Juvenile "Chronic Granulocytic" Leukemia: A Panmyelopathy With Prominent Monocytic Involvement and Circulating Monocyte Colony-forming Cells

By Arnold J. Altman, Catherine G. Palmer, and Robert L. Baehner

Peripheral blood (PB) from two children with so-called juvenile-type (Ph' chromosome negative) "chronic granulocytic" leukemia (CGL) was found to contain cells capable of forming large numbers of exclusively monocytic colonies in vitro. These results are markedly different from those reported for classic adult-type (Ph' chromosome positive) CGL where predominantly granulocytic colonies are produced in vitro from PB. Spontaneously dividing PB and bone marrow (BM) cells from one patient contained a translocation between chromosome 3 and a C-group chromosome; phytohemagglutinin-stimulated PB cells from this patient had normal chromosome morphology, however. Since the translocation was present in 141 of 142 karyotypes from the BM at a time when it contained many dividing erythroid and myeloid cells, the pathologic process is not exclusively confined to the monocyte line, but involves all hematopoietic cells. So-called juvenile CGL is neither a chronic nor a granulocytic leukemia; it is a panmyelopathy with monocyte predominance and should thus be classified as a variant of myelomonocytic leukemia.

Between 1% and 5%, of children with leukemia are said to have chronic granulocytic leukemia (CGL). However, it has been noted that the disease seen in infants and young children differs in several important parameters from that noted in older children and adults: (1) absence of the Ph' chromosome, (2) increased frequency of thrombocytopenia and hemorrhagic manifestations, especially early in the course, (3) unresponsiveness to busulfan, and (4) a much more acute course with significantly poorer median survival. Consequently, it has been postulated on clinical grounds that so-called juvenile CGL represents a form of leukemia distinct from the adult type. The purpose of this paper is to present experimental evidence that supports the concept that juvenile-type CGL is not a true granulocytic leukemia.

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Supported by grants from The American Cancer Society (IN-46-M), Department of Health, Education, and Welfare (Maternal and Child Health Project 924), Riley Memorial Association, and Children's Cancer Study Group A.

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but is a variant of myelomonocytic leukemia with predominant involvement of the monocyte line.

CASE REPORTS

**Case 1**

O.L. had normal health until 16 mo of age when he developed daily fever spikes, purpura, generalized lymphadenopathy, and splenomegaly. Hematologic parameters at that time were as follows: hemoglobin 11.8 g%, WBC 23,900 /cu mm (9% metamyelocytes, 6% bands, 5% polymorphs, 71% lymphocytes, 6% monocytes, 3% eosinophils), platelet count 33,000/cu mm. The bone marrow was strikingly hypercellular with megaloblastosis, erythroid and myeloid hyperplasia, paucity of megakaryocytes, and 5.5% blast cells present. Serum lactic dehydrogenase was 1350. Leukocyte alkaline phosphatase score was 2 and fetal hemoglobin was 38%. The patient was initially given only supportive therapy, but when at 18 mo of age, he continued to spike daily fevers, and had further deterioration of his hematologic parameters (hemoglobin decreased to 6.9 g%, platelets to 6000 /cu mm, WBC 15,000 /cu mm with 2% blasts), he was started on busulfan in increasing doses up to 3 mg/day for approximately 1 mo. No improvement was noted and vincristine and prednisone were substituted for busulfan, again with no effect. The patient was then put on bimonthly pulses of vincristine, prednisone, cytosine arabinoside, thioguanine, and cyclophosphamide, and his spleen was irradiated. He failed to attain remission and died 3 mo after this regimen was initiated.

**Case 2**

J.B. had normal health until 5 yr of age, at which time he developed periorbital edema, petechiae, and purpura. Splenomegaly and lymphadenopathy were initially absent. Hematologic parameters at this time were as follows: hemoglobin 11 g%, WBC 15,800 /cu mm with 1% bands, 11% polymorphs, 33% lymphocytes, 55% monocytes, 18 nucleated red cells/100 WBC, platelets 900 /cu mm. Bone marrow was hypercellular with 35% blast cells and absence of megakaryocytes. The interpretation at this time was maturation arrest of myeloid series accompanied by numerous monocytoid forms. Fetal hemoglobin was 45% and leukocyte alkaline phosphatase was absent by histochemical staining. At 7 yr of age, he was seen at Riley Hospital for the first time and was found to have generalized adenopathy, massive splenomegaly, and the following hematologic values: hemoglobin 8.8 g%, WBC 41,400 /cu mm (15% blasts, 15% myelocytes, 20% metamyelocytes, 27% bands, 3% polys, 20% lymphs); platelet count was 9000 /cu mm and 21 nucleated red cells/100 white cells were present. At 7 years of age, busulfan therapy was initiated but failed to have any effect, and he was begun on bimonthly pulses of vincristine, prednisone, thioguanine, cytosine arabinoside, and cyclophosphamide. He exhibited some response to these medications, but failed to attain true remission and died 6 mo after their initiation.

