be due to incidental factors. There may be differences in the average age of blood lymphocytes of leukemic and non-leukemic patients. It it conceivable that the media used lacks some chemical needed by normal lymphocytes for long time survival. It has not been possible to test these or other incidental factors.

In vivo studies by several investigators have indicated that lymphocytes in patients with chronic lymphocytic leukemic have a longer life span than normal lymphocytes. The similarity between *in vitro* and *in vivo* findings suggests strongly that there is an intrinsic difference in the life span of normal and leukemic lymphocytes.

The present work showed a correlation between *in vitro* survival capacity of leukemic lymphocytes and the absolute lymphocyte count in patients with chronic lymphocytic leukemia. From this finding, it appears that the observed *in vitro* survival percentages may be a function of the *in vivo* life span of leukemic lymphocytes. Furthermore, the finding suggests the hypothesis that the high lymphocyte count in patients with chronic lymphocytic leukemia may be a result of a prolonged survival time of leukemic lymphocytes.

Summary. Purified suspensions of human blood lymphocytes in 50% normal human serum were incubated at 37°C in small test tubes and the number of viable lymphocytes were counted weekly with a phase contrast microscope. The tubes were not shaken and the medium not changed. In suspensions from hematologically normal individuals, 24% of the lymphocytes survived 2 weeks and 5%, 3 weeks. In blood cell suspensions derived from 13 patients with chronic lymphocytic leukemia, 29% of the lymphocytes survived 3 weeks and some of the cells survived 7 weeks. The percentages of leukemic lymphocytes that survived 3 weeks varied considerably for the 13 patients and were directly correlated with the absolute lymphocyte counts of the patients.

1. Schrek, R., Donnelly, W. J., *Blood,* 1961, v18, 561.

2. Schrek, R., *J. Lab. & Clin. Med.,* 1963, v61, 34.

3. Schrek, R., Leithold, S. L., Friedman, I. A., Best, W. R., *Blood,* 1962, v20, 432.

4. Schrek, R., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 328.

5. Schrek, R., Stefani, S., *J. Nat. Cancer Inst.,* 1964, v32, 507.

6. Schrek, R., *Arch. Path.,* 1958, v66, 569.

7. Hamilton, L. D., in *Homeostatic Mechanisms,* Brookhaven Nat. Laboratory, Upton, 1957, p52; and Weisberger, A. S., Levine, B., *Blood,* 1954, v9, 1082.

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