Studies of the Changes in Leukocyte Alkaline Phosphatase Following Pyrogen Stimulation in Chronic Granulocytic Leukemia

By Pasquale E. Perillie

The alkaline phosphatase activity of the granulocytes (LAP) in patients with chronic granulocytic leukemia (CGL) usually is not increased following stimulation by either adrenocortical hormones or pyrogen, whereas the LAP of normal subjects and most patients with nonleukemic disorders shows a definite rise following similar stimulation. An occasional patient with well-documented CGL, however, will demonstrate an increased LAP during acquired infection or following either steroid or pyrogen administration.4

In nonleukemic subjects it has not been possible to determine with certainty the mechanisms leading to the rise in LAP which follows either acquired infections or the administration of corticosteroid or pyrogen. It is not clear whether the rise in LAP is due to the enhancement of enzyme synthesis by the mature circulating cells, or whether such stimuli exert their prime effect on the less mature marrow granulocyte precursors with subsequent delivery of such cells into the circulation. It has been suggested that the rise in LAP following pyrogen stimulation may be due to an initial selective sequestration of enzyme rich circulating cells followed by a subsequent redelivery of such cells into the circulation.

In addition to these basic problems it is uncertain whether in CGL the appearance of granulocytes with increased LAP denotes the presence of a dual population of normal and leukemic cells or represents a temporary alteration in an aberrant biochemical process of the leukemic cell.

This report concerns further attempts to elucidate the mechanism of LAP change in the granulocytes of a patient with CGL who transiently increased his LAP concomitant with an acute bacterial infection. Following recovery from the infection, gram negative endotoxin was injected in order to restimulate LAP. A histochemical method for demonstrating LAP seemed most suitable for the proposed study since it was felt that the appearance of granulocytes with increased LAP would serve as unique natural markers in the setting of the reduced enzyme activity present in the patient’s granulocytes prior to stimulation.

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MATERIALS AND METHODS

Routine hematologic investigations were carried out following accepted methods. Leukocyte alkaline phosphatase activity was measured by the histochemical method of Kaplow. A total of 500 mature neutrophilic granulocytes was counted and scored for enzyme activity on dual coverslip preparations of each sample of bone marrow and peripheral blood obtained. Results were expressed as the "score" or sum of the ratings per 100 neutrophilic polymorphonuclear granulocytes. In addition, after scoring, each coverslip was thoroughly scanned with the low power objective of the microscope to detect the presence of any cells showing alkaline phosphatase activity which might not have been included in the microscopic fields examined for scoring.

After establishing that the LAP scores of the circulating and marrow granulocytes were stable and reduced and that careful scanning of all slides revealed the absence of any granulocytes showing greater than 1+ alkaline phosphatase activity, baseline total and differential peripheral blood white cell counts and LAP scores of marrow and peripheral blood granulocytes were obtained followed by the intravenous injection of a single dose of 0.2 gamma Pyrexal, a lipopolysaccharide from Salmonella abortus equi. In the case of the peripheral blood similar studies were then obtained at hourly intervals for the first 7 hours following Pyrexal administration and then daily for the next 4 days. LAP scores of marrow cells were measured 24 and 48 hours and 4 days after pyrogen stimulation.

Cytogenetic studies of peripheral blood and marrow leukocytes were performed as follows: Cells were incubated with colcemide (5 x 10^-5M) and media 199 for 2 hours, treated with Hanks hypotonic salt solution, fixed in methyl acetic acid and air dried.

CASE HISTORY

A. F., a 43-year-old Negro male, was admitted to the WHVAH in May 1964 for drainage of an ischiorectal abscess. Except for a fever of 104.5°F. and findings in the anorectal area, physical examination was unremarkable. Admission hematologic data revealed: Hb 14.6 Gm. per cent, Hct 44 per cent, WBC 72,350/mm. with 51 per cent segmented neutrophils, 13 per cent bands, 7 per cent myelocytes, 11 per cent metamyelocytes, 10 per cent lymphocytes, 4 per cent monocytes, 3 per cent basophils, and 1 per cent myeloblasts. Platelets were 530,000/mm. Bone marrow aspiration revealed marked myeloid hyperplasia with an increased number of basophils and was considered consistent with the diagnosis of CGL. However, when the LAP score of the peripheral blood was found to be elevated at 110 (normal 10-75), some doubt arose as to the specific hematologic diagnosis. The presence of increased numbers of basophils in the peripheral blood was considered to be more consistent with a myeloproliferative disorder rather than with a leukemoid reaction, but the absence of anemia or splenomegaly and the elevated LAP made the diagnosis of CGL questionable.

Recovery from infection was prompt, and serial measurements of LAP of peripheral blood granulocytes demonstrated a progressive decline so that by the sixth hospital week the LAP score had fallen to zero. Cytogenetic studies of peripheral blood and marrow cells at that time revealed the presence of the Ph1 chromosome in all readable metaphases, thus firmly establishing the diagnosis of chronic granulocytic leukemia (Fig. 1). No antileukemic therapy was instituted so as to carry out the studies employing Pyrexal stimulation described above.

