Culture of Bone Marrow Reveals More Cells With Chromosomal Abnormalities Than the Direct Method in Patients With Hematologic Disorders

By Sakari Knuutila, Pekka Vuopio, Erkki Elonen, Martti Siimes, Riitta Kovanen, Georg H. Borgström, and Albert de la Chapelle

The effect of short-term culture on the type and frequency of chromosomal aberrations found in bone marrow cells from patients with hematologic disease was evaluated. The classic direct method (1–2 hr incubation) was compared with methods involving culture for 1–4 days with or without methotrexate. Data on 32 samples from 21 patients in whom an abnormal clone was found indicated that on 18 occasions, culture yielded a higher proportion of cells belonging to the abnormal than to the normal clone. In 10 of those cases, the difference was statistically significant ($p < 0.05$). The reverse situation was seen on six occasions, none of which was statistically significant. On four occasions, the culture methods disclosed a clonal abnormality that would not have been detected if the direct method alone had been used. The phenomenon occurred in chronic myeloid leukemia, myeloid and lymphoid acute leukemia, and proleukemic conditions. It affected clones with different cytogenetic abnormalities such as $5q-$, t(8;21), and i(17q). In one instance, sister chromatid staining after BuDR incorporation indicated that both cytogenetically normal and abnormal ($5q-$) mitoses were in their second division after 2 days in culture. The implication of these studies is that to improve the chances of detecting cytogenetically abnormal cells, bone marrow cells should be cultured. Examination of as many mitoses as possible after culture for 1–4 days may reveal abnormal clones that are sparsely represented. Finally, results obtained with different methods are not directly comparable.

RESULTS of three recent investigations have indicated that the proportion of cells belonging to abnormal clones among bone marrow cells from patients with leukemia or preleukemia is significantly higher in preparations made after culture for 1–2 days than in those made by the so-called direct method (1–2 hr). Carbonell et al. found the most obvious difference in a clone with a Ph chromosome plus an isochromosome for the long arm of chromosome 17. In patients with acute promyelocytic leukemia, Berger et al. found a higher frequency of cells with the 15;17 translocation after culturing the cells for 24 hr or longer. Finally, in our laboratory, the frequency of a clone with a $5q-$ chromosome from a patient with refractory idiopathic sideroblastic anemia or preleukemia ranged from 0% (direct method) to 90% (culture method). The aim of the present investigation was to determine whether the phenomenon described applies only to certain hematologic malignancies or to all, and whether it occurs preferentially in certain chromosomal abnormalities. In addition, some of the experiments were designed to distinguish between different mechanisms that might lead to the observed phenomenon.

MATERIALS AND METHODS

Experimental Material

Bone marrow samples from patients with leukemia, suspected leukemia, or preleukemia were used to elucidate the relative proportions of cells belonging to cytogenetically different clones in preparations obtained by the "direct" and "culture" methods. The series also included a number of patients with nonleukemic diseases known to be associated with chromosomal aberrations. These were patients with essential thrombocytosis, aplastic anemia, polycythemia vera, multiple myeloma, and malignant lymphoma infiltrating the bone marrow. Altogether, 332 bone marrow samples from 270 patients were studied.

A meaningful result could, of course, be obtained only when the methods to be compared yielded sufficient numbers of analyzable mitoses on the same occasion from the same sample. It is well known that bone marrow samples vary greatly with respect to the number and quality of mitoses produced, the reasons for this variation being only partly understood. Our criteria for minimum numbers and quality of mitoses studied were as follows. On each occasion, both or all methods to be compared must yield a minimum of seven analyzable mitoses. The great majority of such mitoses had been banded. However, when a particular clonal abnormality that had been characterized by banding was also detectable without banding, un.banded cells were occasionally included in the analysis. Furthermore, patients having slides with fewer than seven mitoses were included in the series whenever a previous examination (or another method used on the same occasion) had shown the presence of abnormalities.

Of the 332 samples considered, 56 could be analyzed further, and these form the basis of this report.

