Chronic Granulocytic Leukemia Complicated by Ulcerative Colitis: Elevated Leukocyte Alkaline Phosphatase and Possible Modifier Gene Deletion

By Robert B. Rosen and Raymond L. Teplitz

LEUKOCYTE ALKALINE PHOSPHATASE (LAP) determinations have proved of clinical value in the differential diagnosis of certain myeloproliferative disorders. Low or absent enzyme activity of mature neutrophils is characteristic of chronic granulocytic leukemia, in contradistinction to the usual high levels in polycythemia vera and myelofibrosis with myeloid metaplasia. There are also a number of other conditions in which LAP may be abnormal, one of the more notable, from a theoretical standpoint, being the elevation lately demonstrated in mongolism.

Although the significance of such deviations remains obscure recent cytogenetic findings have fostered considerable speculation about the control of LAP production. In this regard the small acrocentric chromosome 21 has occupied a central position following the discoveries of its triplication (trisomy) in mongolism and diminution (Philadelphia or Ph chromosome) in chronic granulocytic leukemia. From correlations between these and the respective LAP abnormalities has emerged the hypothesis that the phosphatase deficiency in chronic granulocytic leukemia may be the result of loss (presumably by deletion) from chromosome 21 of a segment containing the gene(s) responsible for LAP synthesis. Conversely, in mongolism the heightened LAP activity could be attributable to the supernumerary chromosome 21 and a surfeit of functioning genes.

However, a simple gene-dose interpretation of this nature cannot easily be reconciled with some pertinent facts, as has been ably underscored by King and her co-workers.

In the following report of chronic granulocytic leukemia in a boy with concurrent ulcerative colitis, LAP was greatly elevated in spite of the presence in his bone marrow of the typical minute Ph chromosome. Only after the colitis entered a quiescent phase did the initial enzyme activity decline to the low levels compatible with chronic granulocytic leukemia. This lability of LAP offers additional evidence against the view that loss of a structural gene from chromosome 21 is the basis for the diminished alkaline phosphatase content of neutrophils in chronic granulocytic leukemia.

METHODS

Standard hematologic procedures were employed.

Cytogenetic analysis was carried out on the bone marrow aspirate by a modification of the direct method of Sandberg et al.

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LAP determinations were performed by the semiquantitative histochemical technic of Kaplow.\textsuperscript{11} Capillary blood smears were fixed within an hour and stained no later than 3 hours after preparation. A minimum of 300 neutrophils were counted from duplicate thin blood films by the same observer (R. B. R.). Positive controls (children with infection or pregnant women) were run simultaneously. Negative controls (other patients with chronic granulocytic leukemia or blood smears inactivated by boiling in water for 1 minute) were usually also included. Results were expressed as the "score" or sum of the ratings per 100 polymorphs. In this laboratory values ranging from 20–80 are obtained in apparently healthy adolescents and adults by this method.

**Case Reports**

M. McK., a 15-year-old white male, was referred to the City of Hope Medical Center in December, 1963. He had been well until about 3 weeks earlier when he started feeling tired and complaining of abdominal "fullness." During the week prior to admission, he had fever and profuse watery diarrhea. There had also been a 10-pound weight loss and increasing pallor. Several days before his initial visit he entered another hospital and was found to have a hemoglobin of 7.1 Gm. per cent and a leukocyte count of 162,000/mm.\textsuperscript{3} Bone marrow examination was consistent with chronic granulocytic leukemia. Following blood transfusion, he was transferred to this Pediatric Clinic. By then, the abdominal pain and diarrhea had completely subsided.

His past health had been excellent. He had had diagnostic x-rays of the hand, knee, neck, and back between 1959 and 1962 in connection with athletic injuries. Intrauterine or neonatal exposure to irradiation was denied. The family history was not medically significant.

On admission, the patient weighed 130 pounds and was 68 inches tall. His mucous membranes were pale and the spleen tip was barely palpable. He had no abdominal tenderness, purpura, lymphadenopathy, or hepatomegaly. The hematocrit was 34 per cent and white blood cell count 173,700/mm.\textsuperscript{3} Representative hematologic data are summarized in table 1. Bone marrow aspiration yielded a hypercellular specimen displaying intense activity of the myeloid series with an orderly maturation sequence. Basophilia and eosinophilia were prominent, erythroid precursors diminished and megakaryocytes increased. The serum iron was 36 \textmu g. per cent, iron binding capacity 220 \textmu g. per cent, uric acid 10.2 mg. per cent, and total protein 7.0 Gm. per cent with a normal electrophoretic pattern.

