Hairy Cell Leukemia Is Characterized by Clonal Chromosome Abnormalities Clustered to Specific Regions

By Ulla Haglund, Gunnar Juliiusson, Birgitta Stellan, and Gösta Gahrton

Cytogenetic analysis was performed on B-cell mitogen-stimulated cells from 36 patients with symptomatic hairy cell leukemia. Evaluable metaphases were achieved from 30 patients, and 20 (67%) showed clonal abnormalities. Recurrent chromosomal aberrations involving chromosomes 1, 2, 5, 6, 11, 19, and 20 were found. The abnormalities were mostly deletions and inversions, whereas translocations and numerical abnormalities, except trisomy 5, were rare. Fourteen patients showed multiple clones, which were related and found in different combinations in individual cells. Cells with non-clonal abnormalities identical to those found in clonal changes in other patients were common. Chromosome 5 was involved in clonal aberrations in 12 of 30 (40%) patients, most commonly as trisomy 5 (n = 4), or pericentric inversions (n = 6) and interstitial deletions (n = 4) involving band q14. Three patients showed two and 1 patient three different clones that involved chromosome 5. In addition, 1 patient had a rare constitutional inversion of chromosome 5 with breakpoints at p13.1 and q13.3. Pericentric inversions and interstitial deletions of chromosome 2 occurred clonally in 4 patients (13%) and in single cells of another 6 patients. Deletions of chromosome 1 at band q42 was found in 5 patients, and 1 patient had a translocation between q14q2 and a supernumerary chromosome 5. Deletions of 6q and 11q were similar to those commonly found in other lymphoproliferative disorders. Trisomy 5, structural abnormalities involving the pericentromeric regions of chromosomes 5 and 2, and t(4q2 abnormalities were findings distinguishing the karyotypes in hairy cell leukemia from those of other hematologic malignancies.

© 1994 by The American Society of Hematology.

From the Division of Clinical Hematology and Oncology, Department of Medicine, Karolinska Institute at Huddinge Hospital, S-141 86 Huddinge, Sweden.

Submitted October 5, 1993; accepted January 3, 1994.

Supported by the Swedish Cancer Society, The Swedish Society for Medical Science, The Karolinska Institute’s Research Funds, and The Medical Research Council.

Address reprint requests to Gunnar Juliiussson, MD, Department of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.

0006-4971/94/$3.00/0


2637
<table>
<thead>
<tr>
<th>Patient Age/</th>
<th>Previous Treatment</th>
<th>Cell Source/ Mitogen</th>
<th>No. of Metaphases: Abnormality/Total</th>
<th>No.</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.90 TÖ§</td>
<td>B/</td>
<td></td>
<td>2/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49 yrs/S, I, C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Constitutional karyotype: 46,XY,inv(5)(p13.1q13.3)c</td>
</tr>
<tr>
<td>Patients with clonal abnormalities involving chromosome 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.92 IK</td>
<td>BM, B/</td>
<td>14/37</td>
<td>5 del(11)(q23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38 yrs/U</td>
<td>MP6</td>
<td></td>
<td>2 +5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.91 RH§</td>
<td>BM, B/</td>
<td>4/6</td>
<td>1 del(11)(q23)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39 yrs/S, I</td>
<td>LPS, TPA, CB</td>
<td></td>
<td>16 +5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.91 GE</td>
<td>BM/</td>
<td></td>
<td>1 +inv(5)(p13q13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 yrs/S, I</td>
<td>MP6, LPS, TPA, CB</td>
<td></td>
<td>3 del(11)(q13q21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.92 GS</td>
<td>BM/</td>
<td>23/25</td>
<td>8 +inv(5)(p13q13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64 yrs/U</td>
<td>MP6</td>
<td></td>
<td>8 del(11)(q23)+5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.90 LH§</td>
<td>B/</td>
<td>3/13</td>
<td>2 +5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51 yrs/l</td>
<td>LPS, TPA, CB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.92 KK</td>
<td>BM, B/</td>
<td>17/43</td>
<td>1 del(5)(1q13q14)(q13q21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44 yrs/U</td>
<td>MP6</td>
<td></td>
<td>1 del(5)(1q23)(q11q13)(q13q14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.91 GP§</td>
<td>BM, B, LN/</td>
<td>14/36</td>
<td>2 del(12)(p11q22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73 yrs/S, I, P, C</td>
<td></td>
<td></td>
<td>3 t(5;15)(q33-35;q22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.91 DK</td>
<td>BM, B/</td>
<td>21/89</td>
<td>1 del(12)(p11q22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 yrs/l, dCF</td>
<td></td>
<td></td>
<td>2 t(14;7)(q32;7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.90 HEJ§</td>
<td>BM, B/</td>
<td>15/29</td>
<td>1 del(12)(q23;25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 yrs/l</td>
<td>LPS, CB, TPA</td>
<td></td>
<td>1 del(12)(q23;25)+5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 inv(5)(p13q13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 del(11)(q13q21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 add(11)(p7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued on following page)
Table 1. Patients With Constitutional, Clonal, and Nonclonal Recurrent Chromosome Abnormalities (Cont’d)

