Report of a Three-Year Study of Red Cell Mobility and the Slowing Factor in Serum of Persons with Lymphoma

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With the technical assistance of Agnes Dool

In 1961 the literature pertaining to microelectrophoresis of the red blood cell was reviewed, the technic for its performance described, and mobility values given for a large series of normal persons. Later it was shown that mobility was significantly lowered during pregnancy, during chronic inflammatory processes, and in patients with malignant neoplasm, while in patients with benign tumor it was normal. Studies designed to give understanding of this phenomenon led to the discovery that there were factors in the blood serum responsible for retarding the cell mobility—factors that could be differentiated from each other. Thus erythrocytes from normal persons, with the characteristic mobility of 1.33 μ/sec./volt/cm., when incubated with serum from a cancer patient, were altered so that mobility fell to 0.89 μ/sec./volt/cm., but no lower; mobility of normal cells incubated with serum from a pregnant woman or from a patient with nonmalignant disease, both characterized by retarded mobility, fell to 1.14μ, but no lower. The serum of patients with benign neoplasm contained no slowing factor. The slowing factor in cancer serum retained activity when heated to 50 C., while that from other diseases lost all activity at this temperature; it persisted in high dilutions, while that of the other diseases was not detectable at 1:8 dilution. It was further shown that retarded cells from malignant neoplasmic disease and retarded cells from non-neoplastic disease, when incubated with serum from a healthy person with red cell mobility of 1.33 μ, showed a quantitatively different reaction, the former being accelerated to 1.21 μ, the latter all the way to normal—1.33 μ.

On the basis that at least two factors could be identified, the “0.89” factor and the “1.14” factor, a series of patients with a variety of diseases were then investigated for the presence or absence of either factor. These studies showed the 0.89 factor to be present in persons with malignant neoplasm and absent from those without it.

The questions next considered were: the incidence of the 0.89 factor in malignant neoplasm of a given type; how early it occurs and how long it persists; whether it varies with the varying course of the disease; what effect therapy has upon it. It was thought that answers to some of these questions could be obtained by study of a large series of patients with Hodgkin’s disease. This choice was influenced by the availability of Hodgkin’s disease patients in our clinic, for whom follow-up data for 15 years were available.

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RBC MOBILITY AND SLOWING FACTOR IN LYMPHOMA

Method of Study

The technic employed has been described in detail elsewhere.1 Briefly, the vertical cell and method described by Ponder and Ponder2 were used. With a veronal buffer system of ionic strength 0.172, determinations were made at pH 9.0. Red blood cells were collected either into a plain tube and allowed to clot or into a tube containing the anticoagulant ethylenediaminetetraacetic acid, the cells were washed three times in 1 per cent isotonic sodium chloride solution, and mobility determined the same day or 24 hours later. Identification of the serum factor was achieved by determining the mobility of normal type O blood cells with 1.33 \( \mu \) mobility after incubating them with the test serum, undiluted and diluted 1:8, unheated and heated to 50 C.

Aliquot portions of the same sera were also incubated with red cells of low mobility (1.18-1.14) obtained from cancer patients. These cells were useful because in the presence of serum devoid of slowing factor their mobility rose to 1.33 \( \mu \) and when the cancer slowing factor was present, mobility fell to 0.89 \( \mu \).

The final test was determination of the mobility of red blood cells of lymphoma patients following incubation with serum from normal persons with red cell mobility of 1.33 \( \mu \).

The groups studied comprised 20 persons with lymphosarcoma, 10 with lymphatic leukemia, 3 with chronic myeloid leukemia and 125 with Hodgkin's disease established by biopsy and followed by us in our clinic up to 15 years. There were approximately the same number of males and females. Four per cent of the patients were younger than 20, 60 per cent were between 20 and 50 years, and the remainder were from 51 to 70 years old at the time the test was performed. Thirteen per cent of the patients had had the disease less than one year; 28 per cent from 1 to 5 years; 25 per cent from 6 to 10 years; 7 per cent from 11 to 16 years. At the time the test was performed, all degrees of clinical activity, a diversity of symptoms, and every stage of the disease were represented. A fair number of patients had been in remission up to 10 years and appeared to have arrested disease. This made it possible to attempt a correlation of electrophoretic mobility with the broad diversity of clinical findings so characteristic of Hodgkin's disease. Numerous laboratory tests were performed, or had been performed, on most of these patients (serum electrophoresis, properdin, complement and ribonuclease determination, hematologic studies, etc.) and correlation of these also was attempted.

Results

Table 1 gives mean mobility values for normal persons, pregnant women and patients with lymphoma. It will be observed that mobility of red cells of persons in the second and third groups was below normal, being lower in the third than in the second. Table 2 shows that mobility of all but one patient with Hodgkin's disease was below normal.

In Hodgkin's disease the value was low regardless of age, sex, age at onset, duration of disease, current intensity or activity of the disease, state of nutrition, presence or absence of fever. Patients in remission 9-12 years from onset of disease still exhibited the same degree of slowing as did all other patients. Also, there was no correlation with the presence or absence, type or severity of anemia or with the C-reactive protein test. There was very frequent association of reduced mobility and rapid sedimentation rate. However, the data did not justify the conclusion that this linkage was due to the same basic mechanism, since the sedimentation rate of two patients was normal despite low mobility values.