Busulfan, 4 mg. daily, was instituted. However, when the initial high LAP scores of 160–180 were verified a few days later, therapy was halted pending clarification of these apparently anomalous results. Shortly after his clinic visit, because of resumption of diarrhea and appearance of blood and pus in the stools, a barium enema was performed. This showed tubulation of the large bowel and serration of the colon extending from above the cecum through the rectosigmoid junction. Sigmoidoscopy and biopsy revealed diffuse ulceration, hemorrhage, and intense inflammation.

He was treated with ferrous sulfate, a low residue diet, multivitamins, Pro-Banthine, and Azulfidine. His general condition improved progressively with decreased frequency of stools, steady weight gain, and correction of anemia. The relationship between serial LAP scores and total leukocyte counts is shown in figure 1.

In April, 1964 the Philadelphia chromosome was demonstrated in all evaluable metaphase plates prepared by one of us (R. L. T.) directly from his bone marrow (fig. 2). On repeat sigmoidoscopy the following month, the mucosa grossly appeared near-normal; histologically, renewal of the epithelium and minimal infiltration with neutrophils were noted.

By June, 1964 the patient was virtually asymptomatic. He weighed 139 pounds and was having 2–3 bowel movements daily, only one of which contained any mucus. However, the spleen had enlarged and the leukocyte count and immature granulocytes in the peripheral blood had risen (table 1). He was started on a course of busulfan in...
Fig. 1.—Leukocyte alkaline phosphatase scores and total white blood cell counts during treatment for ulcerative colitis. The size of the spleen, not shown here, gradually increased from 0.5 to 4-5 cm. below the left costal margin over this period.

July, 1964 and has since entered remission. There has been no exacerbation of the colitis and his general health remains excellent.

COMMENT

Although from the outset the blood and bone marrow of this patient were easily consistent with chronic granulocytic leukemia, the minor degree of splenomegaly and, above all, the increased LAP activity were disturbing features. After the diagnosis of ulcerative colitis was established, the possibility arose that he had either an extraordinary leukemoid reaction alone, or a combination of two uncommon chronic diseases. Demonstration of the Ph1 chromosome favored the simultaneous existence of the separate conditions. The high LAP and white blood cell count originally obtained were in retrospect attributed to a summation of the two diseases, the full-blown colitis contributing in greater part to the elevations. With satisfactory control of the inflammatory component both the enzyme level and leukocytes decreased, leaving primarily a residue of the early leukemia. The later divergence of these parameters (fig. 1) reflected increasing activity of the chronic granulocytic leukemia at a time when the ulcerative colitis was relatively quiet.

DISCUSSION

In 1962 Alter et al. reported findings of a 3 to 2 ratio between mean LAP values in children with mongolism and normal controls, interpreting the elevation as a quantitative expression of trisomy 21. They and others reasoned that the same chromosome was duplicated in mongolism as was
Fig. 2.—Metaphase plate and karyotype prepared from direct squash of bone marrow. The arrows indicate the minute Ph1 chromosome.

partially deleted in chronic granulocytic leukemia and that in the latter disease a missing gene was responsible for the known lack of LAP activity.

A number of observations, however, indicate greater complexity of the control mechanism for LAP than that implied by a straightforward gene-dose rela-
Table 1.—Hematologic Data

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<th>Myelocytes</th>
<th>Pro-myelocytes</th>
<th>Myeloblasts</th>
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*“Increased” to “markedly increased” on inspection of blood smear.
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tionship. Thus, confirmation of the increased LAP levels in mongolism\textsuperscript{5-7} quickly followed the original report but agreement on the critical ratio of activities between affected and normal children was not unanimous.\textsuperscript{6,7} In addition, assorted physiologic states and clinical conditions\textsuperscript{12,13} are known to be associated with LAP increases whereas reductions may occur in various hematologic and miscellaneous disorders.\textsuperscript{14,15} It is inconceivable that a specific cytogenetic abnormality could be common to these heterogeneously constituted groups.

Difficulties are also encountered in applying the Alter hypothesis to chronic granulocytic leukemia. As measured by cytochemical methods, LAP activity is either totally absent in the untreated disease or falls substantially below the 50 per cent level predicted if two genes confer 100 per cent activity and the patient possesses only one. Even more damaging to the assumption of a structural gene deletion in chronic granulocytic leukemia are the documented elevations of LAP during remission,\textsuperscript{15} acute transformation,\textsuperscript{5,6} and intercurrent infection.\textsuperscript{17,18}

