Clinical Evaluation of an in Vitro Test for Radiosensitivity of Leukemic Lymphocytes

By ROBERT SCHREK, STANLEY L. LEITHOLD, IRVING A. FRIEDMAN AND WILLIAM R. BEST

A PREVIOUS PAPER described a slide-chamber method for the measurement of the radiosensitivity of lymphocytes from leukemic patients. The method was used to study 31 patients with chronic lymphocytic leukemia or with lymphosarcoma in leukemic phase. The patients showed considerable variability with respect to the radiosensitivity of their blood lymphocytes. Of particular interest were a few patients with lymphocytes which were resistant to irradiation with 1000 roentgens. This high degree of resistance was quite impressive since the lymphocytes of other leukemic patients and of non-leukemic individuals showed significant responses to dosages as low as 2 roentgens.

An evaluation of the clinical significance of the slide-chamber method requires the determination of the correlation of radiosensitivity of the leukemic lymphocytes in vitro with clinical response of the patients. Unfortunately, this is a difficult problem in view of the many uncontrolled variables in a clinical study and because of the lengthy period required to collect sufficient patients and study their clinical progress.

The clinical histories of 80 leukemic patients were thoroughly reviewed. In this report we will consider a few objective clinical findings of 19 patients with radioresistant lymphocytes and 61 patients with sensitive cells.

METHODS AND PATIENTS

The slide-chamber method has been described previously and is summarized only briefly in this paper. The leukocytes from the blood of a patient were concentrated, washed, and resuspended in 50 per cent normal human serum. The suspension in proper dilutions was distributed in special slide-chambers, irradiated, and incubated at 37°C. The viable lymphocytes were counted in a measured area once a day by means of an inverted phase microscope provided with special equipment. A lymphocyte was considered viable if it had a distinctly outlined nucleus with dark, coarse, chromatin masses and light gray nucleoplasm (fig. 1). A cell was considered dead or dying if it had a fragmented or pyknotic nucleus or an intranuclear vacuole surrounded by dark chromatin material (fig. 2). These criteria of viability and death were established by studies of irradiated lymphocytes with time-lapse cinemicrography.

Daily counts of the viable lymphocytes permitted the construction of survival curves and the calculation of 10 per cent survival times (10 per cent ST) for irradiated and non-irradiated lymphocytes. Tests were performed on all patients diagnosed as chronic lymphocytic leukemia or lymphosarcoma in leukemic phase in the Veterans Administration Hospital, Hines, Ill., and Hematology Department, Cook County Hospital, Chicago, Ill.

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AN IN VITRO TEST FOR RADIOSENSITIVE LYMPHOCYTES

Fig. 1.—Viable lymphocytes from patient with chronic lymphocytic leukemia. The cells have been irradiated with 1000 r and incubated at 37 C. for one-half hour. The figure also shows a normal and an enlarged red blood cell and a monocyte (center). The figure is a print made from a 16 mm. time-lapse cinemicrographic film. Magnification 1800 X.

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RESULTS

Sensitivity Tests

Table 1 presents the in vitro findings on the first tests of 80 leukemic patients. In addition, the table shows the results reported previously on 26 non-leukemic control patients.4 The non-irradiated lymphocytes from leukemic and non-leukemic patients had the same median 10 per cent survival time of approximately 9 days. The 10 per cent survival times of normal lymphocytes treated with 1000 r had a range of 1.1 to 2.2 days. The table shows that 61 of the 80 leukemic patients had blood lymphocytes which were at least as radiosensitive as normal lymphocytes. Nineteen patients had lymphocytes which, after irradiation with 1000 r, showed 10 per cent survival times of 2.5 to 11 days. In other words, 24 per cent of the 80 leukemic patients had in vitro radioresistant lymphocytes.

The 19 patients with radioresistant lymphocytes had diverse clinical histories. The ages of the patients ranged from 44 to 84 years. Eleven patients had symptoms for less than 15 months prior to the test. The white blood cell count ranged from 10,000 to 600,000 cells per cu. mm. and the hemoglobin was less than 8.0 Gm. per cent in seven patients. Three patients had very large spleens
Fig. 2.—Dead leukemic lymphocytes with pyknotic, lysed, or vacuolated nuclei. The cells have been irradiated with 1000 r and incubated for 11 hours. The figure is a print from the same cinemicrographic film as in fig. 1. The macrocyte is still present. One lymphocyte (below the macrocyte) is still viable with a morphologically intact nucleus. Magnification 1800 X.

and livers. There did not appear to be any common denominator in the clinical histories of the 19 patients with radioresistant lymphocytes.

