Basophil Production in Myeloproliferative Disorders: Increases During Acute Blastic Transformation of Chronic Myeloid Leukemia

By J.A. Denburg, W.E.C. Wilson, and J. Bienenstock

We have examined basophil production (BP) in suspension cultures of separated peripheral blood cells from patients with chronic myeloid leukemia (CML). Seventy cultures were performed in 47 patients, 33 in chronic and 14 in the accelerated/acute phase of CML. None of 13 normal controls, 0/21 treated chronic phase, 3/33 untreated chronic phase, and 14/18 (p < 0.0001) accelerated/acute phase CML cultures showed at least twofold increases in basophils and histamine in vitro at 14–21 days. A gradient effect of disease phase on BP was observed: maximal histamine content (ng/10^6 cells) was 16 ± 4 for chronic, 56 ± 19 for accelerated, and 183 ± 73 for acute phase CML cultures (mean ± SE, p < 0.002), while basophils (per 10^6 cells) numbered 23 ± 7, 59 ± 20, and 267 ± 81, (mean ± SE, p < 0.0003), respectively. Multivariate analysis of maximal differences in basophils or histamine in vitro and simple linear regression slopes of basophils or histamine on time revealed that disease phase, and not therapy, accounted for the changes in BP in vitro. Serial studies over periods of 6–29 mo in 4 patients followed from chronic through acute phases of disease revealed a reversal of BP in vitro from negative to positive in 4/4, with death supervening within 3 mo of study in 3 cases and refractory leukemia developing in the other. Whole blood histamine values were highest in untreated chronic phase CML (1559 ± 306 ng/ml, mean ± SE), while accelerated/acute phase values were comparable to treated chronic phase (513 ± 131 and 521 ± 101 ng/ml, respectively). Whole blood histamine and BP in CML relate closely to impending acute blastic transformation. The potential role of basophils and histamine in myelopoiesis and acute leukemic transformation of CML is discussed.

The occurrence of increased numbers of circulating blood basophils in CML and related myeloproliferative disorders (MD) has been noted anecdotally by many authors and has been purported to be an indicator of disease severity or impending acute blastic transformation in CML. Several reports have indicated the possible poor prognostic implication of basophilia and hyperhistaminemia in these conditions, although there is not uniformity of opinion on this subject. Such observations suggest that in vivo fluctuations in basophils and histamine in CML are specifically related to the leukemic process and not simply a nonspecific expression of the clonal hematopoietic proliferation in CML. One might thus expect changes in the capacity of leukemic cell populations to differentiate to basophils, containing histamine, as CML progresses from chronic through acute phase.

Until recently, however, it has not been possible to examine BP by in vitro techniques. Miyoshi et al. and we have demonstrated the capacity of CML peripheral blood cells in suspension to differentiate to basophils containing histamine. Using these in vitro techniques, we now report on the relationship between BP and CML disease activity in a group of 47 patients. Our findings indicate that as the acute phase supervenes in CML, basophil counts and histamine concentration in peripheral blood fall, whereas BP in vitro increases. The potential application of whole blood histamine and BP assays to the assessment of CML disease phase is proposed.

Materials and Methods

Study Populations

The studies were performed with approval from the local Ethics Committee and after informed consent. In all, 47 patients were studied; a total of 70 in vitro cultures (including serial studies in some patients), as well as in vivo whole blood histamine and basophil counts, were performed. All but 4 of the patients had Philadelphia (Ph') chromosome positive CML. The exceptions were two patients with juvenile CML (A.D. and J.O.), one with a subacute hyperhistaminemic leukemia (V.G.), and one with accelerated myeloid metaplasia with myelofibrosis (R.J.). The latter two have been grouped with patients having accelerated phase CML (defined as clinical deterioration including at least 3 of the following: splenomegaly, fever, refractoriness to single alkylating agent therapy, and peripheral blood blast cell count ≥20%). Control peripheral blood specimens were obtained by venipuncture from normal asymptomatic healthy volunteers.

Histamine Assay

A single isotopic radiouassay for histamine, utilizing the conversion of histamine to 1H-S-methylhistamine by 1H-S-adenosylmethionine in the presence of histamine-N-methyl-transferase, was performed on either diluted (1:20) whole blood or cell suspensions in supplemented McCoy’s 5A medium and has been described elsewhere. Histamine assays performed on cultured cell suspensions reflect cell-associated histamine, since cell supernatants contained negligible amounts when compared to cell pellets. A significant positive correlation between histamine content and absolute basophil counts in both whole blood and cell cultures was obtained in these and other studies (see Results).

