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<td>Author(s)</td>
<td>Tomonaga, Masanobu; Watanabe, Bunro; Kamochi, Yasuro; Ozono, Noboru; Toyoda, Shigeki</td>
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<tr>
<td>Citation</td>
<td>Acta medica Nagasakiensia. 1960, 5(2-3), p.90-98</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1960-10-25</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/15435">http://hdl.handle.net/10069/15435</a></td>
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Leukocyte Alkaline Phosphatase in Hematological Disorders

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Received for publication July 16, 1960

Leukocyte alkaline phosphatase activity was studied biochemically and histochemically in various blood diseases. 1) N-APA is preferable to L-APA as a unit of biochemical measurement. 2) The low content of leukocyte AP in CGL was confirmed. The determination of leukocyte AP is useful in the diagnosis and treatment of CGL. 3) The low N-APA in CGL rises to normal as a rule after successful treatment and this normalization is consistent with the double cell population theory in leukemic processes. 4) The high content of leukocyte AP in acute leukemia, aplastic anemia, leukemoid reaction, idiopathic eosinophilia, myelosclerosis and infection may be explained by the hypothesis that it represents increased neutropoiesis. 5) A close correlation was confirmed between biochemical and histochemical results, indicating that histochemical methods are reliable and suitable for adoption as a routine test.

The important role of leukocytes in physiological and pathological metabolism of a living body is easily imagined from their numbers and varieties, and their morphology and chemical composition would be in close correlation with the metabolism in or outside of leukocytes. It is therefore valuable to study the chemical composition of leukocytes in hematological disorders, in which the changes of blood cells are predominant. Such studies have proceeded along two lines: biochemical and histochemical methods. Both of these have their own advantages and difficulties. The biochemical investigations have become reliable and exceedingly advanced in recent years, because of the progress of microanalytic methods and leukocyte separating procedures. The pertinent information is likely to be obtained where both methods are used conjointly on the same problems as DAMESHEK *1) emphasizes.

Although many substances of leukocytes such as glycogen, histamine, amino acids and inorganic salts have been investigated, the most actively studied substances are enzymes and more than thirty have been already demonstrated. Excellent reviews of biochemical studies have been published by VALENTINE *14), *15), *16), and LAWRENCE, *5).

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and of histochemical investigations by TAKEUCHI, WISLOCKI and RHEINGOLD.

The present report is concerned with leukocyte alkaline phosphatase (AP) in several hematological disorders which have been studied biochemically and histochemically in our laboratory.

METHODS

Leukocytes were separated by our dextran method previously reported. Biochemical determination of leukocyte AP was performed using VALENTINE and BECK’S method and leukocyte alkaline phosphatase activity (L–APA: mg. P liberated/hour/10" total white cells), neutrophil alkaline phosphatase activity (N–APA: mg. P liberated/hour/10" neutrophils) were calculated. TAKEUCHI’s method was used as a histochemical measurement of leukocyte AP and both AP percentage (AP–P) and AP index (AP–I: almost same with KAPLOW’s scoring method) were calculated.

RESULTS

The distribution of N–APA in normal persons and in those with various diseases is shown in Fig. 1 and mean values of L–APA, N–APA, AP–P and AP–I shown in Table 1.

Fig. 1. N–APA in normal persons and in those with various diseases. AGL: acute granulocytic leukemia, AML: acute monocytic leukemia, ALL: acute lymphocytic leukemia, CLL: chronic lymphocytic leukemia, CGL: chronic granulocytic leukemia.

※ 1. Leukocytosis after splenectomy.
※ 2. Hypoplasia after nitrogen mustard therapy.
### Table 1.