The first question that arises is whether the radioresistance of lymphocytes was the result of previous therapy. It is seen in table 2 that of the 19 patients with radioresistant lymphocytes, 10 (53 per cent) had no significant previous treatment and only 7 (37 per cent) had x-ray therapy. There was no significant difference in the previous therapy of the two groups of patients. The data indicate that radioresistance of lymphocytes occurred in patients who had had no prior x-ray therapy.

The establishment of a group of patients with radioresistant lymphocytes requires further characterization of the group. Most important would be the number of patients with chronic lymphocytic leukemia as compared to those with lymphosarcoma in leukemic phase, since the latter disease is thought to have a more rapid course and shorter survival. Lymphosarcoma-cell leukemia was diagnosed in nine patients (47 per cent) of the 19 with resistant lymphocytes and in 22 patients (36 per cent) of the 61 with sensitive cells (table 1). The difference in the percentages is not statistically significant. Could there be a difference in the cytology that would correlate with the results of the sensitivity? The cytology of the lymphocytes was studied both in blood films and by phase microscopy. No differences could be ascertained in the cells which would identify them as radioresistant or radiosensitive. The resistance
Table 1.—Radiosensitivity of Lymphocytes from Leukemic and Non-leukemic Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>0 r Median</th>
<th>1000 r Median</th>
<th>10% Survival Time Range</th>
<th>No. and % of Patients with LSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 non-leukemic</td>
<td>9.2</td>
<td>1.7</td>
<td>1.1–2.2</td>
<td></td>
</tr>
<tr>
<td>80 leukemic</td>
<td>8.8</td>
<td>1.5</td>
<td>0.6–11</td>
<td></td>
</tr>
<tr>
<td>61 leukemic</td>
<td>8.6</td>
<td>0.6–2.4</td>
<td></td>
<td>22 (36%)</td>
</tr>
<tr>
<td>19 leukemic</td>
<td>9.1</td>
<td>2.5–11</td>
<td></td>
<td>9 (47%)</td>
</tr>
</tbody>
</table>

*The 10 per cent survival time of x-irradiated (1000 r) and non-irradiated lymphocytes from 26 non-leukemic patients and of 80 patients with chronic lymphocytic leukemia or with lymphosarcoma in leukemic phase (LSa). Only the first test on each patient is given in the table.

Table 2.—The Treatment Prior to First in Vitro Test of 19 Leukemic Patients with Radioresistant Lymphocytes and of 61 with Sensitive Lymphocytes

<table>
<thead>
<tr>
<th>Prior Treatment</th>
<th>Radioresistant Lymphocytes</th>
<th>Sensitive Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10 (53%)</td>
<td>29 (48%)</td>
</tr>
<tr>
<td>X-rays</td>
<td>7 (37%)</td>
<td>21 (34%)</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>61</td>
</tr>
</tbody>
</table>

seemed to be a physiologic rather than a morphologic characteristic of the lymphocyte.

The resistant and sensitive groups were compared further to determine if an extraneous factor might be responsible for the observed difference in response to therapy. The factors considered were 1) hospital in which therapy was given, either Cook County Hospital or Hines Veterans Administration Hospital; 2) race and sex of treated patients; 3) initial leukocyte count; 4) site of treatment, such as trunk, abdomen, spleen or lymph nodes; and 5) diagnosis, whether chronic lymphocytic leukemia or lymphosarcoma in leukemic phase. The two groups did not differ significantly with respect to any of these factors.

Another question to be considered is: Does the sensitivity test change in the course of the disease? Twenty-two patients had two or more tests in the course of 11 to 40 months. Of this group, 19 patients had radiosensitive lymphocytes in repeat tests without any appreciable change in sensitivity. Of the 19 patients, five are dead including three patients who were found to have sensitive lymphocytes 1 to 2 months before death. The 19 patients had various types of therapy including x-rays and nitrogen mustard. In these patients, the treatment did not cause any change in the in vitro sensitivity test.