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Cell Cultures

In vitro liquid culture of separated CML or control peripheral blood cells was performed in a modified Marbrook system as originally described by Golde and Cline or in Falcon 35 cu mm plastic flasks (Falcon plastics) as previously described. Cells were first separated by Ficoll-Hypaque density gradient (specific gravity, 1.079) centrifugation of ethylenediaminetriacetic acid or heparin (preservative-free, GIBCO, Grand Island, N.Y.) anticoagulated peripheral blood removed in a sterile fashion by venipuncture. The cells were washed three times in McCoy’s 5A medium, then suspended in the same medium supplemented with 15% fetal calf serum, 1% penicillin/streptomycin, and 2-mercaptoethanol at a final concentration of 5 x 10^-5 M. One milliliter of a final cell suspension at a concentration of 1-3 x 10^6/ml was suspended over a dialysis membrane within a sterile Ehrlenmeyer flask or 5 ml were placed in sterile Falcon plastic flasks. Cultures were performed in duplicate at 37°C in a moist 100% humidified incubator with 5% CO2, and replicates harvested for cell counts, cytocentrifuge smears, and cell-associated histamine assay after 1-4 wk in vitro. Leukocyte conditioned media were not used for BP cultures; in fact, use of such media in these suspension cultures abrogated basophil growth and differentiation (Denburg, J.A., unpublished observations).

Cell Morphology

Cell counts were performed by Coulter Zb counter. Cytochemical staining with 0.05% toluidine blue, May-Grünewald-Giemsa, or Astra blue-acridine orange was performed on air-dried cytocentrifuge smears. Viability was assessed by trypan blue dye exclusion. Differential counts of 500-1000 cells per slide were performed and absolute basophil counts calculated from the total viable cell count and the percent basophils on stained slides. Cells counted as basophils contained metachromatic granules on toluidine blue or May-Grünwald-Giemsa staining, and emerald green granules on Astra blue-acridine orange staining; similar histochemical techniques for the identification of blood basophils have been previously described.

Statistical Methods

Descriptive statistics including means, standard errors (SE), and product moment correlations (r) were used to summarize the univariate and bivariate relationships. Simple linear regression was used to assess the relationship between basophil numbers or histamine concentration responses and time of incubation. The slope of the regression line was used to differentiate among the disease phases. Two-tailed Student’s t test was used to compare univariate outcomes from independent groups of patients. Chi-square tests were used to compare proportions of responses. The predictive relationship between cultures and disease phase was summarized with standard measures of sensitivity, specificity, and positive and negative predictive value. Multivariate analyses of basophils and histamine at multiple time points and simultaneous comparisons using slopes and intercepts from simple linear regression equations were analyzed with Hotelling’s statistic. Although exact p values are quoted when the computer programs provided them, significance was judged at p = 0.05. The SPSS statistical package on the HP3000 and CDC6400 computers at McMaster University was used for all of these analyses.

RESULTS

Patient Groups

Overall, 41 cultures (32 in chronic, 9 in accelerated/acute phase) were performed in patients who were receiving no therapy at the time of study; the remaining 29 cultures (20 in chronic, 9 in accelerated/acute phase) were done in patients receiving busulfan or combinations of this and/or other chemotherapeutic agents for control of their disease. Eighteen patients in whom cultures were performed were in accelerated/acute phase of CML at the time of study.

Whole Blood Histamine

Mean whole blood histamine levels in different patient groups and controls are shown in Table 1 and are depicted for all individual patients to demonstrate ranges of values in Fig. 1 (including some patients whose cells were not cultured). As can be seen, there were significant elevations of histamine in the blood of all CML patient subgroups when compared to controls (p < 0.0001). A significantly lower whole blood histamine value was observed in treated, chronic phase CML as well as in acute/accelerated phase when compared to untreated chronic phase CML (p < 0.01). This almost invariably was a reflection of a lower total white blood cell and absolute basophil count, although in some cases of accelerated/acute disease it reflected the latter only. The course of patient J.G., depicting a fall in whole blood histamine during busulfan therapy (Fig. 2) was representative of that found for all patients in whom therapy produced a gradual fall in total WBC and absolute basophil values; this fall in basophils and histamine was observed for in vitro studies as long as chronic phase of disease was maintained (Fig. 2 and see below).