**Mean Values of Leukocyte AP in Normal Persons and in Those with Various Hematological Disorders**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>L-APA Min.</th>
<th>L-APA Max.</th>
<th>L-APA M ± m</th>
<th>N-APA Min.</th>
<th>N-APA Max.</th>
<th>N-APA M ± m</th>
<th>AP-P Min.</th>
<th>AP-P Max.</th>
<th>AP-P M ± m</th>
<th>AP-I Min.</th>
<th>AP-I Max.</th>
<th>AP-I M ± m</th>
</tr>
</thead>
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<tr>
<td>Normal</td>
<td>M</td>
<td>30</td>
<td>10.0</td>
<td>34.8 ± 21.8</td>
<td>26.4 ± 1.55</td>
<td>*1</td>
<td>*1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17</td>
<td>15.0</td>
<td>35.0 ± 23.6</td>
<td>30.9 ± 1.71</td>
<td>49.4</td>
<td>72.6 ± 2.27</td>
<td>0.65</td>
<td>1.02</td>
<td>0.82 ± 0.044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>47</td>
<td>10.0</td>
<td>35.0 ± 22.4</td>
<td>28.0 ± 1.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AGL</td>
<td>10</td>
<td>0.7</td>
<td>12.2</td>
<td>4.2 ± 1.15</td>
<td>54.3 ± 7.35</td>
<td>*2</td>
<td>*2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>10</td>
<td>1.6</td>
<td>12.2</td>
<td>4.8 ± 0.88</td>
<td>70.5 ± 14.5</td>
<td>62.0</td>
<td>99.0 ± 3.38</td>
<td>0.65</td>
<td>2.64</td>
<td>1.56 ± 0.212</td>
<td></td>
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</tr>
<tr>
<td>ALL</td>
<td>3</td>
<td>2.3</td>
<td>12.3</td>
<td>6.90</td>
<td>63.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGL</td>
<td>16</td>
<td>0.9</td>
<td>8.0</td>
<td>3.6 ± 0.38</td>
<td>5.7 ± 0.53</td>
<td>2.0</td>
<td>16.5</td>
<td>7.3 ± 1.31</td>
<td>0.02</td>
<td>0.16 ± 0.08</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CLL</td>
<td>2</td>
<td>16.6</td>
<td>20.2</td>
<td>18.4</td>
<td>60.2</td>
<td>54.1</td>
<td>83.1</td>
<td>93.5</td>
<td>88.3</td>
<td>1.84</td>
<td>1.58</td>
<td></td>
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<tr>
<td>Aplastic Anemia</td>
<td>3</td>
<td>44.2</td>
<td>87.5</td>
<td>65.6</td>
<td>110.5</td>
<td>96.5</td>
<td>97.5</td>
<td>97.0</td>
<td>2.13</td>
<td>2.60</td>
<td>2.31</td>
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<tr>
<td>Leukemoid Reaction</td>
<td>2</td>
<td>22.2</td>
<td>169.6</td>
<td>95.9</td>
<td>105.7</td>
<td>169.8</td>
<td>85.9</td>
<td>86.3</td>
<td>1.10</td>
<td>1.77</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloma</td>
<td>1</td>
<td></td>
<td></td>
<td>58.4</td>
<td></td>
<td></td>
<td>96.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.04</td>
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* 1 Mean value of ten normal persons
* 2 Mean value of nine cases
* 3 Mean value of nine cases

1. **Chronic granulocytic leukemia (CGL)**

The most striking finding in Fig. 1 is the extremely low value of N-APA in 16 cases of CGL, which is consistent with previous observations by Takeuchi, Wachstein, and Mitus histochemically and by Valentine, Kenny, and Wiltshaw biochemically. It is interesting that three patients, who were detected in the preclinical stage of their disease by the authors in a mass survey of atomic bomb survivors, had low N-APA in spite of a very small percentage of immature cells as Moloney's cases. The changes in N-APA in 12 cases of CGL after treatment are summarized in Fig. 2. These cases were classified into four groups according to the response to treatment.

In group 1 consisting of five cases, N-APA returned to normal levels after treatment and one of them showed temporarily a remarkable elevation of N-APA during a leukopenic phase induced by treatment and thereafter returned to normal. This N-APA value was accompanied by histaehemical increase of AP-P and AP-I. The remission was long as shown by the number (months) in brackets in Fig. 2. Relapse occurred in only one case and the decrease of N-APA occurred about two weeks before the appearance of immature cells and the rise of the leukocyte count. On the other hand, N-APA returned to normal later than the return of the normal blood picture when the remission occurred as shown in Fig. 3 a representative case.