MATERIALS AND METHODS

Twenty milliliters peripheral blood (PB) or 1–2 ml bone marrow (BM) was collected in plastic syringes containing approximately 0.1 ml heparin without preservative (sodium heparin, Grand Island Biological Co.). After collection, the specimens were harvested by sedimentation, washed twice in modified McCoy’s 5A medium, and then suspended at 10^5 nucleated cells/ml in 0.8% methylcellulose (Dow Methocel viscosity 4000 pcu) containing modified McCoy’s 5A medium and cultured in 1-ml aliquots in 35-mm Petri dishes (Falcon). Petri dishes were placed in a humidified atmosphere containing 10% CO₂ at 37°C.

Starting at day 6, when colonies were first microscopically visible, 5–10 colonies were removed by Pasteur pipette every other day and Wright-stained smears of single colonies were prepared. Two additional colonies were removed every other day and peroxidase stained smears were prepared. This procedure was followed until day 20.

Calculation of the monocyte-colony/granulocyte-colony (M/G) ratio was performed as follows: A colony consisting purely of monocytes (or macrophages) was counted as a monocyte (M) colony, while any colony which contained more than 10% granulocytes was counted as a granulocyte...
(G) colony. Since granulocyte colonies were not apparent before day 8 and all cells had transformed
to macrophages by day 20, only colonies obtained between days 8 and 18 were used in calculating
M/G ratio. Thus, 25-50 colonies were counted for each M/G ratio.

The number of colonies per 10^3 cells plated was determined by counting them at day 14; a colony
was defined as having more than 50 cells at this time.

**Glass Adherence (PB Cells of O.L. and J.B. Studied)**

Cells used in these studies were obtained in three ways. (1) 22 x 22-mm glass cover slips were
placed in Petri dishes prior to addition of the methylcellulose and cells. At varying intervals
after day 6, the cover slips were removed, washed several times with culture medium and then
Wright-stained. (2) Two milliliters of culture medium was placed above agar containing 10^6
cells per ml; after 10 days this medium was removed by Pasteur pipette and was found to contain
large numbers of mononuclear cells. (3) Colonies were removed from methylcellulose by Pasteur
pipette and added to 0.5 ml warm culture medium. The culture medium was then rinsed through
the pipette several times. Glass adherence by cells derived by method (1) could be directly
assessed by examining the Wright-stained cover slips for evidence of adherent cells. Cells obtained
by methods (2) and (3) were incubated with glass cover slips for 3 hr at 37° C. The cover slips
were then washed several times with warm culture medium, Wright-stained, and examined for
adherent cells.

**IgG Receptors**

Rh+ human RBC were washed twice with culture medium and resuspended to a vol/vol con-
centration of 5%. They were incubated with anti-Rh typing serum (Ortho) at 37° C for 30 min
and then washed four times with warm culture medium to remove excess antibody before resus-
pension in culture medium to a vol/vol concentration of 5%. Subsequently, they were incubated at
37° C for 30 min with mononuclear cells of 10- and 14-day-old colonies derived from the PB of
O.L. obtained by each of the three methods described above. The incubation took place above
glass cover slips in Petri dishes. Following the incubations, the cover slips were washed several
times with warm culture medium, Wright-stained, and examined for rosette formation.

**Phagocytosis**

A suspension of zymosan particles in 10%, human pooled serum was added to a cell suspension
(from 10 and 14 day PB cultures) in a ratio of 3:1. The suspension was then incubated in a 37° C
water bath for 30 min. Wright-stained smears of the suspension were evaluated by light microscopy
for uptake of zymosan particles by cells.

**Chromosome Studies**

PB was cultured both with and without phytohemagglutinin (PHA). PB (whole blood or buffy
coat) was cultured either for 72 hr using Gibco chromosome medium 1A containing PHA, or for
24 hr in F-10 medium with 20% calf serum without the addition of PHA.