In Vitro Studies

Normal Cultures

Basophils and histamine in peripheral blood cultures of asymptomatic volunteers were both found to decrease sequentially over 4 wk; the pertinent aspects of these studies have been previously reported.9 Morphological examination of normal peripheral blood mononuclear cells cultured in this manner for up to 4 wk in suspension almost invariably reveals large mononuclear cells, often syncytial, with a smaller proportion of granulocytes (<5%).

Table 1. Basophil Production in Peripheral Blood Cultures in CML and controls

<table>
<thead>
<tr>
<th>Culture Result</th>
<th>Therapy</th>
<th>No Therapy</th>
<th>Accelerated/Acute</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) ve*</td>
<td>0</td>
<td>3</td>
<td>14†</td>
<td>0</td>
</tr>
<tr>
<td>(−) ve</td>
<td>21</td>
<td>30</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

*Basophil and histamine rise of at least twofold over 2-3 wk in vitro.
†p < 0.00001 compared to all other subgroups.
Patient Cultures

Morphological observations of CML cultures. Cultures from chronic phase CML patients invariably demonstrated large numbers (50%-80% differential count) of neutrophilic myelocytes and small numbers of mature basophils at 2–3 wk. In contrast, as patient disease status changed to accelerated/acute phases, increasing numbers of basophils, both mature and immature, were noted (up to 80% differential count) as has been previously shown.9

Colony-forming cells (CFU-C) in CML cultures. In general, assayable myeloid precursors (CFU-C) in methylcellulose were present in chronic phase CML cultures at the start and in decreasing numbers at subsequent times in vitro. In accelerated/acute phase CML peripheral blood, there were significantly fewer CFU-C and more clusters compared to colonies, as has been well documented,21 over the liquid culture period, some accelerated/acute phase cultures became autonomous, establishing cell lines that continued to contain CFU-C (Denburg, J., unpublished observations). These lines are currently being further characterized. In the presence of conditioned medium containing colony-stimulating activity, liquid cultures of all phases of CML grew less impressively, demonstrating lower numbers of all differentiated myeloid cell types.

Correlation of in vitro histamine with basophils. The relationship of histamine values to absolute basophil counts was quite constant in both CML and control cultures. There was little variation, and there were no statistically significant differences in histamine content, calculated on a picogram per basophil basis, between starting and subsequent times in vitro. In CML cultures, these values ranged from 1.01 ± 0.16 to 1.30 ± 0.38 pg/basophil (mean ± SE).
BP-positive cultures according to CML disease phase and therapy. Table 1 lists the numbers of cultures positive for BP as assessed by changes in basophils and histamine in vitro over a 2–3-wk period, according to CML disease phase or therapy with cytotoxic drugs. In chronic phase CML, treated or untreated, only 3/54 cultures demonstrated increases in basophils and histamine greater than twofold at 2–3 wk in vitro; the rule was a decrease in total basophils and histamine by 3 wk, as in normal cultures, although the levels of basophils or histamine (the latter as an absolute value or calculated as pg/100 viable cells) were much higher than in control cultures and did demonstrate some rises early in vitro in some patients (Fig. 2).9 Large decreases in in vitro basophil and histamine values were regularly observed in cultures from patients in chronic phase CML and/or within 1 mo of discontinuation of alkylating agent (usually busulfan) therapy. However, in vitro basophil and histamine values at 2–3 wk in 14/18 (p < 0.0001) cultures of cells from patients in accelerated or acute phase of disease demonstrated progressive, large increases of basophils and histamine in vitro (Tables 1 and 2), after which time significant numbers of eosinophils were seen in vitro, associated with falling basophil and histamine values by 4 wk (data not shown).

BP assay in serially studied CML through accelerated/acute phase. Maximal basophil and histamine values in cultures of individual accelerated/acute phase CML patients are shown in Table 2. In some, increases of 20–30-fold of basophils/histamine were noted. Four patients who have been serially followed from chronic through accelerated or acute phases of disease also demonstrated much greater basophil and histamine increases in vitro in the accelerated/acute phase of their disease when compared to chronic phase (Fig. 3). This occurred whether or not alkylating agent therapy had been discontinued before entering accelerated/acute phase (Table 2).