One patient, C733, had lymphocytes that were resistant to x-rays according to two in vitro tests in December 1957. The lymphocytes, however, were found to be radiosensitive in four repeat tests during the period September 1958 to October 1959. The evaluation of the change in radiosensitivity of the lympho-
cytes is complicated by the fact that the patient had cryptococcosis of the meninges at the time of the first tests. The infection was successfully controlled by use of amphotericin B.

Of the 22 patients retested, two showed a change from sensitive to resistant lymphocytes. This change occurred 11 and 15 months after the initial test. In one patient (H690) the initial test on September 30, 1957 showed that the 10 per cent survival time of the 1000 r treated lymphocytes was 1.4 days. In two additional tests during the next 15 months the results were 1.3 and 1.8 days. The 10 per cent ST-1000 r rose to 4.7 days on January 6, 1959 and remained at this level. The other patient (H1399) had an initial 10 per cent ST-1000 r of 2.1 days on November 26, 1958. The test was repeated 2 months later with the finding of 1.5 days. On October 14, 1959, the 10 per cent ST-1000 r rose to 7.8 days. In eight additional tests during the next 4 months, the 10 per cent ST-1000 r varied from 6.2 to 8.9 days. Prior to the change in sensitivity one patient had received x-ray therapy. However, it is not possible to say that the therapy was responsible for the observed change. The two patients died 22 and 23 months after the change in sensitivity.

Prognosis

The survival time of the patients provided an objective means of studying the clinical significance of the in vitro test. As the test was being evaluated in this study, the survival time of the patients was measured from the time of the initial in vitro test to the date of death or the date of the last follow-up.

The actuarial method as reviewed by Merrell and Shulman13 was used to calculate the survival curves of 19 patients with radioresistant and 61 patients with radiosensitive lymphocytes (fig. 3). The two survival curves in the figure are significantly different. The 50 per cent survival time or life expectancy was 22 months for the radiosensitive group and only 4 months for the radioresistant group. The results showed that the in vitro finding of radioresistant lymphocytes was correlated with a short survival time of the patient.

Minimal Leukocyte Count Following X-ray Therapy

Detailed studies were made on the response of the leukocyte count to x-ray treatment of any kind, whether given over the trunk to reduce the white blood cell count or over enlarged lymph nodes. The effect of each course of therapy on the leukocyte count is shown in figure 4, which presents the initial count before therapy and the lowest count after therapy. The lowest count is expressed as a percentage of the initial count. Patient H690 showed a change in his radiosensitivity test from sensitive to resistant lymphocytes. Two courses of x-ray therapy given before the observed change are included in the sensitive group (points: 182,000 cells, 25 per cent; and 350,000 cells, 25 per cent) and two courses after the change are shown in the resistant group (399,000 cells, 29 per cent; and 306,000, 96 per cent). The figure presents the effects produced by 10 courses of x-ray treatment given to eight patients with resistant lymphocytes and by 18 courses to 12 patients with sensitive lymphocytes. The geometric means of the final leukocyte count were 12.0 per cent of the initial count in the sensitive group and 54.5 per cent for the resistant group. The difference in the means
is statistically significant. Further analysis of the data in figure 4 showed that
the correlation index of the leukocytic response (lowest count as per cent of
the initial count) and the radiosensitivity index (the in vitro survival time of
irradiated lymphocytes) was 0.60, which is statistically significant.

It may be concluded from this analysis that the in vitro finding of radio-
resistant lymphocytes may be correlated with a poor response of the leukocyte
count to x-ray therapy.

Additional detailed statistical studies were made to take into consideration
the rate of decline of leukocyte count and the intensity and duration of ther-
apy. In addition, another method of measuring the in vitro radiosensitivity of
lymphocytes was used. These studies, which will be published at a later date,
confirmed the present findings of a distinct correlation between the in vitro
test for radiosensitivity and the leukocyte response of the patient to x-ray
therapy.

