Differences Between Lymphocytes of Leukemic and Non-Leukemic Patients with Respect to Morphologic Features, Motility, and Sensitivity to Guinea Pig Serum

By ROBERT SCHREK AND WILLIAM J. DONNELLY

A SLIDE-CHAMBER method has been developed to study and compare the morphologic, physiologic, and pharmacologic characteristics of leukemic and non-leukemic human lymphocytes. The term "leukemic lymphocytes" refers to lymphocytes from the blood of patients with chronic lymphocytic or lymphosarcoma-cell leukemia. A previous study with this method showed that heterologous sera were more toxic to leukemic than to non-leukemic lymphocytes. In addition, concomitant microscopic observations and time-lapse cinemicrographic studies suggested that leukemic and non-leukemic lymphocytes differ in morphology and motility. The studies led to the conclusion that viable leukemic lymphocytes have distinctive characteristics. To test the validity of this thesis, observations were made on 22 specimens of peripheral venous blood obtained from 20 selected patients. The viable lymphocytes in the blood specimens were studied with respect to morphology, motility, and sensitivity to inactivated sera of guinea pigs. These observations were made without knowledge of the clinical and conventional laboratory findings. This report presents the findings on the viable lymphocytes in the blind tests and compares these observations with the clinical diagnoses of the patients.

METHODS

To maintain objective conditions for testing, the work was done in two separate stages. The first stage consisted of selection of patients, collection of blood, and standard counts on the blood. This was performed by the Hematology Section and its laboratory. The second stage, which consisted of concentrating the leukocytes and studying the viable lymphocytes, was done in the Tumor Research Laboratory.

Twenty hospitalized patients were selected for these tests by one of us (W. J. D.). The patients selected included those with the following diseases: chronic lymphocytic leukemia, lymphosarcoma with and without lymphocytosis, diseases with relative or absolute lymphocytosis, and diseases with normal lymphocyte counts.

Personnel of the Hematologic Section obtained specimens of 20 ml. of heparinized venous blood from the selected patients. In addition, a leukocyte count and differential count were done on the same day in the Hematology Section.

The blood specimens were labeled with a code number and submitted to the Tumor Research Laboratory. In this laboratory, the leukocytes were concentrated by sedimentation. The cells were then washed and resuspended in equal parts of tissue culture medium TC199.*

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and normal human serum or heat-inactivated serum (56 C. 30 min.) of guinea pigs. The suspensions were then distributed to slide-chambers described previously. The chambers were incubated at 37 C. The lymphocytes were examined daily with an inverted phase microscope and were photographed with a time-lapse cinemicrographic camera.

Twenty blood specimens from 20 patients were studied. An additional specimen was obtained from each of two patients at the request of the Tumor Research Laboratory. Results of the study of the viable lymphocytes in all specimens were tabulated in the Tumor Research Laboratory without knowledge of clinical or conventional hematologic data. After study of all 22 specimens had been completed, results were compared with the clinical and laboratory findings of the Hematologic Section.

Criteria Used for Diagnosis of Living Lymphocytes

When the present study was undertaken, the Tumor Research Laboratory had previously completed studies on the blood lymphocytes of 130 patients with chronic lymphocytic or lymphosarcoma-cell leukemia and of 227 non-leukemic individuals. The studies included direct observation of the viable lymphocytes by phase microscopy. In many cases, the lymphocytes were studied by time-lapse cinemicrography. These observations suggested that viable leukemic lymphocytes differ from non-leukemic lymphocytes. The impressions obtained from these observations led to the formulation of the following criteria for evaluating viable lymphocytes in the specimens submitted in the present study.

Morphology. Blood lymphocytes from non-leukemic patients were, shortly after incubation, round and approximately 7\(\mu\) in diameter (fig. 1). The nuclei were relatively large and round. Some nuclei were indented, lobated or irregular in shape. The chromatin material consisted of small, light gray, inconspicuous masses. After one or more days of incubation, most of the lymphocytes elongated so that the long diameters were 2 or more times the short diameters (figs. 2a and 2b). This elongation, according to cinemicrographic studies, was a sign of motility. The anterior end consisted of a hyaline pseudopod; the posterior end was elongated and contained a few, dark mitochondria. The nuclei usually showed slight elongation.