Group 2 consisted of three mild cases. Two were detected by the atomic bomb mass survey. They were all treated as outpatients and discontinued taking medicine...
When subjective complaints disappeared. Accordingly slight abnormal blood pictures have remained and N-APA has showed a slight increase. If the medicine had been taken regularly, it would be expected that N-APA of this group would rise to the normal range as in group 1.

Group 3 consisted of two cases, which showed a complete remission in the blood picture and clinical signs and in which N-APA were always below the normal range. In compared this group with group 1 from various viewpoints, a noticeable difference was the fact that each case in group 1 was treated with busulfan at first, but two cases of group 3 had been treated with 6-MP repeatedly for over one year with incomplete remissions, and then complete remission with busulfan.

Group 4 consisted of two cases which were atypical. N-APA of the first case was abnormally high ever before treatment. The patient had a relative low leukocyte count, a very large spleen, numerous erythroblasts in the peripheral blood and an abnormally high platelet count (one million), suggesting a diagnosis of myelosclerosis with myeloid metaplasia. High N-APA values in most cases of myelosclerosis have
been reported by Valentine and Mitus. Although splenomegaly disappeared, the blood picture returned to normal and remission has continued for eight months by the treatment with busulfan, N-APA has remained high. The second case presented a typical picture of CGL in the beginning of the disease, showed initially hematological and clinical improvement with busulfan therapy, with accompanying elevation of N-APA, but before long there occurred an acute exacerbation with sudden fever, myeloblastosis beyond 50% and "hiatus leukemicus". The N-APA was elevated just as in acute leukemia in contrast to the low values of the usual advanced phases of CGL.

In Fig. 4 the relationship between leukocyte AP and the percentage of immature cells is shown. There is a close negative correlation between them (r = 0.72, t = 3.99, p < 0.01) indicating that N-APA is related to the severity of the disease.

2. Acute leukemia
The mean value of N-APA is significantly high in acute leukemia compared with the normal mean (p < 0.01) and no cases show low values below normal range as in CGL. The low L-APA seems to be due to the predominance of immature cells, mostly blast cells which contain only a little or no AP. This relation would be well understood when we know there is a positive correlation between neutrophil percentage and L-APA as shown in Fig. 5 (r = 0.59, t = 3.23, p < 0.01). No significant difference is noticed between N-APA of each type of acute leukemia. Furthermore, there is a close positive correlation between N-APA and immature cells (r = 0.55, t = 2.94, p < 0.01) as shown in Fig. 6, indicating that the more severe the disease is, the higher N-APA increases in the opposite direction of CGL.

3. Aplastic anemia
All three cases showed high L-APA and N-APA. Although high L-APA may have no special meaning because the percentage of neutrophils is moderately higher in aplastic anemia, comparing with acute leukemia which had low L-APA and high N-APA, N-APA of aplastic anemia seems to be higher than that of acute leukemia. This corresponds with the histochemical finding that O type with no staining reaction is very uncommon or absent in aplastic anemia, while relatively frequent in acute leukemia.

4. Other diseases
Two cases of chronic lymphocytic leukemia showed normal L-APA and high N-APA in contrast to CGL. Two cases of panperitonitis and 9 months pregnancy with leukemoid reactions had high L-APA and N-APA and the latter returned to normal after delivery of the baby. High N-APA were also noticed in a case of
multiple myeloma and three cases of idiopathic eosinophilia (greater than 50%). N-APA of Banti's disease were in lower levels of normal range, but elevated strikingly in the leukocytotic phase following splenectomy.

5. Relationship between biochemical and histochemical measurement

In Fig. 7. and 8 the relationship between the biochemical and histochemical determinations is shown. Close correlation is noticed between the log of N-APA and AP-P and AP-I (\( r = 0.92, t = 17.7, p < 0.001 \) and \( r = 0.87, t = 13.8, p < 0.001 \) respectively).

DISCUSSION

The precise knowledge of function, source and structure of leukocyte AP is not known. Therefore a discussion of the meaning of leukocyte AP changes in various diseases must be based on empirical findings and run the risk of useless speculation, but they may be diagnostically useful. In order to form a working hypothesis, it may be helpful to consider various possibilities.