DISCUSSION

Previous work developed an in vitro method of measuring the sensitivity of
normal and leukemic blood lymphocytes to x-rays and chemotherapeutic
agents. The question arose—Does the in vitro test have any clinical signif-
icance? The longevity of the patients and the white blood cell response seemed
useful criteria to evaluate the test. The longevity was expressed from the time
of the first in vitro test rather than from onset of disease. According to several
investigators, the measurement of longevity from onset is fallacious because
the determination of the time of onset is difficult and is dependent on subject-
ive factors in both the patient and the physician.

Our experience seems to be in accord with that of several investigators,
Bethell et al., Pisciotta and Hirschboeck, and Dameshek and Gunz who described a benign and an aggressive form of chronic lymphocytic leukemia. Of 86 patients reported by Pisciotta and Hirschboeck, 18 (21 per cent) had the aggressive form with severe symptoms and signs including fever, weakness, anemia and very large lymph nodes, liver and spleen. Although the aggressive form behaved as a distinct disease, they did not find any cytologic differences in the blood cells of patients with the benign and aggressive variants of disease. Similarly, Gunz and Hough and Leavell reported that some patients diagnosed as chronic lymphocytic leukemia had only a short survival time. Feinleib and MacMahon observed that 18 per cent of 649 patients had a total duration of survival of less than 6 months after onset. Dameshek and Gunz commented that some patients with chronic lymphocytic leukemia are resistant to radiotherapy. These radioresistant patients include some who were previously untreated and some who had previously shown sensitivity to radiation. According to Osgood, patients vary in their sensitivity to therapy with x-rays or radioactive phosphorus and some patients with chronic lymphocytic leukemia are resistant to irradiation. Other workers also reported on the resistance of some patients with chronic lymphocytic leukemia to x-ray therapy. It may be concluded from this brief review of the literature and from our present findings that some patients with the clinical diagnosis of chronic
lymphocytic leukemia may be said to have an aggressive form of disease, and that such cases are resistant to x-ray therapy and have a short survival time.

According to various reports and our present findings, morphologic studies do not help to detect the patients with chronic lymphocytic leukemia who respond poorly to x-ray therapy and have a short survival time. According to our present study, it would seem that the in vitro test for radiosensitivity of lymphocytes may aid in detecting this group of patients before treatment is instituted.

It may seem surprising that a correlation was obtained between the in vitro radioresistance of the peripheral blood lymphocytes and the clinical resistance of a patient to x-ray therapy. The general assumption is that x-ray therapy of a leukemic patient inhibits the formation of leukemic cells but has no effect on the peripheral lymphocytes.18 Recent in vitro experiments have shown that irradiation in vitro with small doses (2 \( r \) or more) produced a direct deleterious effect on normal differentiated, non-dividing human lymphocytes.4 Hulse\(^4\) showed that irradiation in vivo had two effects on the normal lymphocytes of rats. The greater effect was a direct destructive action on the mature lymphocytes in the blood of the irradiated rats. A second effect was an inhibition of formation of lymphocytes. This inhibition was less prominent and was delayed as compared to the first direct destructive effect. A recent study in this laboratory showed that total body irradiation of rats with 50 \( r \) produced a direct deleterious effect on the circulating blood lymphocytes.20 It has also been seen in this study that leukemic lymphocytes of most patients have the same radiosensitivity as normal lymphocytes. These considerations indicate that irradiation of most leukemic patients has a direct and rapid destructive action on blood lymphocytes and a secondary delayed effect on the formation of lymphocytes. It follows that irradiation of a leukemic patient would have less clinical effect if the peripheral lymphocytes are radioresistant. An in vitro test of the radiosensitivity of the blood lymphocytes would therefore be expected to be correlated, at least to some extent, with the in vivo response of the patient with chronic lymphocytic leukemia to irradiation.

Alexander\(^2^1\) observed a change in the radiosensitivity of cells from a transplantable mouse lymphoma. The change in radiosensitivity occurred after the cells had been cultured in vitro for 6 months. Alexander therefore warned of the danger of deductions concerning radiosensitivity of cells in vivo from in vitro studies. Unfortunately, he did not test the in vivo sensitivity of his radioresistant and radiosensitive strains. In any case, the present study measured the in vitro radiosensitivity of the patients' own cells—not descendants of the cells. Alexander's observations are not in conflict with the present findings.