Definitions

The International Workshops on Chromosomes in Leukemia have adopted criteria for the minimum numbers of cytogenetically identical mitoses required to define a clone. One cell with a normal...
Cytogenetic Methods

After aspiration, the bone marrow sample was transferred to an injection bottle containing 5 ml of medium and 167 IU of heparin without phenol. The medium used in all methods contained four parts of Eagle’s minimum essential medium and one part of pooled human serum. Within 90 min, the sample was transported to the cytogenetic laboratory where part of the specimen was prepared by the direct method and the rest was cultured by one or more methods.

Direct method. Bone marrow cells were incubated at 37°C for 1.5 hr in medium containing deacetyl-methylcolchicine (Colcemid, CIBA) (0.05 μg/ml).

Culture methods. The cells were incubated at 37°C for 22, 46, 70, or 94 hr in medium before being subjected to colchicine treatment as above.

 Methotrexate method. This method was adapted for BM use by Hagemeijer et al. and serves to synchronize the cultures. Our modification was as follows. The cells were incubated at 37°C for 17 or 41 hr in medium containing methotrexate (Methotrexate sodium, Lederle, 10⁻⁷ M). They were then washed twice and reincubated at 37°C for 5 hr in medium containing thymidine (10⁻⁸ M). Colchicine was present during the final 1.5 hr of this incubation.

Bromodeoxyuridine labeling (Harlequin) technique. Bone marrow cells were incubated in the dark at 37°C for 44–48 hr with 10 ml of medium and BUdR (5-bromodeoxyuridine; Sigma Chemical Co., St. Louis, Mo.; 5 μg/ml). Colchicine was added 2 hr before the end of the incubation period.

Table 1. Cytogenetic and Clinical Data on Patients With Malignant Blood Diseases in Whom a Chromosomally Abnormal Clone was Detected in the Bone Marrow Sample Studied

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Occasion</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Clone(s)</th>
<th>No. of Cells/Clone</th>
<th>Total</th>
<th>Significance (P) Direct vs Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>24*</td>
<td>F</td>
<td>65</td>
<td>I</td>
<td>RISA</td>
<td>N/abnormal*</td>
<td>9/7 16</td>
<td>0/24 24</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>RISA</td>
<td>36/6 42</td>
<td>N/abnormal</td>
<td>22/41 63</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>RISA</td>
<td>36/16 52</td>
<td>N/abnormal</td>
<td>5/24 29</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>RISA 54/9 63</td>
<td>M</td>
<td>N/abnormal</td>
<td>26/18 44</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>PL</td>
<td>N/5q- 7/0 7</td>
<td>6/18 24</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>70</td>
<td>PL</td>
<td>N/I(11;16) 3/18 21</td>
<td>2/8 10</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>PL</td>
<td>3/0 3</td>
<td>N/I(11;16)</td>
<td>4/0 4</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>57</td>
<td>PL</td>
<td>N/-1, +r 2/31 33</td>
<td>1/12 13</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>59</td>
<td>PV</td>
<td>N/+8 21/6 27</td>
<td>17/5 22</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>44</td>
<td>ALL,RI 6/4 10</td>
<td>N/I(18;21)</td>
<td>0/11 11</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>44</td>
<td>ALL,RI 6/4 10</td>
<td>N/I(18;21)</td>
<td>0/11 11</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>5</td>
<td>APL,D,g 5/0 5</td>
<td>N/I(15;17)</td>
<td>52/1 53</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>43</td>
<td>ALL,RI 5/1 21</td>
<td>D/V,P</td>
<td>N/I(44 abn./46 abn.) 17/25/2 21</td>
<td>5/24/3 32</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>ALL,RI 6/4 10</td>
<td>D/V,P</td>
<td>N/I(44 abn./46 abn.) 3/21 16</td>
<td>0/0/2 2</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>4</td>
<td>ALL,D,g 7/0 7</td>
<td>N/58</td>
<td>16/2 18</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>ALL,RI 18 18</td>
<td>P.O.A</td>
<td>N</td>
<td>21 21</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>M</td>
<td>62</td>
<td>ML</td>
<td>N/-5, -14, + mar 1-3 6/3 8</td>
<td>15/1 26</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>ML</td>
<td>N/-5, -14, + mar 1-3 3/3 6</td>
<td>4/0 4</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>66</td>
<td>ML</td>
<td>N/-Y 5/3 8</td>
<td>11/6 17</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>M</td>
<td>67</td>
<td>MM</td>
<td>Mel N/-Y 13/13 26</td>
<td>33/22 55</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*This patient had several different cytogenetically abnormal clones, which have been described in detail previously. In this table all cells belonging to any of the abnormal clones are considered together ("abnormal"). For a detailed account of the karyotypes and frequencies of the different clones, see Table 2. For similar data in CML, see Table 3.

