Electrophoretic Patterns of Human Leukocyte Alkaline Phosphatase in Polycythemia Vera

By Marion F. Baxter and Ralph F. Reinfrank

Electrophoretic patterns of leukocyte alkaline phosphatase have been examined in seven normal and four erythrocytosis control subjects and in eight patients with polycythemia vera to determine whether changes occurred after chemotherapy. One major fast band and two narrow slow and intermediate bands of enzyme activity were found in both groups. There was no significant difference between the normal and erythrocytosis subjects. Mean values for the control group revealed that the slow band extended from the origin to 0.5 cm, the intermediate band from 0.5 to 1.0 cm, and the fast band from 1.5 to 5.0 cm, with the fast peak apex located 3.4 cm from the origin. In the eight polycythemia vera patients studied both before and after chemotherapy, there was a consistent and significant shift of the fast band from a location extending from 0.9 to 4.2 cm to a near normal position of 1.4 to 4.8 cm with a shift of the fast peak apex from 1.9 to 3.3 cm from the origin, after a remission was obtained. The slow and intermediate bands did not appear to be influenced by therapy.

In 1966, Trubowitz and Miller described for the first time an abnormal leukocyte alkaline phosphatase electrophoretic pattern in polycythemia vera, but made no mention of the stage of the disease or therapy. Lyons et al. have reported the existence of three isoenzymes in polycythemia patients treated previously with $^{32}$P, and subsequently these authors proposed that the patterns probably varied with the clinical status of the patient.

The present study was undertaken to characterize further the electrophoretic patterns of the leukocyte enzyme from patients with polycythemia vera and to determine if changes occurred with chemotherapy.

Materials and Methods

Peripheral blood leukocytes were obtained from seven normal subjects, four patients with erythrocytosis, and from eight patients with polycythemia vera both before and after chemotherapy. The diagnosis in the polycythemia vera group was based on the presence of an elevated hematocrit, increased red cell volume, hypercellular marrow with a pancytopenia.
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tosis, and peripheral blood leukocytosis and thrombocytosis. The leukocyte alkaline phosphatase histochemical score determined by the method of Rutenberg et al. was elevated. Seven of the eight patients had splenomegaly. Patients were considered in remission when the hematocrit, white blood count, and platelets stabilized at normal levels after chemotherapy without requiring phlebotomy. Red cell mass determinations and marrow aspirations were not repeated while in remission. The term “relapse” was used to indicate patients who, after a remission period, reverted to their original abnormal peripheral blood status. In the erythrocytosis group, three subjects had the relative type due to a decrease in plasma volume and one had the absolute type with hypoxemia secondary to chronic pulmonary disease. In this group, splenomegaly, leukocytosis, and thrombocytosis were absent and the LAP score was normal.

After blood collection in heparinized containers, separation of leukocytes was accomplished by the rapid red cell sedimentation method of Skoog and Beck, with residual erythrocyte contamination eliminated by the hypotonic lysis method of Fallon et al., as modified by Trubowitz and Miller. After suspension in an activating buffer recommended by Boyer, the leukocytes were either frozen and stored at -20°C or homogenized as described by Robinson, Pierce and Goldstein.

Butanol extraction of the enzyme was carried out by the method of Morton, as modified by Trubowitz et al. and by Robinson et al.

Vertical starch gel electrophoresis was done using the method of Warnock, and staining of the zymogram by the method of Taswell and Jeffers. Pattern tracings were made with a Densicord Recording Electrophoresis Densitometer (Photovolt Corp.). The distance of migration and the total width of each band and the apex of the fast peak were recorded as centimeters from the point of origin. This apical point did not necessarily coincide with the midpoint of the fast band.

Statistical analysis of the data was done using the t test of significance between two sample means.

Results

One wide and two narrow bands of leukocyte alkaline phosphatase activity were found in both the polycythemia vera and the control groups. The terms “major” and “minor” bands, respectively, were chosen to describe the relative width of these enzyme bands, and “slow,” “intermediate,” and “fast” were chosen to denote the relative mobilities of the enzyme bands from the origin. No attempt was made to equalize enzyme levels or to relate these levels to the number of leukocytes in the suspension, because neither dilution nor concentration of any extract produced changes in electrophoretic characteristics, only intensity of staining. As previously demonstrated, the total enzyme content by biochemical assay, like that of the histochemical score, was higher in the polycythemia vera patients than in the control group.