Cells were treated for 2 hr with colcemide (0.6 μg/ml), treated hypotonically with KCl
(0.075 M) and fixed in methanol acetic acid (3:1). Air dry slides were prepared, dried at 65° C,
and stained directly with Giemsa or subjected to trypsin banding using 0.02% twice recrystallized
tryptsin (Worthington Biochemicals, Freehold, N.J.) at pH 8.0 for 6 sec at 4° C. Slides were
stained with Harleco Giemsa (diluted 1:50) in pH 6.8 buffer for 5 min.

BM was aspirated and an aliquot suspended in 0.03 μg/ml colcemide in 0.85 NaCl solution for
1½ hr, and treated subsequently with hypotonic citrate (1%) for 37 min. The slides were fixed
and stained as described above.

Cells were obtained by Pasteur pipette aspiration from microcolonies for chromosomal study
according to the method of Lawson and Boggs.

**RESULTS**

Hematologic status of patients at the time of study is summarized in Table 1.
Table 1. Hematologic Status of Patients at Time of Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>WBC</th>
<th>% Blasts (PB)</th>
<th>% Blasts (BM)</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.L.</td>
<td>12/1</td>
<td>18,000</td>
<td>0</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>12/16</td>
<td>6,900</td>
<td>4</td>
<td>4</td>
<td>Prednisone (Vincristine 1 wk before)</td>
</tr>
<tr>
<td>J.B.</td>
<td>11/16</td>
<td>115,000</td>
<td>28</td>
<td>54</td>
<td>Busulfan</td>
</tr>
<tr>
<td></td>
<td>3/12</td>
<td>700</td>
<td>0</td>
<td>19</td>
<td>Had 5 drug therapy 10 days prior to study; see text</td>
</tr>
</tbody>
</table>

Peripheral Blood

PB from both patients produced large numbers of in vitro colonies even in the absence of feeder layers or CM (Table 2). PB from controls developed no colonies when inoculated at the same concentration (10⁵/ml). When inoculated at a concentration tenfold higher, normal PB has been reported to produce in vitro colonies⁹; however, relatively few colonies per 10⁵ cells were obtained when compared with our patients' PB (Table 2).

Colonies were visible microscopically at 6 days after inoculation. All cells at this time appeared as undifferentiated blasts with deeply basophilic cytoplasm, diffuse nuclear chromatin, and visible nucleoli. At 7–9 days, a few azurophilic granules appeared; these granules were weakly peroxidase positive. Nuclear morphology at this time was monocytoid in nature with typical folding; these cells were apparently promonocytes. Within the next few days, the granules were no longer visible and the cytoplasm assumed a blue-gray appearance. By day 14, vacuoles and ingested granules of methylcellulose were visible in the cytoplasm; shortly thereafter, the cells transformed into macrophages (Figs. 1A–1D). After day 18, all colonies were composed totally of macrophages. At no time was nuclear morphology suggestive of granulocyte maturation nor did any neutrophilic or eosinophilic granules develop in the cytoplasm. These results are in marked contrast to those reported for normal⁹ or adult CGL¹⁰ PB where the majority of colonies were granulocytic in nature.

Bone Marrow

BM colonies were first visible at day 6. Monocyte colonies developed as described above. Granulocyte colonies were first recognizable as such by day

Table 2. In Vitro Leukocyte Colony Production From Peripheral Blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>Colonies/10⁵ Nucleated Cells</th>
<th>Monocyte Colonies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.L.</td>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>J.B.</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>Controls (5)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Chervenick and Boggs⁹*</td>
<td>0.1–2</td>
<td>32</td>
</tr>
</tbody>
</table>

* Used 10⁶ cells/ml in their incubation mixture.
Fig. 1. Cells from PB colonies obtained from a patient with juvenile CGL. (A) Days 7–9, all were initially monocytic with folded nuclei. (B) Days 10–14, cells were intermediate between monocytes and macrophages. (C) Day 14, cytoplasm filled with vacuoles and ingested methylcellulose. (D) Day 18, all were vacuolated macrophages.

7–8, at which time many azurophilic granules were noted in a majority of the cells; these granules gave a strong reaction when stained for peroxidase activity. By day 13, nuclear indentation and neutrophilic granule formation were evident in some of these colonies while others transformed to macrophage colonies.