Lymphocytes from known leukemic patients were highly variable. One cell type consisted of a small lymphocyte approximately 5\(\mu\) in diameter (fig. 3). The nucleus had large dark prominent chromatin masses, some of which were attached to the nuclear wall. In some cells the attached chromatin masses were arranged as spokes of a wheel. Many of these cells remained round even after several days incubation. In some instances, these cells became slightly elongated or irregular in shape. After incubation for a few days, the leukemic lymphocytes seemed to enlarge with an increase in the diameter of the cells (compare figs. 3a and 4). It is not known whether this observed increase in size of the lymphocytes represents a true increase in the volume and dry weight of the cells or whether it is dependent on flattening of the cell. Both round and irregular shaped cells from some patients acquired, in 1 to 3 days, thin flagella-like projections measuring as long as 4\(\mu\) (fig. 4). “Flagella,” 1 to 5 per cell, appeared to be distinctly different from pseudopods of normal lymphocytes (compare figs. 2 and 4). In time-lapse cinemicrographic film, it was seen that the presence of flagella did not indicate motility of the cell.
Fig. 1.—Normal viable human lymphocytes as seen in a slide-chamber with phase microscopy. The cells were incubated at 37 C. for 2 days. The chromatin masses are small and light gray. 1700x.

Figs. 2a & 2b.—Normal blood lymphocytes incubated 2 days. The cells are elongated and have anterior hyaline pseudopods (pointing upward) and thin posterior tails. 1700x.

Figs. 3a & 3b.—Blood lymphocytes from 2 patients with chronic lymphocytic leukemia. Cells incubated 2 hours (fig. 3a) and 5 hours (fig. 3b). The lymphocytes have large dark chromatin masses, some of which are attached to the nuclear wall. Fig. 3a, 1700x; fig. 3b, 2000x.

Fig. 4.—Leukemic lymphocytes incubated at 37 C. for 5 days. The cells are from the same slide-preparation as fig. 3a. The cells have a larger cross-sectional area than in fig. 3a, are slightly irregular in shape and have several thin, elongated flagella-like structures, which differed morphologically from pseudopods of normal lymphocytes (fig. 2). 1700x.

Fig. 5.—Lymphocytes from a patient diagnosed as lymphosarcoma-cell leukemia. The cells were incubated for 2 days. The lymphocytes are large with large dark nucleoli. 1700x.

Fig. 6.—Abnormal cell that developed from blood specimen 17 after 5 days of incubation. The cell is very large with a large nucleolus. The specimen was obtained from 2 patients with hemochromatosis (17a and 17b, table 1). 1700x.
Another leukemic cell type was, shortly after incubation, round and enlarged (8 to 10 μ). The nucleus had few or no visible chromatin masses but had a large dark nucleolus (fig. 5). The nucleoplasm was clear and a lighter gray than the cytoplasm. After 1 day of incubation, these cells frequently became irregular in shape. Usually they had anterior pseudopods with one or more thin, elongated, flagella-like projections. The posterior end usually consisted of a short tail containing a few granules. The nucleus remained round with a conspicuous nucleolus. Blood lymphocytes of patients with lymphosarcoma-cell leukemia usually were of this cell type. Some of these cells were, however, seen in the blood of many patients with chronic lymphocytic leukemia.

The abnormal cell types described above seemed to be two extreme variations from normal. In most patients, the leukemic lymphocytes had various mixtures of the morphologic characteristics of the 2 cell types. After 1 hour of incubation, a cell was considered to be abnormal if it had large chromatin masses, a prominent nucleolus or was small or large in size. After 1 day or more of incubation, additional abnormal signs were: the presence of flagella, the failure to elongate, and irregularity in shape.