In order to see whether or not low mobility values of lymphoma patients remained constant, two to ten determinations were made on each of 50 patients
Table 1.—Red Cell Mobility of Normal Persons, Pregnant Women and Patients with Lymphoma

<table>
<thead>
<tr>
<th>pH</th>
<th>9.0</th>
<th>7.0</th>
<th>5.5</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (200)</td>
<td>1.33 ± .05*</td>
<td>1.27 ± .05</td>
<td>1.16 ± .04</td>
<td>1.08 ± .08</td>
</tr>
<tr>
<td>Pregnancy (100)</td>
<td>1.21 ± .02</td>
<td>1.13 ± .03</td>
<td>1.06 ± .03</td>
<td>1.00 ± .03</td>
</tr>
<tr>
<td>Hodgkin's disease (125)</td>
<td>1.13 ± .11</td>
<td>1.02 ± .10</td>
<td>0.95 ± .14</td>
<td>0.84 ± .17</td>
</tr>
<tr>
<td>Lymphatic leukemia (10)</td>
<td>1.09</td>
<td>1.08</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>Myeloid leukemia (3)</td>
<td>1.09</td>
<td>1.12</td>
<td>1.02</td>
<td>0.87</td>
</tr>
<tr>
<td>Lymphoma (20)</td>
<td>1.03</td>
<td>1.06</td>
<td>0.97</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Standard deviation.

Table 2.—Number of Normal Persons, Pregnant Women and Hodgkin's Disease Patients in Various Ranges of Red Cell Mobility at pH 9.0

<table>
<thead>
<tr>
<th>Mobility in Microns</th>
<th>121 Normal Persons</th>
<th>100 Pregnant Women</th>
<th>101 Hodgkin's Disease Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67-0.87</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>0.90-0.98</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>1.00-1.12</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>1.14-1.19</td>
<td>0</td>
<td>36</td>
<td>43</td>
</tr>
<tr>
<td>1.21-1.23</td>
<td>1</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>1.26</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1.28-1.35</td>
<td>112</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1.38</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data is presented for intervals of 2 weeks to 18 months, for 3 years. Values remained low and in general the variation was within the range of experimental error. In several instances there was a significant rise shortly after nitrogen mustard, Meticorten, x-ray therapy, transfusion or splenectomy, but values had returned to the original low level when the test was repeated a week or more later. For other patients the same types of therapy did not produce even a transitory rise in mobility.

Sera of 20 patients with Hodgkin's disease were next tested for the slowing factor. Since the results were uniformly the same for all patients, data are presented in table 3 for one patient with this disease, also for one patient each from various other disease categories. The table shows clearly the contrasting reactions of the various slowing factors present in a variety of diseases. That of cancer and lymphoma fall into one group; pregnancy, pneumonia, chronic cervicitis, myocardial infarct, cerebral infarct into another. The type of reaction pattern observed when no slowing factor is present is illustrated by the patients with fatty liver. Column "a" shows that sera of patients with lymphoma and cancer have a slowing factor whose maximum effect is to reduce the mobility of normal red cells from 1.33 \( \mu \) to 0.89 \( \mu \), while the maximum effect of the factors of all the other diseases enumerated is a retardation to 1.14\( \mu \); column "b" shows that at 1:8 dilution, activity of the cancer and lymphoma factor is retained, while in other diseases it is lost at this dilution; column "c"
Table 3

<table>
<thead>
<tr>
<th>Characteristic Mobility</th>
<th>4 ml. serum undiluted</th>
<th>4 ml. serum diluted 1:8</th>
<th>Serum heated 50° C.</th>
<th>4 ml. test serum + ca. cells</th>
<th>Normal serum + test cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer of lip</td>
<td>1.14</td>
<td>0.89</td>
<td>0.89</td>
<td>0.89</td>
<td>1.21</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>1.12</td>
<td>0.89</td>
<td>0.89</td>
<td>0.89</td>
<td>1.21</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>1.23</td>
<td>1.14</td>
<td>1.35</td>
<td>1.33</td>
<td>1.20</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1.21</td>
<td>1.14</td>
<td>1.33</td>
<td>1.35</td>
<td>1.18</td>
</tr>
<tr>
<td>Chronic cervicitis</td>
<td>1.17</td>
<td>1.14</td>
<td>1.35</td>
<td>1.35</td>
<td>1.18</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1.19</td>
<td>1.14</td>
<td>1.35</td>
<td>1.35</td>
<td>1.18</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>1.14</td>
<td>1.14</td>
<td>1.30</td>
<td>1.35</td>
<td>1.17</td>
</tr>
<tr>
<td>Fatty liver</td>
<td>1.33</td>
<td>1.33</td>
<td>1.33</td>
<td>—</td>
<td>1.33</td>
</tr>
<tr>
<td>Normal persons*</td>
<td>1.33</td>
<td>1.33</td>
<td>1.33</td>
<td>1.33</td>
<td>1.33</td>
</tr>
</tbody>
</table>

a) 4 ml. serum, undiluted + 0.01 ml. RBC with mobility of 1.33 μ
b) 4 ml. serum diluted 1:8 + 0.01 ml. RBC with mobility of 1.33 μ
c) Serum heated to 50 C. + 0.01 ml. RBC with mobility of 1.33 μ
d) 4 ml. test serum + 0.01 ml. cancer cells with mobility of 1.18 μ
e) 2.5 ml. normal serum + 0.01 ml. test cells

*Normal serum was tested against cancer cells with mobility of 1.19 μ. Note acceleration.
†Normal sera lost ability to accelerate cancer cells at 1:128 dilution.