Colonies were obtained from BM of juvenile CGL patients and controls both in the presence and absence of CM (Table 3). Although colony size was not quantitated, colonies from the CGL patients were significantly larger than those derived from controls. The M/G ratio in Table 3 reflects the proportion of pure mononuclear colonies to colonies containing granulocytes. BM from patients with CGL consistently produced more pure mononuclear colonies than granulocyte-containing colonies. Controls, on the other hand, consistently produced more granulocyte than monocyte colonies; this was also true of the studies reported in the literature.10-12
### Table 3. In Vitro Leukocyte Colony Production From Bone Marrow

<table>
<thead>
<tr>
<th>CGL Patients</th>
<th>Source of CM</th>
<th>Colonies/10⁵ Nucleated Cells</th>
<th>M/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.L.</td>
<td>Normal PB</td>
<td>85</td>
<td>2.1/1</td>
</tr>
<tr>
<td></td>
<td>Normal PB</td>
<td>91</td>
<td>2/1</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>86</td>
<td>2.4/1</td>
</tr>
<tr>
<td>J.B.</td>
<td>Normal PB</td>
<td>35</td>
<td>25/1</td>
</tr>
<tr>
<td></td>
<td>Normal PB</td>
<td>35</td>
<td>2.5/1</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Normal PB</td>
<td>30</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>23</td>
<td>1/1.5</td>
</tr>
<tr>
<td>B</td>
<td>Normal PB</td>
<td>32</td>
<td>1/3.3</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>28</td>
<td>1/2.8</td>
</tr>
<tr>
<td>C</td>
<td>Normal PB</td>
<td>21</td>
<td>1/3.1</td>
</tr>
<tr>
<td></td>
<td>CGL Pt. O.L.</td>
<td>27</td>
<td>1/2</td>
</tr>
<tr>
<td>D</td>
<td>Normal PB</td>
<td>14</td>
<td>1/3.5</td>
</tr>
<tr>
<td></td>
<td>CGL Pt. O.L.</td>
<td>20</td>
<td>1/1.5</td>
</tr>
<tr>
<td>E</td>
<td>Normal PB</td>
<td>100</td>
<td>1/5.5</td>
</tr>
<tr>
<td></td>
<td>CGL Pt. J.B.</td>
<td>62</td>
<td>1/3.8</td>
</tr>
<tr>
<td>Brown and Carbone&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Embryo kidney</td>
<td>4–24</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>1–10</td>
<td></td>
</tr>
<tr>
<td>Robinson and Pike&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Normal PB</td>
<td>16–52</td>
<td>1/20</td>
</tr>
<tr>
<td>Iscove et al.&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Normal PB</td>
<td>38–130</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>11–128</td>
<td></td>
</tr>
</tbody>
</table>

Although the M/G ratio for patients' BM usually varied between 1.1/1 and 2.5/1, on one occasion J.B. produced a ratio of 25/1. This occurred when he was in blast cell crisis with 28% blasts in PM and 56% blasts in BM. He was taking busulfan at this time and the markedly increased M/G ratio may thus be attributable either to suppression of granulocyte precursors by busulfan or replacement of myeloblasts by monoblasts as part of the blast-cell crisis.

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**Fig. 2.** BM granulecyte colony. Myelocytes, metamyelocytes, and a poly are evident.
Fig. 3. (A) Karyotype from unstimulated BM and PB cells of patient O.L. Note translocation of C-group chromosome to chromosome 3 (arrows). (B) Banding pattern showing the translocated material on chromosome 3 (arrows).
Functional Studies of Monocyte-Like Cells Derived from PB Colonies of O.L.

Glass adherence. Although the percentage of glass adherent cells was not calculated, many cells obtained by all three of the methods described above were found to be glass adherent. Whole colonies of monocyte/macrophage-like cells were found on glass cover slips when method 1 was used.

Phagocytosis. Of the cells which had migrated from the agar layers into the overlying culture medium, 65% contained ingested zymosan particles (method 2). In contrast, 52% of cells grown on glass cover slips (method 1) and 40% of cells pipetted from colonies (method 3) had demonstrable phagocytosis.

IgG receptors. Over 85% of cells obtained by all three methods had demonstrable IgG receptor sites by virtue of rosette formation.

Results of Chromosome Studies

Patient O.L. BM was obtained twice, on 10/9/72 and 11/15/72. The initial sample had 41 cells bearing a translocation between chromosome 3 and a C-group chromosome (Fig. 3). One cell did not appear to carry the translocation. Cells from BM obtained at the later day all had the translocations (100 cells) and several cells evidenced chromosome breakage (this was post busulfan therapy). Differential counts on mitotic figures in Wright-stained smears of BM showed that 53% of mitoses on the former occasion and 60% on the latter involved erythroid cells while 44% and 36%, respectively, involved granulocytes; the remainder either could not be classified or appeared to involve undifferentiated blasts.