The question remains—whether in vitro radioresistance is a symptom of a terminal stage rather than a significant factor leading to early death. In this study, 19 patients continued to have radiosensitive lymphocytes on repeat tests and three of these patients were found to have sensitive lymphocytes 1 to 2 months before death. In these patients, at least, a terminal stage per se was not associated with radioresistant lymphocytes.
SUMMARY

A recently developed slide-chamber method was used to test the radiosensitivity of blood lymphocytes from 80 patients with chronic lymphocytic or lymphosarcoma-cell leukemia. The objective of this study was to determine whether these in vitro tests on sensitivity to x-rays had any clinical significance.

Two objective criteria were used to measure the clinical reactions of the leukemic patients. The first was the duration of survival of patients following the in vitro test. The second was the minimal leukocyte count of a patient following x-ray therapy; the minimal count was expressed as a percentage of the count before therapy.

The in vitro radiosensitivity was measured by the 10 per cent survival time of lymphocytes irradiated with 1000 r. Blood lymphocytes from non-leukemic individuals were highly radiosensitive with indices of 1.1 to 2.2 days. In initial tests, the lymphocytes of 61 leukemic patients had the same high sensitivity to x-rays as lymphocytes from non-leukemic individuals. In contrast, the lymphocytes of 19 leukemic patients were radioresistant to irradiation with indices of 2.5 to 11 days.

The 61 patients with radiosensitive lymphocytes had a median survival time of 22 months after the in vitro test. In contrast, the 19 patients with radioresistant lymphocytes had a median survival time of only 4 months. Clinical x-ray therapy caused a greater decline in leukocyte counts in patients with radiosensitive lymphocytes than in those with radioresistant cells.

A significant index of 0.60 was obtained for the correlation of in vitro radiosensitivity of lymphocytes and the in vivo decrease in leukocyte counts of patients after x-ray therapy.

It is concluded that an in vitro finding of radioresistant lymphocytes is correlated with a poor response of the leukocyte count to x-ray therapy and a short survival time of the patient.

SUMMARIO IN INTERLINGUA

Un recentemente disveloppate metodo de “cameras a laminas” esseva usate pro testar le radiosensibilitate de lymphocytos de sanguine ab 80 patientes con chronic leucemia lymphocytic o a cellulas de lymphosarcoma. Le objectivo de iste studio esseva determinar si iste tests in vitro in re le sensibilitate a radios X habeva ulle signification clinic.

Duo criterios objective esseva usate pro mesurar le reactiones clinic del patientes leucemic. Le prime esseva le duration del superviventia del patientes post le test in vitro. Le secunde esseva le minime numeration leucocytic de un patiente post le therapia a radios X. Le numeration minime esseva exprimite como un procentage del numeration ante le therapia.

Le radiosensibilitate in vitro esseva mesurate per le tempore del superviventia de 10 pro cento del lymphocytos irradiate con 1000 r. Le lymphocytos del sanguine de non-leuemic subjectos de controlo esseva altemente radiosensible, con indices de 1.1 a 2.2 dies. In tests initial, le lymphocytos de 61 patientes leucemic habeva le mesme alte sensibilitate pro radios X como
lymphocytos ab individuos non-leucemic. Per contrasto con isto, le lymphocytos del 19 altere patientes leucemic esseva radioresistente in le irradiation con indices de 2,5 a 11 dies.

Le 61 patientes con radiosensibile lymphocytos habeva un longevitate median de 22 menses post le test in vitro. Per contrasto con isto, le 19 patientes con radioresistente lymphocytos habeva un longevitate median de solmente 4 menses. Therapia a radios X causava un plus marcate declino del numeration leucocytic in patientes con radiosensibile que in patientes con radioresistente cellulas.

Un significative indice de 0,60 esseva obtainite pro le correlation del radiosensibilitate in vitro del lymphocytos con le declino in vivo del leucocytosis per therapia a radios X.

Es concludite que le constatation de radioresistentia in vitro del lymphocytos es correlationate con un poco favorabile responsa del leucocytosis al therapia a radios X e un curte superviventia del patiente.

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