BP assay gradient effect according to CML disease phase. As can be seen (Table 3), in vitro histamine values in cultures from patients in accelerated or acute phase were markedly different from those in chronic phase of CML. Morphological evidence in support of this included large numbers (20%–80%) of metachromatically staining cells at 2–3 wk in vitro. Nine cultures in accelerated/acute phase were done while patients were receiving chemotherapy with alkylating agents or other cytotoxic drugs at the time of study, and one of these was performed after a bone marrow transplant; 4/9 did not show BP in vitro and in all 4, multiple agent chemotherapy had been administered (Table 2). Of the remaining 9 accelerated/acute phase cultures, only 3 were performed in the absence of any previous chemotherapy; in all the remaining cases, therapy had been discontinued at least 1 mo prior to
study. Analysis of variance revealed that therapy could not account for differences in basophils and histamine in vitro by clinical subgroups (Fig. 4, Table 3).

**BP as a Predictor of Survival in CML**

The serial studies in patients M.O., J.H., R.S., and A.D. were done at intervals of 6–29 mo between phases of disease. Gradient effect of quantity of basophil differentiation in vitro from accelerated through acute phase is seen in studies done serially in these patients (Fig. 3) and by overall comparison of 5 accelerated phase cultures versus 13 acute phase cultures (Table 3). Furthermore, death has supervened within 2 mo of positive culture in 6 patients whose follow-ups are complete (C.H., M.O., I.S., A.D., J.O., V.G.) while 2 others are presently in refractory leukemic phase and candidates for bone marrow transplant (Fig. 3). Of the 3 patients last studied in accelerated phase (R.J., J.S., N.P.), 2 are still alive within 6 mo of study (J.S. and N.P.), one (J.S.) after having undergone splenectomy and multiple agent chemotherapy, and other (N.P.) multiple agent chemotherapy only.

**Analysis of Regression Equations for BP: Possible Predictor Model for CML Disease Phase**

Analysis of linear regression equations of basophils and/or histamine on time of incubation in vitro revealed that the mean slope of either basophils or histamine on time changed dramatically, from negative to positive, from chronic through acute phases of CML \((p < 0.02\) and \(p < 0.0003\) for basophils and histamine, respectively; Fig. 4). Similar analysis for sex and therapy variables revealed no differences in mean slopes of basophil and/or histamine curves; however, Ph\(^1\) status had a statistically highly significant effect in this analysis \((p < 0.001\) for basophils and \(p < 0.0001\) for histamine), reflecting the fact that 4 Ph\(^1\)-negative patients were all studied in accelerated or acute phases of disease (Table 2).

**DISCUSSION**

Using peripheral blood liquid culture techniques and a sensitive histamine assay, we have demonstrated serial changes in basophils and histamine in vitro that relate to CML disease activity and, ultimately, to blastic transformation (Tables 2 and 3, Figs. 3 and 4). These findings further extend our initial observations of increased growth of basophils from CML peripheral blood. Others have reported the presence of several morphologically distinct cell types after several weeks of suspension cultures of separated normal bone marrow cells;\(^12,16\) in neither the latter reports nor in our current studies have increases in basophils been identified in normal peripheral blood cultures.

Of significant interest is our observation of very large increases in in vitro basophils and histamine when cells are cultured from patients approaching or in CML blast crisis (Tables 2 and 3). These findings are...
in keeping with the description of in vivo basophilia and hyperhistaminemia occurring in patients with CML as perhaps portending a poor prognosis\textsuperscript{1-3} and suggest that basophil precursors may be present in increased numbers in peripheral blood as CML changes from chronic to accelerated or acute phase. Our studies on four patients examined serially in chronic through accelerated/acute phase (Fig. 3) as well as the consistent finding of BP in vitro in accelerated/acute phase CML (Table 3, Fig. 4) support this hypothesis. In this connection it is interesting to note that basophilia in vivo may be the first peripheral blood sign to follow the Ph\textsuperscript{1} chromosome in impending radiation-induced CML;\textsuperscript{4} thus, our in vitro BP assay may reflect a biologically important event in the natural evolution of CML.