The cytology of blood samples was classified as 3+, 2+, 1+, or 0, according to the degree and number of abnormalities. If most of the lymphocytes had several abnormal leukemic cytologic features, the blood was classified as 3+. A classification of 0 indicated no or very few abnormal lymphocytes; and of 2+ and 1+, intermediate degrees of abnormality in the morphologic features of the cells.

Motility. When observed with time-lapse cinemicrography, most lymphocytes of non-leukemic patients became motile after 2 or more days of incubation. In the projected films, the cells moved across the field, usually in a straight line. All motile cells were, as stated previously, elongated with anterior pseudopods. Furthermore it was seen that elongated cells were nearly always motile except if their tails were adherent to an adjacent mass of cells or to the tail of another lymphocyte.

As indicated previously, some leukemic lymphocytes remained small and round after incubation. These cells were seen to be non-motile when studied with time-lapse cinemicrography. Other leukemic lymphocytes developed irregularities in shape with anterior pseudopods and flagella and short posterior tails. In projected cinemicrographic film, these cells seemed to thrash about and might even change their positions slightly but few of these cells moved across the field. These cells had impaired or no motility.

It should be noted that an irregular, or hand-mirror shape did not necessarily denote motility of the lymphocyte although highly elongated lymphocytes were nearly always motile.

In this study, blood cells were usually photographed on the 4th day. The motility of lymphocytes in each sample was classified by study of the cinemographic film and by consideration of the shape of the cell on direct microscopy. A blood sample was classified as 0 if no abnormalities or decrease in motility were observed, i.e., if most of the lymphocytes moved across the field; a classification of 2+ was given if few or no lymphocytes were motile; and 1+ indicated a small number of motile cells.
**Sensitivity to guinea pig sera.** To measure the sensitivity of lymphocytes to guinea pig sera, the leukocytes were suspended in 50 per cent inactivated (56°C. 30 min.) sera of guinea pigs. Each suspension was tested against sera from 3 to 7 guinea pigs. The sera were not pooled but were tested separately. The cells were also suspended in 50 per cent serum from a non-leukemic, ambulatory patient. Each guinea pig serum was first tested for toxicity to non-leukemic lymphocytes from a known patient. A few sera were found to be toxic to the non-leukemic lymphocytes and were not used in tests on the unknown specimens.

The daily counts of the viable lymphocytes permitted the calculation of the 10 per cent survival time of the lymphocytes in the test sera. Previous work\(^3\) has shown that non-leukemic lymphocytes in normal human sera had a 10 per cent survival time of 9.3 days with a standard error of 0.3 days. Leukemic lymphocytes had approximately the same 10 per cent survival time. The sensitivity of the cells to a guinea pig serum or the cytotoxic effect produced by the serum was expressed by the following index:

\[
100 \left( 1 - \frac{10\% \text{ survival time of lymphocytes in guinea pig serum}}{10\% \text{ survival time of lymphocytes in human serum}} \right)
\]

With this index, 100 represents the maximum sensitivity, and 0 or less represents no toxic effect or sensitivity. For each suspension, an index of sensitivity was obtained for each guinea pig serum, and then the indices for 3 to 7 guinea pig sera tested were averaged.

In previous unpublished work, guinea pig serum produced an approximate index of 25 with lymphocytes from non-leukemic patients, and 75 with leukemic lymphocytes. The blood specimens were classified as 3+, 2+, 1+ and 0, depending on the average index of sensitivity. An average index of 65 or more was arbitrarily classified as 3+; 48 or less was classified as 0. The classifications of 1+ and 2+ were used loosely and depended on the average sensitivity, the number of sera tested, and the consistency of the effects produced by the different sera.

**Total diagnostic index.** It has been seen that 3 factors were used to evaluate the blood specimens: morphology, motility, and sensitivity to guinea pig sera. The degree of deviation from normal was classified as 3+ to 0 for cytology and for sensitivity to guinea pig sera and 2+ to 0 for motility. Accordingly, each specimen has 3 indices representing the degree of abnormality of the 3 factors. The summation of the 3 indices gave a total diagnostic index which varied from 0 to a maximum of 8. Arbitrarily total indices of 7 and 8 were considered to be diagnostic of leukemic lymphocytes; an index of 6, probably leukemic; indices of 0 and 1, non-leukemic; and indices of 2 and 3, probably non-leukemic.