Apparently the first to directly associate a phosphatase increase with the chromosomal aberration of chronic granulocytic leukemia were King, et al.\textsuperscript{6} These workers, in 1962, alluded to observations on the myeloblast crisis suggesting that "a rise (in LAP) may occur even in cells derived from precursors carrying the Ph\textsuperscript{1} chromosome." The patient who is the subject of this report validates their findings under circumstances of an inflammatory process. A closely related situation with high enzyme activity and the Ph\textsuperscript{1} chromosome has very recently come to our attention as "an unusual case of myeloproliferative disorder."\textsuperscript{19}

The existence of a dual population of leukocytes in chronic granulocytic leukemia has been advanced as an explanation for the rise of LAP with infection.\textsuperscript{18} It has also been suggested that busulfan therapy may inhibit leukemic granulocytes and result in a new line of cells containing normal amounts of alkaline phosphatase.\textsuperscript{16} Neither these possibilities nor the untenability of the gene-dose hypothesis preclude genetic modulation of LAP. Genes on chromosome 21 could represent one determinant of a complex control system probably involving physiologic mechanisms as well as other factors.\textsuperscript{6}

The intensive studies on regulation of protein synthesis at the genetic level in bacteria may afford insight into this problem. In 1961, Jacob and Monod\textsuperscript{20} summarized the work on adaptive enzyme formation in \textit{E. coli}. The outgrowth was a model based on the concept of a "unit of genetic expression" (termed the operon) and incorporating the notion of regulation by repression. Others\textsuperscript{21,23} have found that the interaction of several regulator substances together with environmental metabolite ultimately controls bacterial alkaline phosphatase production.

The behavior of LAP in chronic granulocytic leukemia may be considered in this framework, assuming protein synthesis in man to be analogous to that in \textit{E. coli}.\textsuperscript{20,23} Most of the requirements would be satisfied if, in addition to the structural gene(s) for LAP, chromosome 21 carried a set of regulators, each able to exert negative control. One regulator of LAP would suppress the
corresponding structural gene locus but would in turn be neutralized by repressor substance produced by the second regulator. If the latter, a modifier gene, were positioned on the distal third of the long arm of chromosome 21, its deletion would allow unregulated inhibition of LAP, accounting for the usual enzyme depression. If the repressor substance were a diffusible protein or micromolecule\textsuperscript{20-23} it would also interfere with modifier from the paired (trans) chromosome, further reducing LAP output to the very low levels of chronic granulocytic leukemia.

To provide for increases of LAP activity even in the presence of the Ph\textsuperscript{+} deletion, it is postulated that products of the inflammatory process and acute transformation effect high levels of modifier. The diffusible modifier substance elaborated through the mediation of genes on the intact chromosome 21 may then neutralize the regulators and release from inhibition the structural genes for LAP of both chromosomes.

Whether a scheme involving a multiplicity of regulators can be extrapolated to LAP formation in man is presently unknown. The theory outlined seems compatible with a number of observations on LAP in chronic granulocytic leukemia and may be a useful point of departure for further study.

**Summary**

A boy with Ph\textsuperscript{+}-positive chronic granulocytic leukemia and coincidental active ulcerative colitis presented with high leukocyte alkaline phosphatase (LAP) scores. Recession of the inflammatory process was accompanied by a marked fall of enzyme activity to expected levels for chronic granulocytic leukemia.

The extreme variations of LAP under these and related circumstances appear inconsistent with a simple gene-dose hypothesis as applied to the partial deletion of chromosome 21.

A system of control based on studies of bacterial enzyme synthesis and involving sequential repression by a pair of regulatory genes on chromosome 21 is proposed. Loss of a regulator (modifier) rather than the structural gene for LAP is offered as being in closer accord with the known behavior of this enzyme in chronic granulocytic leukemia.

**Summario in Interlingua**

Un puero con chronic leucemia granulocytic Ph\textsuperscript{+}-positive in coincidentia con active colitis ulcerative se presentava con alte valores de leucocytic phosphatase alcalin. Le recession del processo inflammatori esseva accompagnate de un declino maracte in le activitate de enzyma usque al nivello de valores a expectar in chronic leucemia granulocytic.

Le extreme variationes in le valores pro leucocytic phosphatase alcalin sub iste e relationate circumstantias non pare esser compatibile con le hypothese de un simple relation inter gen e dose applicabile al deletion partial de chromosomes 21.

Es proponite le these de un sistema regulatori basate in studios del synthesese bacterial de enzyma e stipulante un repression sequential per un par
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de genes regulatori in chromosoma 21. Perdita de un regulator (modificator) plus tosto que le existentia de un gen structural pro leucocytic phosphatase alcalin es hypothetisate con le explication que iste these se trova in melior correspondentia con le observate comportamento de iste enzyma in chronic leucemia granulocytic.

ACKNOWLEDGMENTS

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REFERENCES


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