The patient was discharged from the hospital in June 1964 and followed in the hematology outpatient clinic. During the next 6 months progressive splenomegaly and anemia developed associated with a further rise in the total white cell count. Accordingly, he was started on busulfan therapy in January 1965. Shortly after commencing busulfan therapy he was readmitted to the hospital with fever (103°F.), cough and shortness of breath. Chest x-ray revealed a right lower lobe pneumonia. The total white cell count on admission was 140,000 with 40 per cent segmented neutrophils, 10 per cent bands, 10 per cent promyelocytes.
10 per cent myelocytes, 15 per cent metamyelocytes, 10 per cent lymphocytes, 3 per cent monocytes and 2 per cent basophils. LAP score of the peripheral blood was reduced with a score of 4 and remained low or absent during the remainder of that hospitalization. The patient responded to antibiotics and was discharged. He expired in December 1965 during an acute blastic crisis of his leukemic process.

Results

As can be seen from Figure 2 a definite rise in the LAP of the morphologically mature marrow granulocytes was noted 24 hours after pyrogen stimulation while no significant change in the LAP of circulating cells was noted until 24 hours later or 48 hours after pyrogen stimulation. In both the marrow and peripheral blood, all of the enzyme activity was accounted for by approximately 25 per cent of the total cells scored. Enzyme activity was demonstrable only in the mature neutrophilic granulocytes (band and polymorphonuclear) of the peripheral blood or bone marrow.

Discussion

The results of the present studies demonstrate that in the patient under discussion the increased LAP which developed following pyrogen administration appeared initially in the late marrow granulocyte precursors and was followed within 24 hours by the appearance of a similar population of cells in the peripheral blood. The absence of circulating granulocytes containing demonstrable LAP both prior to pyrogenic stimulation and at a time when such cells
were demonstrable in the marrow suggests that the neutrophils with increased alkaline phosphatase were probably derived from the marrow. Consistent with this interpretation is the known mechanism of action of administered endotoxin. Perry et al.9 and Athens et al.10 have shown that endotoxin administration produces an absolute increase in the size of the total circulating granulocyte pool mainly by promoting the delivery of maturing marrow granulocyte elements into the circulation. Of additional significance was the observation that the increased cellular enzyme activity which appeared following pyrogenic stimulation was confined to approximately 25 per cent of polymorphonuclear leukocytes scored in the bone marrow and peripheral blood, suggesting that the double population of enzymatically active cells which ultimately appeared in the peripheral blood probably originated in the marrow. Kenny and Maloney5 and Rosen and Nishiyama11 have also noted that the increased LAP levels which developed in their patients with CGL following infection were accounted for by a small number of the total cells scored for such activity.

The question of whether the granulocytes in CGL are capable of spontaneously increasing their LAP or whether such an occurrence in CGL implies the presence of a separate population of nonleukemic granulocytes cannot be answered directly by the present study. While the demonstration of the Ph1 chromosome in all readable metaphases in the present patient and the patient of Rosen and Nishiyama would be more consistent with a single leukemic population hypothesis, most of the remaining data available from the present study and from similar reported cases would seem to favor the dual population hypothesis.5-11

A significant rise of LAP in CGL patients following infection or the administration of steroid or pyrogen, although well documented, is still quite unusual. It is therefore of interest that most CGL patients so described have at the time
been atypical in that they were either in complete drug-induced remissions of their disease or more commonly, like the patient reported here, were in an apparently early stage of their disease without anemia or splenomegaly.\textsuperscript{2-4} It seems logical to assume that such patients would be likely to have foci or normal myeloid tissue remaining within their marrow. Further, if this were so, one would expect that as the disease advanced in such patients they would lose their ability to raise their LAP in response to infection or other forms of LAP stimulation. In line with this reasoning was the observation that our patient was unable to raise his LAP when he developed an infection at a time when his leukemic process appeared to be well advanced.

In most normal individuals following infection and steroid or pyrogen administration, the majority of mature neutrophilic granulocytes become strongly positive for LAP. The appearance of a small number of enzymatically active cells following pyrogen stimulation in our patient and those of Kenny and Moloney and Rosen and Nishiyama is therefore also suggestive that a dual population of normal and leukemic granulocytes best explains the rise of LAP seen in such patients.\textsuperscript{5-11}

**Summary**

Studies of changes in leukocyte alkaline phosphatase (LAP) in a patient with chronic granulocytic leukemia (CGL) following infection and pyrogenic stimulation revealed that increased LAP appeared in circulating mature neutrophilic forms 24 hours later than in similar cells of the marrow, suggesting that the cells containing increased LAP originated in the marrow. It was reasoned that the LAP responsiveness in CGL probably requires the presence of foci of normal myeloid tissue.

**Summario in Interlingua**

Studios del alterationes in le phosphatase alcalin del leucocytos in un patiente con chronic leucemia granulocytic post infection e stimulation pyrogenic revelava que un augmento del phosphatase alcalin del leucocytos appareva in circulante formas neutrophilic matur 24 horas plus tarde que in simile cellulas del medulla, un facto que suggestiona que le cellulas continent un augmento de phosphatase alcalin habeva lor origine in le medulla. Esseva arguite que le responsivitate a phosphase alcalin leucocytic in chronic leucemia granulocytic require probahilemente le presentia de focos de normal tissu mycloide.

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