Control Subjects

Figure 1 illustrates the electrophoretic patterns obtained in this control group. The minor intermediate band is discernible in most normal patterns only with densitometry analysis. By visual means alone, it appears to merge with the slow minor band at the origin.

Table 1 shows the location of the three bands of enzyme activity, the total width of each band, and the location of the apex of the peak of the major fast band. There is no significant statistical difference between the hematologically normal and the erythrocytosis subjects. The mean values obtained for this group show that the slow minor band extends from the origin to 0.5 cm, the
intermediate band extends from 0.5 cm to the 1.0-cm mark, and the major fast band is located between 1.5 and 5.0 cm, with the apex of this fast band peak located 3.4 cm from the origin.

Table 1.—Analysis of Leukocyte Alkaline Phosphatase Electrophoretic Patterns of Eleven Control Subjects

<table>
<thead>
<tr>
<th>Control Subjects</th>
<th>Bands of Enzyme Activity (cm From Origin)</th>
<th>Fast Peak Apex (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slow</td>
<td>Intermediate</td>
</tr>
<tr>
<td>1. Normal</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>2. Normal</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>3. Erythrocytosis</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>4. Erythrocytosis</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>5. Normal</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>6. Normal</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>7. Normal</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>8. Normal</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>9. Normal</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>10. Erythrocytosis</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>11. Erythrocytosis</td>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Normal Controls (7 subjects)
Mean values
± SE
0 ± 0.1 0.6 ± 0.2 1.5 ± 0.3 5.1 ± 0.3
3.4 ± 0.3

Erythrocytosis Control (4 subjects)
Mean values
± SE
0 ± 0.1 0.4 ± 0.3 1.4 ± 0.5 4.9 ± 0.2
3.3 ± 0.3

Combined Group (11 subjects)
Mean values
± SE
0 ± 0.1 0.5 ± 0.3 1.5 ± 0.4 5.0 ± 0.2
3.4 ± 0.2

Fig. 1.—Leukocyte alkaline phosphatase patterns on starch gel electrophoresis of seven normal (*) and four erythrocytosis subjects (†). Distance and direction of migration from origin indicated in centimeters at left and band locations at right.
Table 2.—Effects of Chemotherapy on the Electrophoretic Patterns of Leukocyte Alkaline Phosphatase in Polycythemia Vera

<table>
<thead>
<tr>
<th>Case</th>
<th>Therapy</th>
<th>Fast Band (cm From Origin)</th>
<th>Apex of Fast Peak (cm)</th>
<th>Change in Fast Peak Mobility (cm)</th>
<th>Hematologic Data*</th>
<th>LAP Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>Myleran</td>
<td>1.0</td>
<td>5.7</td>
<td>1.3</td>
<td>5.2</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>Leukeran</td>
<td>1.2</td>
<td>4.6</td>
<td>2.5</td>
<td>6.0</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>Myleran</td>
<td>1.2</td>
<td>4.3</td>
<td>1.3</td>
<td>5.5</td>
<td>1.9</td>
</tr>
<tr>
<td>4†</td>
<td></td>
<td>1.3</td>
<td>5.0</td>
<td>1.1</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.5</td>
<td>4.4</td>
<td>1.7</td>
<td>5.8</td>
<td>1.8</td>
</tr>
<tr>
<td>6†</td>
<td></td>
<td>1.3</td>
<td>3.5</td>
<td>1.2</td>
<td>4.0</td>
<td>1.7</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.7</td>
<td>2.9</td>
<td>1.4</td>
<td>3.7</td>
<td>1.4</td>
</tr>
<tr>
<td>8†</td>
<td></td>
<td>0.5</td>
<td>3.5</td>
<td>1.0</td>
<td>3.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Mean Values:

|         | 0.9 ± 0.2 | 1.4 ± 0.4 | 1.9 ± 0.3 | 0.9 ± 0.1 |
|         | ± 0.3     | ± 0.9     | ± 0.5     | ± 0.4    |

* Hct as per cent PCV. WBC and platelets per cu mm.
† No prior chemotherapy.
†† Incomplete remission.
↑ = increased platelet estimate on smear.
N = normal estimate.
ND = not done.
Polycythemia Vera Patients

Of this group five were in hematologic relapse after prior $^{32}$P therapy. In the remaining three patients, the diagnosis had just been established.