†2-day culture in the presence of 5-bromodeoxyuridine.

§Tests of significance were performed comparing the proportion of abnormal mitoses detected by different methods. In patients with several abnormal clones, the superscripts indicate which clones were compared.

Abbreviations: A, asparaginase; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; D, doxorubicin; Dg, diagnosis; M, methenolone; Mel, melphalan; ML, malignant lymphoma; MM, multiple myeloma; O, oncovin; P, prednisone; PL, preleukemia; PV, polycythemia vera; RISA, refractory idiopathic sideroblastic anemia; RI, relapse; Rm, remission; V, vincristine.
MARROW CULTURES REVEAL ABNORMAL KARYOTYPES

Table 2. Frequency of Cytogenetically Different Cell Clones in the Bone Marrow Cells on Four Occasions (I–IV) and by Different Methods in a Female Patient With Refractory Idiopathic Sideroblastic Anemia (Patient 24, Table 1)

<table>
<thead>
<tr>
<th>Clone</th>
<th>Direct</th>
<th>Culture</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 hr</td>
<td>46 hr</td>
<td>22 hr</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>46,XX*</td>
<td>9</td>
<td>36</td>
<td>54</td>
</tr>
<tr>
<td>46,XX,5q-†</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>47,XX,+8</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>46.X.-X.+8</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>47,XX,+14</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>47,XX,+19</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>42</td>
<td>52</td>
</tr>
</tbody>
</table>

*Including occasional cells with apparently random chromosome losses.
†Mitoses (46,XX,5q-1) otherwise cytogenetically normal, but including occasional cells with apparently random chromosome losses.

RESULTS

Of the 44 patients, 23 yielded samples in which no abnormal clone was found by any method. However, the number of cells studied was often so small (less than 10 cells in several instances) that actual absence of abnormal clones cannot be inferred. These patients will not be further dealt with.

Tables 1–3 and Fig. 1 summarize data on 32 samples from 21 patients (24–44) in whom an abnormal clone was found. There was one patient with refractory idiopathic sideroblastic anemia, four with preleukemia, one with polycythemia vera, two with ANLL, two with ALL, two with malignant lymphoma infiltrating the bone marrow, one with multiple myeloma, and eight with CML. Comparisons of the results obtained with the direct and the culture methods showed that the proportion of cells belonging to the abnormal clone was higher after culture on 18 occasions. The difference was statistically significant \( (p < 0.05) \) in 10 of those 18 comparisons. The reverse situation was seen on 6 occasions, none of the differences being statistically significant. Cases in point are, for instance, patients 24 and 25, in whom the major abnormal clone was 46,XX,5q-.