TAKEUCHI reported that AP in neutrophils appears in promyelocyte, increasing thereafter in parallel with maturation, and that there is very little or none in eosinophils, lymphocytes and erythrocytes. The findings of other researchers in the histochemical field are substantially same. Therefore N-APA is more reliable as a unit of biochemical determination than L-APA. The close correlation between L-APA and neutrophil percentage in acute leukemia indicates this relationship.

Previous observations indicating a striking decrease of AP in CGL were confirmed.
This low N-APA in CGL is almost pathognomonic of the disease and has an important diagnostic meaning. How to explain the normalization of N-APA in CGL after successful treatment is very difficult. Although Dameshek, Valentine and Moloney have considered such normalization as exceptional, we want to consider the normalization as a rule from our experience above mentioned, and it would be a beneficial evidence for the hypothesis that there may be two normal and leukemic neutrophils-series in leukemia, which are identical morphologically, but distinct from each other in their biochemical composition. This double population theory has been also speculated upon by Valentine and Dameshek. The later normalization of leukocyte AP following that of the blood picture after the successful treatment of CGL indicates that the remaining number of leukemic neutrophils can be estimated precisely by the determination of leukocyte AP, that is not possible morphologically. Accordingly it is reasonable to continue treatment until N-APA returns to normal even after the disappearance of immature cells. In our cases, which were treated according to this principle, the remission periods were long. It is our standard belief that the determination of leukocyte AP would present a numerical and objective for discontinuation or maintenance of chemotherapy.

In contrast to the great interest directed to leukocyte AP in CGL and allied diseases such as myelosclerosis and polycythemia vera, the content of leukocyte AP in acute leukemia and chronic lymphocytic leukemia has been relatively neglected.

For example, Wachstein paid no special attention to the high AP scoring in acute leukemia described on his report. Based on the double cell-population theory, it is our belief that most mature neutrophils in acute leukemia belong to normal series and...
therefore have capacity to increase their AP content. From this viewpoint it is interesting that the correlation between N-APA and immature cells percentage, namely the severity of the disease, is reversed in CGL and acute leukemia. Moreover, the findings of (1) some neutrophils show a strong positive (Type 3) reaction in the beginning phase of CGL and seldom in the advanced phase, (2) AP staining of neutrophils becomes very strong rapidly just after an acute exacerbation of CGL, and (3) N-APA values in the leukopenic phase of CGL after treatment are very high as in the leukopenic phase of Hodgkin's disease after nitrogen mustard, are consistent with the two cell-population theory.

Can the high content of leukocyte AP in several diseases be explained monistically? The elevation of N-APA in infection is well known and complications of infection are often observed in acute leukemia and aplastic anemia. However it is impossible to explain the high N-APA in these diseases by infection alone. We would propose a hypothesis that the elevation of N-APA represents the hyperfunction of normal neutrophoiesis. Namely the high N-APA in infection, leukemoid reaction and leukocytotic stage after splenectomy may be due to general stimulation of neutrophoiesis. The high N-APA in acute leukemia, myeloma, eosinophilia and aplastic anemia may be results of compensatory hyperfunction of residual neutrophoiesic areas which are replaced with leukemic, eosinophilic and tumor tissue or survive in case of aplastic anemia. The high values in myelosclerosis may be due to hyperfunction of metaplastic neutrophoiesis.

The establishment of a new precise method to differentiate acute leukemia from aplastic anemia is urgently needed, because morphological methods are limited. In our experiment, N-APA in aplastic anemia has a tendency to be higher than in acute leukemia and the distribution of histochemical scoring has a different appearance in both diseases, although the number of cases is small. These results suggest the possibility that extensive biochemical and histochemical investigations of leukocyte may settle this problem.

Biochemical methods seem to be preferable to histochemical methods because they are more accurate. However the procedures of the former are in general complicated. Our results showing a close correlation between both methods are consistent with results of other authors* and indicate that histochemical methods may be used as a reliable semi-quantitative method. The histochemical methods have the advantages of simpler procedure, rapidity and less equipment. It is therefore desirable that they be adopted more widely, because of the significance of leukocyte AP changes in the diagnosis and treatment of blood diseases.

REFERENCES