**Observations**

Table 1 summarizes the significant clinical diagnoses and conventional laboratory findings on the 20 selected patients. For convenience of exposition the patients listed in table 1 have been classified into 3 groups according to
Table 1.—Clinical and Conventional Laboratory Data on 20 Patients Used in Study

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Clinical diagnosis</th>
<th>Age</th>
<th>Blood leukocyte count per mm.³ x 10⁶</th>
<th>Total</th>
<th>Percentage of lymphocytes* in stained blood film</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymphoblasts</td>
</tr>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>Chronic lymphocytic leukemia</td>
<td>69</td>
<td>15.0</td>
<td>59</td>
<td>57</td>
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<td>3</td>
<td>Chronic lymphocytic leukemia</td>
<td>85</td>
<td>10.0</td>
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<td>71</td>
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<tr>
<td>5</td>
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<td>200.0</td>
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<td>7</td>
<td>Chronic lymphocytic leukemia</td>
<td>46</td>
<td>20.0</td>
<td>89</td>
<td>87</td>
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<td>14</td>
<td>Lymphosarcoma-cell leukemia</td>
<td>54</td>
<td>300.0</td>
<td>94</td>
<td>89</td>
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<td>92.0</td>
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<td>5.0</td>
<td>47</td>
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<td>10.2</td>
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<tr>
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<td>Diabetes mellitus</td>
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<tr>
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<td>Hodgkin's disease</td>
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<td>7.0</td>
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<td>8.0</td>
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<tr>
<td>13</td>
<td>Lymphosarcoma</td>
<td>21</td>
<td>4.0</td>
<td>28</td>
<td>25</td>
</tr>
</tbody>
</table>

*The cytologic criteria summarized by Wintrobe1 were used to define lymphoblasts and mature, lymphosarcoma-cell, and atypical lymphocytes.

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the clinical diagnoses. Group I consists of 7 patients with diagnoses of chronic lymphocytic or lymphosarcoma-cell leukemia. The white blood cell counts in these patients were usually high but 2 patients had counts of 10,000 and 15,000 with 74 and 59 per cent lymphocytes respectively (table 1). Of the 7 leukemic patients, 5 had received no prior treatment for leukemia and 2 had received x-ray therapy 6 and 18 months prior to the tests.

Another group consisted of 3 patients with lymphosarcoma (group III, in table 1). In these 3 patients, the white blood cell count and the lymphocyte percentage were not elevated at the time of testing although the patients were found to have some lymphosarcoma cells in stained blood films. One of these patients (number 9) had had white blood cell counts up to 10,400 with 50 per cent lymphocytes. Radiation therapy had resulted in a decrease in lymphocyte count. At time of testing, the leukocyte count was 8,000 with 37 per cent lymphocytes. This group of patients may be labeled "lymphosarcoma without lymphocytosis."

A third group consisted of 10 patients who had neither leukemia nor lymphosarcoma (group II, table 1). The white blood cell counts were 8,000 or less.
OBSERVATIONS ON LEUKEMIC LYMPHOCYTES

Table 2.—An Analysis of the Frequency of the Diagnostic Indices Obtained in 3 Groups of Patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chronic lymphocytic or lymphosarcoma</th>
<th>Non-leukemia and non-lymphosarcoma</th>
<th>Lymphosarcoma without lymphocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Number of patients 7 10 3
Number of blood specimens 9 10 3
Number of specimens with total diagnostic indices* of:
- Number of specimens with morphologic indices* of:
  - Number of specimens with motility indices* of:
  - Number of specimens with sensitivity indices* of:

*Indices are defined in text under Criteria Used for Diagnosis of Living Lymphocytes. The indices represent the degree of abnormality in the characteristics of the viable lymphocytes with 0 representing no significant abnormality in the characteristic.

except in one patient with a count of 10,200 cells. The percentage of lymphocytes was 47 to 59 per cent in 4 instances. Morphologically abnormal lymphocytes were observed in routine blood films of 1 patient diagnosed infectious mononucleosis.