Discussion

It has been established that microelectrophoretic mobility of the red blood cell is significantly retarded in 99 per cent of cases of Hodgkin’s disease, and cells from all other lymphomas tested were also slowed. The responsible factor in all 25 instances of Hodgkin’s disease tested and in the 10 instances of other lymphoma was identified as the “0.89” factor. This same factor has previously been identified in the blood of some 200 persons with other forms of malignant neoplasm. From this it may be concluded that all forms of cancer and lymphoma resemble each other with respect to this one factor.

Since all patients we studied had established disease at the time the test was performed, there is no way of telling how soon the factor appears. The answer may be forthcoming from animal investigation now under way. Studies of AK leukemic mice obtained from Gross show that the change is present at 4 months of age, a time at which leukemia has not yet established itself anatomically.8 The same is true of Tannenbaum’s strain of C3H mice in which mammary tumor occurs in 100 per cent of animals. In this strain we found slowing established by the 4th month, at which time mammary tumor had not yet appeared. These mice will be followed until tumors make their appearance.8

In Hodgkin’s disease, once the factor appears it persists to the end, however long the disease may remain quiescent. It was present in two patients in complete clinical remission for 10 years, and we have made the same observation in cancer patients free of recurrence 6 to 7 years after complete removal of the tumor. It was found at autopsy in an individual completely free of detectable recurrence of cancer.
As to the specificity of the 0.89 factor, it has been demonstrated in the blood serum of practically all cancer and lymphoma patients tested. It has been found also in infectious mononucleosis. Preliminary studies show, however, that whereas in the former it has been shown to have the mobility of alpha-1 protein, in infectious mononucleosis the mobility lies in the alpha-2 region.9

Concerning the chemistry of the slowing factors we know very little. We do know that they are heat sensitive—the 1.14 factor more so than the cancer 0.89 factor.6 The factors also withstand freezing, thawing and freeze-drying.

The identification of the 0.89 factor may make possible its utilization in differential diagnosis of obscure cancer. If the technic can be simplified, its use as a screening test for the cancer process should be considered. To this end, efforts are under way to find out whether the factor is antigenic; if so, immunologic identification would be practicable.

An important question raised by the 0.89 factor concerns its role in the disease in which it is found. Is it related to cause or defense, or is it a patho-metabolic byproduct? The answer will, we believe, be forthcoming from continued investigation.

**Summary and Conclusions**

1) A variety of pathologic conditions retard the electrophoretic mobility of the red blood cell.

2) Two retarding factors have been found in the blood serum, one that reduces the mobility of the red cell from normal (1.33 μ/cm/sec/volt) to 0.89 μ, and one that reduces it to 1.14 μ.

3) The “0.89” factor and the “1.14” factor can be differentiated and identified.

4) The 0.89-factor has been found in the serum of 99 per cent of patients with cancer and Hodgkin’s disease studied and has been lacking from the serum of all persons with benign tumor.

5) The 0.89-factor has also been found in patients with infectious mononucleosis.

6) A few physical characteristics that enable differentiation of the two slowing factors are described.

7) Clarification of the role played by the 0.89-factor in malignant neoplasm is deemed important.

8) Determination of the presence or absence of the 0.89-factor in the blood serum may become of value in differential diagnosis of malignant neoplasm.

**Summario in Interlingua**

1) Varie conditiones pathologic retardar le mobilitate electrophoretic del erythrocytos.

2) Esseva trovate in le sero sanguinee duo factores retardatori. Le un reduce le mobilitate del erythrocytos ab le norma de 1,33 μ/cm/sec/volt ad 0,89 μ, le altere ad 1,14 μ.

3) Le factor “0,89” e le factor “1,14” pote differentiaye e identificaye.

4) Le factor “0,89” esseva trovate in le seros de 99 pro cento del patientes con cancere e morbo de Hodgkin studiate e esseva absente ab le sero de omne subjectos con benigne tumores.
5) Le factor "0,89" esseva etiam trovate in patientes con mononucleosis infectiose.
6) Es describite certe caracteristicas physic que permitte le differentiation del duo factores retardatori.
7) Es reguardate como importante le clarification del rolo del factor "0,89" in neoplasma maligne.
8) Determination del presentia o absentia del factor 0,89 in le sero sanguinee es possibilemente de valor in le diagnose differential de neoplasma maligne.

REFERENCES

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