PB cultures grown for 72 hr (with PHA) had two populations of cells, those with normal male karyotype (five karyotypes), and those with the translocation which could be identified as 46, XY, 3q+, 7q-, t(3q7q) (three karyotypes). The point of breakage appeared to be proximal to band 7q2 and distal to 3q8-26. Twenty-four-hour cultures carried predominantly the abnormal population (13 karyotypes) and only one cell was observed to have a normal karyotype.

Few dividing cells were obtained from the microcolonies of a quality that could be scored for the extra-long chromosome No. 3. Six cells appeared to carry the translocation and one cell appeared to have normal chromosomes.

Patient J.B. had normal chromosome studies.

DISCUSSION

There is a growing body of evidence which implicates the monocyte as the predominant cell type involved in juvenile CGL. (1) Large numbers of monocyteid cells are frequently present in the BM and PB of patients with juvenile CGL.2-3 (2) Granulocyte hyperplasia, low LAP, and early onset of thrombocytopenia are characteristic of classical Schilling-type monotypic leukemia,14,15 as well as juvenile CGL. (3) Serum and urinary muramidase levels are markedly elevated in monotypic leukemia,15 myelomonocytic leukemia,15 and juvenile CGL.17 By contrast, muramidase levels in classical Ph1 chromosome positive CGL are only slightly elevated.

In support of the above clinical evidence, we now have demonstrated experimentally the production of large numbers of pure monocyte colonies from PB
of patients with juvenile CGL and of a predominance of pure monocytic colonies from their BM. Our criteria for pure monocytic colonies were strict, since any colony with greater than 10%, granulocytes was excluded and classified with the granulocytic colonies. We interpret these studies as indicating that all the circulating colony-forming cells and a majority of BM colony-forming cells in these patients are monocyte precursors. These results are in marked contrast to those obtained in adult type CGL,10,18 where mainly granulocyte colonies are obtained in vitro; they thereby provide further evidence that these two diseases are entirely different entities, the adult variant being a true granulocytic leukemia and the juvenile variant being predominantly a monocytic one.

Although the monocyte appears to be the cell predominantly involved in this syndrome, there are data which document involvement of other marrow elements as well. Involvement of the erythroid line is manifested by ineffective erythropoiesis, megaloblastic changes in RBC precursors, high fetal hemoglobin levels,19-21 subnormal hemoglobin A2 levels,20,21 a decreased titer of the erythrocyte I antigen,20,21 and reversion to a fetal type of erythropoiesis.21 Granulocyte involvement is suggested by low LAP and recurrent infections16 in the presence of granulocyte hyperplasia. Thrombocytopenia is accompanied by paucity of megakaryocytes which cannot be accounted for merely by marrow infiltration with blast cells, since the marrow blast count may be quite low early in the course; functional platelet defects have been reported in adults with a similar condition.22

The chromosome studies on patient O.L. provide further evidence that this condition is a panmyelopathy. His peripheral blood contained two populations of cells, spontaneously dividing cells which contained the marker chromosome and PHA-stimulatable cells which had a normal chromosome complement; the former presumably represents the circulating blast cells and the latter, lymphocytes. Virtually all spontaneously dividing cells of his marrow contained the marker chromosome at a time when a large proportion of mitoses were seen in erythroid and granulocyte precursors. Thus, all hematopoietic cells appear to contain the abnormal chromosome, whereas nonhematopoietic cells (e.g., lymphocytes) have a normal chromosome pattern.

Recently, Saarni and Linman23 reported their finding in 132 adults with leukemia involving two or more marrow lines and noted the frequent involvement of monocytes or monocytoid cells in these patients. They went on to state that when one applied strict criteria, few cases of “monocytic leukemia” fail to reveal abnormalities of other marrow cell lines. They therefore defined this proliferative process involving all marrow elements as myelomonocytic leukemia, “myelo” to indicate that all myelo (i.e., marrow) elements are involved, and “mono” to emphasize the prominent monocytoid features. Since juvenile CGL has evidence of involvement of all marrow elements, as well as prominent monocytoid features, we feel that this condition should be classified as a variant of myelomonocytic leukemia.

ACKNOWLEDGMENT

We wish to thank Mr. Walter J. Rierden, Miss Judith Morris, and Mrs. Lillian Wang for technical assistance, and Mrs. Joyce Partlow and Mrs. Beverly Slater for assistance in typing this manuscript. We are also grateful to Miss Vita Ozolins for photographic assistance.
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