Table 2 summarizes the observations in the 3 groups of patients. Lymphocytes in 9 specimens from the 7 leukemic patients (group I) had total diagnostic indices of 6 to 8 and were correctly characterized as leukemic or probably leukemic. Lymphocytes in 9 specimens from the non-leukemic, non-lymphosarcoma group (group II) had total indices of 0 to 3 and were all correctly characterized as non-leukemic. The 3 specimens from the 3 patients with lymphosarcoma without lymphocytosis had diagnostic indices of 1 to 3 and were, therefore, considered to have non-leukemic lymphocytes.

The morphology of the viable lymphocytes was considered to be abnormal
in all 9 blood specimens of the leukemic patients (all rated as 3+ or 2+ abnormal, table 2). In the 13 other blood specimens, only 2 had a 2+ abnormality.

The cytologic characteristics of specimen 17 (table 1) were of particular interest. The lymphocytes appeared to be normal during the first 4 days of incubation. On the 5th day, the cell suspension contained a small number of large cells with large clear nuclei and prominent nucleoli (fig. 6). In addition, a small number of cells were in mitotic division. The large abnormal cells were not considered to be leukemic as they had not been seen previously in known leukemic blood specimens. The cytology of specimen 17 was, however, classified as 1+. The specimen proved to be a mixture of bloods from 2 patients with hemochromatosis.

The motility of the lymphocytes was considered to be reduced in all blood specimens from the leukemic patients (table 2). Only 1 blood specimen from the non-leukemic patients showed reduced motility (1+). Table 2 shows furthermore that all the leukemic patients had a considerable increase in sensitivity to guinea pig sera (3+ increase in 8 specimens and 2± in 1). In contrast, a 2+ increase was observed in only 1 non-leukemic blood specimen.

It may be concluded that the viable lymphocytes of patients in group I were distinctively different from the lymphocytes of patients in groups II and III.

DISCUSSION

Three criteria were used to study living lymphocytes: morphology, motility, and sensitivity to guinea pig sera. These criteria were applied to a study of lymphocytes in 22 blood specimens. The findings were then compared with clinical reports.

The morphologic abnormalities that were considered to be suggestive of leukemic lymphocytes were: unusually small or large cells; large, prominent chromatin masses; large nucleoli; and flagella-like structures. On the basis of the present and previous findings we believe that the morphologic appearance of viable leukemic lymphocytes in the slide-chamber is characteristically different from that of normal lymphocytes.

Phase microscopy has been used to study viable leukemic and non-leukemic lymphocytes by several investigators including Bessis, Ackerman, and Moeschlin. In a review of phase microscopy, Hayhoe concluded that leukemic lymphocytes do not have any characteristic features. The observations reported in this study are not in accord with Hayhoe's conclusion. It should be noted that the present study employed a slide-chamber in which the lymphocytes are under more or less physiologic conditions and remain viable for 5 to 10 days. The previous workers used the wet smear in which the lymphocytes are compressed between a cover slip and slide and in which the cells remain morphologically intact only for a few hours. Further, in the slide-chamber method, the lymphocytes are concentrated, washed, and resuspended in 50 per cent normal human serum. In the wet smear, the whole blood is used for the
preparation. The differences in the present and previous findings may be dependent on differences in methods.

The motility of the lymphocytes was studied on the fourth day of incubation by time-lapse cinemicrography. In non-leukemic blood, most of the lymphocytes elongated and showed active motility. A decrease in the number of motile lymphocytes was considered to be suggestive of leukemia. The motility was estimated crudely and was represented by an index of 2+, 1+, or 0 depending on the degree of abnormality of motility. Further studies are planned to obtain a quantitative index of the motility of normal and leukemic lymphocytes. The observed decreased motility of the leukemic lymphocytes is not considered to be due to injury of the leukemic lymphocytes during processing. It should be noted that on the average the leukemic lymphocytes survived as long in vitro as the normal lymphocytes.