Table 3. Cytogenetic Data on Patients With Philadelphia-Chromosome-Positive Chronic Myelocytic Leukemia: The Culture Method Was 1-Day Culture in the Presence of Methotrexate, Unless Otherwise Indicated

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Occasion</th>
<th>Phase of Disease</th>
<th>Chemo-therapy</th>
<th>Clone(s) Containing Chromosome</th>
<th>No. of Cells/Clone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>F</td>
<td>59</td>
<td>I</td>
<td>CP</td>
<td>B</td>
<td>Ph1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>38</td>
<td>M</td>
<td>42</td>
<td>I</td>
<td>CP</td>
<td>H</td>
<td>Ph1</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>39</td>
<td>M</td>
<td>39</td>
<td>I</td>
<td>CP</td>
<td>B</td>
<td>Ph1</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>70</td>
<td>I</td>
<td>AP</td>
<td>B</td>
<td>N/Ph1</td>
<td>1/17</td>
<td>0/8</td>
</tr>
<tr>
<td>41</td>
<td>F</td>
<td>28</td>
<td>I</td>
<td>AP</td>
<td>H</td>
<td>Ph1/i(17q)</td>
<td>33/0</td>
<td>33/32/2</td>
</tr>
<tr>
<td>42</td>
<td>F</td>
<td>70</td>
<td>I</td>
<td>AP</td>
<td>H</td>
<td>Ph1/i(17q)</td>
<td>17/0</td>
<td>17/55/2</td>
</tr>
<tr>
<td>43</td>
<td>M</td>
<td>34</td>
<td>I</td>
<td>BT</td>
<td>B</td>
<td>Ph1/i(17q)</td>
<td>21/0</td>
<td>21/3/1</td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>33</td>
<td>I</td>
<td>AP</td>
<td>H</td>
<td>Ph1/i(17q)</td>
<td>87/0</td>
<td>87/36/42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>BT</td>
<td>H</td>
<td>Ph1/i(17q)</td>
<td>47/1</td>
<td>47/34/40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>III</td>
<td>BT</td>
<td>H</td>
<td>Ph1/i(17q)</td>
<td>5/7</td>
<td>5/12/6/7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IV</td>
<td>BT</td>
<td>H</td>
<td>Ph1/i(17q)/+i(17q)</td>
<td>0/28/0</td>
<td>0/87/1</td>
</tr>
</tbody>
</table>

*3-day culture without methotrexate.
†2-day culture in the presence of 5-bromodeoxyuridine.
‡4-day culture without methotrexate.
§2-day culture without methotrexate.

Abbreviations: AP, accelerated phase; B, busulphan; BT, blastic transformation; CP, chronic phase; H, hydroxyurea; V, vincristine.
Fig. 1. Comparison of percentages of cells of the abnormal clone(s) (or of cells with abnormalities in addition to the Ph' in CML) found in preparations made by the direct and the culture method (see Tables 1–3). The figure does not include values for patients 24 and 44 (occasions II–IV and II, respectively) or for those patients in whom fewer than 7 cells prepared by either method were studied. (*) Difference in the proportion of cells of the abnormal clone (or clone with other abnormality plus Ph') statistically significant (p < 0.05) (tested by Fisher's exact test); (z) combined frequency of 3 clones having Ph' chromosome plus other abnormalities. (——) Refractory idiopathic sideroblastic anemia, preleukemia, or polycythemia vera; (----) acute nonlymphocytic or lymphocytic leukemia; (———) chronic myelocytic leukemia; (…………) malignant lymphoma or multiple myeloma.
of investigation in each case, the presence of the 5q−
clone would have passed undetected if the direct
method alone had been employed. The same is true for
patients 31 and 33 with APL and ALL, respectively.
In other patients the abnormal clone was noted in the
“direct” preparations, but was more frequent after
culture. For instance, in patient 30, who had ANLL
and was studied in relapse, the clonal abnormality,
46,XY,t(8;21) was seen in 4 of 10 cells by the “direct”
and 11 of 11 cells by a culture method (p < 0.01).
Similar observations relate to the first occasion of
investigation in patient 32 with ALL in relapse (p <
0.001) and perhaps in patient 34 with malignant
lymphoma (p = 0.3).