Although histamine is found mostly in the peripheral blood basophil, there are small amounts found in other circulating blood cells in animals and in man; rabbit platelets, as well as human and guinea pig platelets to a much smaller extent, contain this vasoamine.\textsuperscript{17} Our cultures did not contain morphologically recognizable megakaryocytes or platelets; it therefore seems likely that most of the histamine we are finding in vitro is derived from basophils. These latter cells may be difficult to recognize morphologically since it is known that histochemical staining of basophils with agents that bring out metachromasia is dependent on the mucopolysaccharide, and not histamine, content of the cell.\textsuperscript{13} Thus, it is possible that in some cultures a morphologically nonbasophilic cell capable of histamine synthesis is present; our previous observations\textsuperscript{9} and relatively good correlation of histamine and basophil values in vitro\textsuperscript{9,11} are consistent...
with active basophil growth and differentiation in cultures of accelerated/acute phase CML peripheral blood cells.

It is not clear from these studies whether the in vitro phenomenon we have described is a cause or effect of the disturbances in myelopoiesis known to occur in CML. However, it is conceivable that CML disease phase-related changes in circulating committed granulocyte progenitors (CFU-C) or loss of granulocyte feedback inhibition may be linked to BP in vitro. Astra blue-acridine orange staining of cells occurring in clusters has been used to demonstrate mast cells in several patients with acute myeloid leukemia in relapse; inasmuch as cluster formation is a hallmark of acute transformation in CML, close morphological examination of clusters in CML would seem warranted, based on our current in vitro findings. Preliminary observations, indeed, reveal Astra blue-staining cell clusters in semisolid cultures of several of our patients with CML in whom parallel liquid cultures demonstrated evidence for basophil differentiation (Denburg, J.A., unpublished observations).

Two possible explanations for the positive association shown in our cultures between BP and CML disease phase are: (1) as CML becomes more acute, there are increased numbers of circulating myeloid precursors committed to differentiation along the basophil lineage; and (2) alternatively, basophilopoietic factors may be secreted by leukemic or other leukemia-associated regulatory cell populations such as T lymphocytes, which have been shown to be necessary for guinea pig BP and to induce mast cell proliferation. In this connection it is worth noting that long-term Dexter cultures of murine nonadherent bone marrow cells have demonstrated the appearance of metachromatic basophil-like cells; furthermore, Schrader has found that a persisting, nonadherent hematopoietic cell propagated in suspension cultures contains histamine and resembles basophils.

In summary, significant changes in circulating basophil precursors and/or histamine have been demonstrated to occur during acute leukemic transformation of CML and related myeloproliferative disorders. It appears from these studies that with in vitro techniques...

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### Table 3. Basophils and Histamine in Peripheral Blood Cultures From Normal Controls and Patients With Chronic Myeloid Leukemia in Different Phases

<table>
<thead>
<tr>
<th>Clinical Subgroup</th>
<th>Basophils (× 10⁴)</th>
<th>Histamine (ng)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Start 2-3 wk</td>
<td>Start 2-3 wk</td>
</tr>
<tr>
<td></td>
<td>(per 10⁶ Cells Cultured)</td>
<td></td>
</tr>
<tr>
<td>Chronic myeloid leukemia (n = 70)</td>
<td>99 ± 18</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>Chronic phase (n = 52)</td>
<td>132 ± 26</td>
<td>90 ± 16</td>
</tr>
<tr>
<td>Accelerated phase (n = 5)</td>
<td>69 ± 3</td>
<td>25 ± 9</td>
</tr>
<tr>
<td>Acute phase (n = 13)</td>
<td>28 ± 13</td>
<td>29 ± 12</td>
</tr>
<tr>
<td>Normal controls (n = 12)</td>
<td>22 ± 4</td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

Mean ± SE.  
*Differences in basophils or histamine in vitro among clinical subgroups by phase of disease, significant at p < 0.0003 and p < 0.002, respectively (Hotellings multivariate test values 0.48147 and 0.38985, respectively); by therapy, not significant at p = 0.05.

Analysis of variance for maximum differences (at 2-3 wk in vitro, from start of culture) revealed significant effect of phase on basophils (F = 5.377, df = 2; p < 0.008) and on histamine (F = 20.575, df = 2; p < 0.0001) and no significant effect of therapy on either basophils (F = 1.873, df = 3) or histamine (F = 2.849, df = 2).
examining BP, new information on staging and possibly prognosis may be obtained in patients with these disorders.

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REFERENCES


ADDENDUM

Since this manuscript was first submitted, another patient serially studied from chronic through acute phase (39 mo) CML has demonstrated the change in liquid culture growth pattern described, with BP in vitro antedating his clinical transformation by 3 mo.
Basophil production in myeloproliferative disorders: increases during acute blastic transformation of chronic myeloid leukemia

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