We have been able to find so far only one reference to the motility of human lymphocytes from patients with chronic lymphocytic leukemia. Rind mentions in his atlas on hematology that the motility of leukemic lymphocytes is increased. He bases this conclusion, apparently, on the finding of many leukemic lymphocytes with hand-mirror and irregular shapes. However, it has been seen in this study with time-lapse cinemicrography that hand-mirror shaped cells did not necessarily have the capacity to move actively across the slide.

The sensitivity of human lymphocytes to inactivated sera of guinea pigs was of particular interest. A previous study indicated that guinea pig sera were slightly toxic to normal lymphocytes but were relatively more toxic to leukemic lymphocytes. The present findings are in agreement with those obtained previously.

In this paper, we present findings that leukemic lymphocytes have reduced motility and increased sensitivity to guinea pig sera. A previous paper showed that blood lymphocytes from some patients with chronic lymphocytic leukemia are resistant to irradiation with x-rays in vitro. Dameshek, Schwartz, and Oliner report that lymphocytes from patients with chronic lymphocytic leukemia may be abnormal in their immune reactions and produce red cell antibody responsible for hemolytic anemia. According to these studies, leukemic lymphocytes may differ from non-leukemic lymphocytes in certain physiologic functions.

**Summary**

Observations were made on viable lymphocytes in 22 specimens of blood from 20 patients to test whether leukemic lymphocytes have distinctive characteristics. Special slide-chambers were used in this study. The features suggestive of leukemic lymphocytes were: large chromatin masses, prominent nucleoli, abnormal size of cells, flagella-like structures, reduced motility, and an increase in the sensitivity to inactivated guinea pig sera. An index summarizing these abnormal features selected correctly 9 blood specimens from 7 patients with chronic lymphocytic or lymphosarcoma-cell leukemia. Distinctive abnormalities of the living lymphocytes were not found in the blood of 3 pa-
tients who were diagnosed lymphosarcoma and had normal lymphocyte counts and some lymphosarcoma-cell lymphocytes in stained blood films. Blood lymphocytes from 8 patients with infectious mononucleosis, diabetes, hemochromatosis, and other diseases were correctly identified as non-leukemic. The findings obtained in this study indicate that viable leukemic lymphocytes are characterized by abnormal morphologic features, reduced motility, and increased sensitivity to heat-inactivated guinea pig sera.

**SUMMARIO IN INTERLINGUA**

Esseva facite observationes in viabile lymphocytos de 22 specimens de sanguine ab 20 patientes, con le objectivo de determinar si o non lymphocytos leucemic possede caracteristicas distinctive. Le metodo empleate—distinguite per le utilisation de cameras de lamina—esseva illo previemente descrbite in un altre publicatio. Le caracteristicas de lymphocytos ab patientes con leucemia pareva esser grande massas de chromatina, prominente nucleolos, anormal dimensiones del cellulas, estructuras del apparentia de flagellas, reducece motilitate, e un augmento in le sensibilitate pro inactivate seros de porco de India. Un indice in que iste anormalitates esseva summarisate seligeva correctemente 9 specimens de sanguine ab 7 patientes con leucemia del typos lymphocytic chronic o a cellulas de lymphosarcoma. Distinctive anormalitates del vive lymphocytos non esseva trovate in le sanguine de 3 patientes con diagnoses de lymphosarcoma e con normal numerationes lymphocytic e alicun lymphocytos lymphosarcomatocytic in colorate frottis de sanguine. Le lymphocytos ab le sanguine de 8 patientes con mononucleosis infectiose, diabete, hemochromatosis, e altere morbosesseva identificate correctemente como non-leucemic. Le constatationes obtenite in iste studio indica que viabile lymphocytos leucemic es characterisate per anormal aspectos morphologic, reducete motilitate, e augmento del sensibilitate pro thermo-inactivate seros de porco de India.

**REFERENCES**

OBSERVATIONS ON LEUKEMIC LYMPHOCYTES


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