For practical purposes, it is important to establish
how often, if ever, the “direct” method yields more
abnormal mitoses than culture. As indicated above,
there were six instances altogether in which this was
noted. None of them showed statistical significance,
however, and their practical implications may not be
important for the following reasons. In cases 26 and 27
the proportion of abnormal mitoses was quite high
(86% and 94%) by the direct method and only slightly
lower after culture (80% and 92%). In cases 35 and 36,
the abnormality was Y, and again the proportions of
the abnormal clone found by the two methods differed
only slightly (38% versus 35% and 50% versus 40%,
respectively). In cases 44 (occasion II) and 34 (occa-
sion II), the numbers of cells studied were very small
by each method (< 14 cells).

As to whether the phenomenon is restricted to
certain diseases, the above data indicate that it occurs
in both acute nonlymphocytic and acute lymphocytic
leukemia. It is also clearly present in preleukemic
conditions. Our data are not large enough to suggest
absence of the phenomenon in any one disease.

As to whether the phenomenon described is
restricted to, or more common in, certain chromoso-
mal abnormalities than in others, the data in Table 1
indicate the following. The phenomenon does occur in
5q−, in t(8;21), in t(15;17), and in abnormalities
associated with ALL. Evidence from patients 24 and
28 indicates the absence of the phenomenon with
respect to +8. Finally, data from patients 35 and 36
do not indicate a higher frequency of Y after culture
than by the “direct” technique.

In Ph1-positive chronic myeloid leukemia, the Phila-
delphia chromosome is mostly present in 100% of the
bone marrow mitoses studied.8 The data in Table 3 are
consistent with this long-known fact. Incidentally, in
patient 40 the Ph1 was not present in 1 of 18 “direct”
mitoses or in 2 of 16 cultured mitoses. Regarding other
chromosomal abnormalities in addition to the Ph1,
some interesting observations were made (Table 3).

Patients 41 and 42 had clinical evidence of acceler-
ation, but had not entered blastic transformation (BT).
By the “direct” method, no additional abnormality
was seen in 33 and 17 mitoses, respectively. In
contrast, culture yielded 7 of 43 and 2 of 57 mitoses,
respectively, with additional abnormalities suggesting
BT (which actually did follow).

Even more striking were the findings in patient 44, a
33-yr-old male with apparent acceleration of his CML
on occasion I. None of 87 “direct” mitoses had any
additional abnormality, while 42 of 78 mitoses
obtained by 1-day methotrexate culture had an i(17q).
Later (occasions II and III 3 and 4 mo later) when BT
actually occurred, the i(17q) was seen in “direct”
mitoses also. Finally, on occasion IV (6 mo after
occasion I) all mitoses seen after both methods had it.

It is necessary to consider whether the methotrexate
used in most of our samples processed by culture was
directly responsible for the higher frequency of abnor-
mal cells or whether the time in culture was the main
factor. We have already presented evidence indicating
that the main factor is not the methotrexate, but the
time in culture.1 Inspection of the data in Tables 1 and
3 reinforces this assessment. Methotrexate was not
used, for instance, in case 25 (Table I) and cases 41
and 43 (Table 3) with striking enrichment after
culture. But more data are clearly needed to fully
evaluate this question.

Bromodeoxyuridine Labeling

A number of samples were incubated with BUdR
for 2–3 days (Tables 1–3). The object of this pro-
dure was to demonstrate by unequal sister chromatid
labeling that the mitoses studied were actually
undergoing their second (or third, etc.) division in
culture.7 However, most chromosomal abnormalities
are difficult to detect in Harlequin-stained mitoses
(which do not have bands). Since the main object was
to study the frequency of clonally abnormal cells, most
of the slides made from cultures incubated with BUdR
were stained by GTG-banding and scored as ordinary
mitoses. To prove that the cells were in their second
division, a small number of slides were sister chroma-
tid (Harlequin) stained in case 24. In all instances in
which such an analysis was possible, the findings
confirmed the sister chromatid staining both in chro-
mosomally normal cells and in cells belonging to the
abnormal clone.