As seen in Table 2, although the peripheral blood values returned to normal in all patients except No. 6, who did not achieve a complete remission, the elevated LAP score seen before chemotherapy did not return to normal range in any of the six patients in whom the score was repeated after therapy.

The mean values for the location of the slow and intermediate bands of alkaline phosphatase activity were essentially normal and not influenced significantly by chemotherapy. However, the fast band showed a definite shift from a more cathodal pretreatment position extending from 0.9 cm to 4.2 cm to a near normal location of 1.4 to 4.8 cm from the origin after chemotherapy. The fast band apical peak of enzyme activity shifted from the mean pretreatment location of 1.9 cm to a normal location of 3.3 cm from the point of origin.
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origin after therapy ($p < 0.01$) with a mean shift of $+1.4$ cm for the whole group. Figure 2 illustrates the electrophoretic patterns obtained in this group.

DISCUSSION

It has been determined that, in man, leukocyte alkaline phosphatase activity is first observed at the myelocyte stage, increasing with cell maturation. As the granulocyte matures, specialized membrane-limited cytoplasmic organelles appear, termed specific granules, which have been demonstrated to contain numerous hydrolytic enzymes characteristic of lysosomes, among which is alkaline phosphatase.

Electrophoretic variants of leukocyte alkaline phosphatase were first demonstrated by Robinson, Pierce and Goldstein in normal subjects who had a faint band at the origin, a second band located from 0.5 to 1.0 cm, and a third band located between 3 to 3.5 cm, with the midpoint of the peak at 3.25 cm. Abnormal patterns were also described in both acute and chronic myelogenous leukemia, and the pattern differences observed were considered to be due to charge differences on the normal enzyme components. The present study reveals mean values for the control group very similar to the normal enzyme pattern described by these authors.

In polycythemia vera, Trubowitz and Miller found that the two slower bands were similar to the normal pattern in location, but that the third major band had shifted to a more cathodal position, with the midpoint located at about 2 cm from the origin. The similarity of this pattern in polycythemia to that found in untreated chronic myelogenous leukemia by Robinson, Pierce and Goldstein raised the possibility that these patterns might represent a similar molecular form of leukocyte alkaline phosphatase in these two diseases.

Lyons, Weaver and Beck proposed that either a selective production of leukocyte clones in normal individual due to an altered environment could be responsible for the changing gel patterns or that the variations are produced by an alteration of synthesis or masking of activity of a particular isoenzyme. In their study, two patients with treated polycythemia vera had bands of enzyme activity in the normal position for their particular electrophoretic system, equivalent to those described by Robinson et al.

The data reported here confirm that there is a cathodal shift of the major fast band of leukocyte alkaline phosphatase activity in untreated or relapsed polycythemia vera. However, the slower mobility of the apex of this fast band peak of enzyme activity is a more consistent and significant finding. Treatment of either situation results in a shift of the fast band back toward a normal position, with the fast peak apex relocating in a normal position in most instances.

Patients with nonleukemic leukocytoses have also been found to have abnormal leukocyte alkaline phosphatase electrophoretic patterns, which are distinctly different from the abnormality described in polycythemia vera, and these findings will be reported in a separate communication.

The finding of significant differences in starch gel patterns of leukocyte
alkaline phosphatase in patients with polycythemia vera before and after chemotherapy offers evidence that some change has occurred in the enzyme which affects its electrophoretic mobility. At this time, it is not clear why the slow and intermediate bands show no consistent change in this study, although a striking change occurs in the fast band.

ACKNOWLEDGMENT

The technical assistance of Miss Joyce E. Jarosz, M.S., Mrs. Margaret Van Why, M.A., and Mrs. Janet R. Emmanuel, B.S., is gratefully acknowledged. The authors express thanks to Robert E. Correll, Ph.D., for his assistance with the statistical analyses.

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