The Philadelphia Chromosome in Human Macrophages

By David W. Golde, Carmen Burgaleta, Robert S. Sparkes, and Martin J. Cline

Three patients with chronic myelocytic leukemia in different phases of the natural history of the disease were studied. Their bone marrow cells were cultured under conditions favoring macrophage proliferation, and parallel cytogenetic and cytochemical studies were performed. All cell metaphases examined contained the Ph1 chromosome at a time when more than 80% of these metaphases were in identifiable macrophages. We conclude that the mononuclear phagocyte cell line contains the abnormal chromosome in Ph1-positive chronic myelocytic leukemia.

The Philadelphia (Ph1) chromosome of chronic myelogenous leukemia results from an acquired chromosomal translocation apparently restricted to hematopoietic cells and involving erythrocytic, granulocytic, and megakaryocytic precursors. This characteristic cytogenetic abnormality is not found in fibroblasts or those lymphoid cells responding to phytohemagglutinin. Although it is probable that monocytes and granulocytes derive from a common stem cell, there has been no direct demonstration of the Ph1 chromosome in cells of the monocyte-macrophage complex. Chervenick and his colleagues and Moore and Metcalf have observed the Ph1 chromosome in colonies grown in vitro from the blood or bone marrow of patients with chronic myelogenous leukemia.

Tissue macrophages derive in part from progenitors in the bone marrow identified as promonocytes. If such macrophage progenitors contain the Ph1 chromosome, then it is of both clinical and biological importance to determine if these cells populate the mononuclear phagocytic elements of the reticuloendothelial system of man during the course of chronic myelogenous leukemia. Using a liquid tissue culture technique and combined cytochemical-cytogenetic analysis, we have obtained evidence that bone marrow-derived macrophages in chronic myelogenous leukemia carry the Ph1 chromosome.

MATERIALS AND METHODS

Three patients with chronic myelogenous leukemia were studied. Patient 1 was clinically stable and well controlled in the chronic phase of the disease. His dividing bone marrow cells contained a typical single Ph1 chromosome. Patient 2 was in the blast crisis phase of disease, and his proliferating hematopoietic cells contained a double Ph1 chromosome and a marker chromosome (Fig. 1). Patient 3 was in an accelerated phase and his cells had a single Ph1 chromosome. All analyzable metaphases from these patients contained at least one Ph1 chromosome. Bone marrow (patient 1) and peripheral blood cells (patients 2 and 3) were obtained, and nucleated cells were isolated by sedimentation and centrifugation. The hematopoietic cells were washed twice in complete α medium with 15% fetal calf serum and antibiotics and cul-

From the Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024.
Submitted June 28, 1976; accepted October 21, 1976.
Supported by USPHS Grants CA 15619, CA 15688, and RR 00865.
Address for reprint requests: Dr. David W. Golde, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024.
tured in Marbrook diffusion chambers in the same medium. Cultures were maintained for up to 25 days and harvested at regular intervals for viable and differential cell counts. This culture system encourages proliferation and differentiation along the macrophage line and permits easy access to the cells for morphological, histochemical, and cytogenetic studies.

Cytogenetic studies were performed at periods when prominent mononuclear phagocyte replication and little granulopoiesis were in evidence. Cultures were prepared for cytogenetic analysis by adding colcemid (0.02 μg/ml), and subsequently processed by routine methods. Standard air-dried chromosome preparations were made, stained with Giemsa, and the metaphases were scored for the presence of the Ph chromosome. Approximately six to ten metaphases were analyzed from each culture, and all well-spread metaphases were scored for the presence of the Ph chromosome. Parallel cultures were examined for the frequency of metaphases in identifiable macrophage precursors. In these cultures, cells were exposed to colcemid for 2 hr and then deposited on glass slides with a cytocentrifuge (Shandon Instruments) without hypotonic exposure. The cytocentrifuge preparations were stained for α-naphthyl butyrase (a histochemical marker for cells in the mononuclear phagocyte complex), and counterstained lightly with Giemsa. Mitotic figures were counted in these preparations and the percentage of mitoses occurring in α-naphthyl butyrase-positive cells determined. Granulocytes lack α-naphthyl butyrase activity. Studies were conducted on duplicate cultures from each patient at three time periods after 7 days in vitro.

RESULTS

The suspension cultures of hematopoietic cells from the three patients demonstrated active cell proliferation with prominent macrophage replication. Macrophage precursors and mature macrophages predominated in culture after

### Table 1. Percentage of Cells Containing α-Naphthyl Butyrase in Diffusion Cultures

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of Culture (Days)</th>
<th>5–6</th>
<th>7–8</th>
<th>11–12</th>
<th>20–25</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>27</td>
<td>74</td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>20</td>
<td>47</td>
<td>82</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>—</td>
<td>53</td>
<td>72</td>
<td>86</td>
</tr>
</tbody>
</table>
Fig. 2. (A) Ten-day Marbrook culture showing mitosis in a macrophage, a nonmitotic macrophage, and two lymphocytes (Giemsa stain). (B, C) Mitoses in macrophages stained for α-naphthyl butyrase (12-day culture).

7 days and comprised more than 70% of the cell population by day 11 (Table 1). At the culture intervals when cytogenetic analyses were performed, more than 80% of the observed metaphases were in α-naphthyl butyrase-positive cells (macrophages and macrophage precursors) (Fig. 2). All analyzable metaphases in cultures contained the Ph<sup>1</sup> chromosome—an observation consistent with our previous experience<sup>9</sup> and that of other investigators.<sup>7</sup>
DISCUSSION

We applied parallel cytochemical and cytogenetic studies on cultured hematopoietic cells from three patients with chronic myelogenous leukemia in order to determine if the Ph1 chromosome was present in the macrophage cell line. There was active proliferation of mononuclear phagocytes in these cultures and, although maturation was defective in some (patient 2), most of the mononuclear cells contained α-naphthyl butyrase activity after 1 wk in vitro. Because the great majority of metaphases after day 7 were in identifiable macrophages, and since all cytogenetic spreads contained at least one Ph1 chromosome, we conclude that this abnormal chromosome is present in macrophages. The alternative possibility, that only granulocyte precursors (less than 20% of metaphases) produce analyzable metaphases, whereas macrophages (greater than 80%) do not, seems unlikely.

In view of the recent demonstration of repopulation of alveolar macrophages by donor cells after allogeneic bone marrow transplantation in man, the present observations suggest the possibility that, in chronic myelogenous leukemia, large segments of the tissue macrophage population may be replaced by cells derived from the leukemic clone. Since tissue macrophages also have proliferative capacity, they may participate in further clonal evolution.

REFERENCES

The Philadelphia chromosome in human macrophages

DW Golde, C Burgaleta, RS Sparkes and MJ Cline