DISCUSSION

In the present study a higher frequency of clonal
abnormalities (or in CML clonal abnormalities in
addition to the Ph1) was detected after culture than by
the direct method in patients with CML, ANLL,
ALL, refractory idiopathic sideroblastic anemia, and other preleukemic conditions and possibly also in one patient with polycythemia vera. We do not yet know precisely why this is so. The fact that in one instance we were able to show by BUdR incorporation that cells with a clonal abnormality detected after culture for 2 days were in their second division in vitro supports the idea that cytogenetically abnormal cells can go on dividing in culture. But cytogenetically normal cells were also in their second division, showing that these cells, too, survive. It should be possible to devise experiments that will determine whether the chief mechanism underlying the observed phenomenon is related to increased mitotic activity and longevity of cytogenetically abnormal cells. Alternatively, there could be decreased mitotic activity and/or death of cytogenetically normal cells. The answer may well be a combination of the two mechanisms.

The results of BUdR incorporation argue against the possibility that the cytogenetic abnormality is an artifact produced by culture in vitro. Since structurally abnormal chromosomes were Harlequin-stained, they must have been present at or near the start of the culture.

Further experiments are needed to elucidate the mechanisms leading to enrichment of the culture in cytogenetically abnormal mitoses. It should be remembered that so far there is no direct evidence of an increase in the actual number of cytogenetically abnormal cells. But this appears likely in view of the results of Berger et al. who found a relative increase in promyelocytic interphase cells (presumably cytogenetically abnormal) studied after cytochemical staining methods for mitotic cells that allow simultaneous assessments to be made of their morphological type and karyotype. Such methods are being developed in our laboratory.

The first conclusion to be drawn from the present results is that cytogenetic analyses of BM cells from patients with hematologic malignancies should be performed after culture rather than by the direct method. The results appear to promise possibilities of earlier detection of abnormal clones and of clonal evolution as well. Whether such earlier detection will prove valuable in indicating impending clinical deterioration cannot yet be fully assessed, but cases like patient 44 (Table 3) with CML was found in over half of mitoses after culture of samples at a time when there was acceleration of the disease but no blastic transformation. Soon afterwards, 1 “direct” mitosis in 48 had i(17q). When blastic transformation occurred, most, and later all, “direct” mitoses had i(17q). During this entire period a high proportion of the “cultured” mitoses had i(17q).

Two points require further discussion: the number of cells that should be studied and the value of very few or even single cells studied in the diagnosis of hematologic malignancies.

Several observations reported in this article may be interpreted to indicate that only after a large number of mitoses have been studied should the patient be regarded as free of any clonal abnormality. Patient 31 (Table 1) with APL is a case in point. Here, t(15;17) was seen in only one of 58 cells studied. In fact, it was the 32nd cell studied, a number higher than the total number of cells studied in most published reports on patients with leukemia. Incidentally, the one abnormal cell was from a cultured sample. Similarly, 1 cell with 47,XY,Ph1,+8,i(17q), was found in patient 43 (Table 3) when his CML had already undergone blastic transformation. Three weeks later, all cells had the additional abnormalities. These findings bear out the observation by Sonta and Sandberg that in some patients with CML, abnormal and normal clones present in low frequency can be detected if very large numbers of mitoses are studied.

The second conclusion to be drawn from our results is that large numbers of mitoses must be studied before it can be confidently assumed that the karyotype is normal. Furthermore, even single cells with abnormalities must be regarded as possible or even probable representatives of an abnormal clone. Finally, in most cases culture methods appear to disclose such cells with a clearly greater probability than the “direct” method. Yet in many cases the two methods yield similar results and in a small number of cases the “direct” method may yield more abnormal mitoses than culture.

Another finding is that extreme caution must be exercised when comparing results obtained by different methods. Geographical variations in the prevalence of chromosomal abnormalities, such as have been noted for t(15;17) in APL and for t(8;21) in ANLL, may or may not turn out to be due to subtle differences in methodology that have so far not been recognized. Renewed efforts are needed to clarify these questions.

REFERENCES


Marrow cultures reveal abnormal karyotypes

Culture of bone marrow reveals more cells with chromosomal abnormalities than the direct method in patients with hematologic disorders

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