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Previous Treatment</th>
<th>Cell Source/ Mitogen</th>
<th>No. of Metaphases: Abnormalities/Total</th>
<th>No. Cells With Constitutional, Clonal, and Nonclonal Recurrent Abnormalities</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.87 KS</td>
<td></td>
<td>B/</td>
<td>7/14</td>
<td>3 inv(6)(p11q11-13)</td>
<td></td>
</tr>
<tr>
<td>74 yrs/U</td>
<td></td>
<td>LPS, CB, TPA</td>
<td>2</td>
<td>1 inv(6)(p11q11-13), del(11)(q42)</td>
<td></td>
</tr>
<tr>
<td>35.92 LS</td>
<td></td>
<td>BM, B/</td>
<td>18/34</td>
<td>4 add(11)(p?)</td>
<td></td>
</tr>
<tr>
<td>69 yrs/U</td>
<td></td>
<td>MP6</td>
<td></td>
<td>2 del(15)(q22)</td>
<td></td>
</tr>
<tr>
<td>6.90 JJ†</td>
<td></td>
<td>BM, B/</td>
<td>20/23</td>
<td>17 inv(5)(p15q15-22), t(1;14)(p22;q32), t(4;10)(q7;q7)</td>
<td></td>
</tr>
<tr>
<td>56 yrs/S, I, dCF</td>
<td></td>
<td>LPS, TPA, CB, MP6</td>
<td>3</td>
<td>3 inv(5)(p15q15-22), t(1;14)(p22;q32), t(4;10)(q7;q7), del(21)(p11)</td>
<td></td>
</tr>
<tr>
<td>7.92 ÅW</td>
<td></td>
<td>BM/</td>
<td>8/27</td>
<td>2 del(2)(q11q13), t(13q21)</td>
<td></td>
</tr>
<tr>
<td>64 yrs/U</td>
<td></td>
<td>MP6</td>
<td></td>
<td>1 del(2)(q11q13), t(13q21), del(6)(q25)</td>
<td></td>
</tr>
<tr>
<td>4.92 SGC</td>
<td></td>
<td>BM, B/</td>
<td>9/25</td>
<td>1 inv(19)(p13.3q13.1)</td>
<td></td>
</tr>
<tr>
<td>61 yrs/U</td>
<td></td>
<td>MP6, LPS, TPA</td>
<td></td>
<td>1 del(1)(q21)</td>
<td></td>
</tr>
<tr>
<td>10.91 RB</td>
<td></td>
<td>BM/</td>
<td>13/20</td>
<td>2 dup(13)(q12q13), t(13q14)</td>
<td></td>
</tr>
<tr>
<td>46 yrs/l</td>
<td></td>
<td>MP6</td>
<td></td>
<td>1 del(13)(q12q13), t(13q14), del(1)(p??)</td>
<td></td>
</tr>
<tr>
<td>9.91 SK</td>
<td></td>
<td>BM/</td>
<td>11/26</td>
<td>2 inv(19)(p13.3q13.4)</td>
<td></td>
</tr>
<tr>
<td>48 yrs/l</td>
<td></td>
<td>MP6</td>
<td>3</td>
<td>1 add(11)(p?)</td>
<td></td>
</tr>
<tr>
<td>4.91 NM</td>
<td></td>
<td>BM, B/</td>
<td>9/33</td>
<td>3 inv(19)(p13.3q13.3)</td>
<td></td>
</tr>
<tr>
<td>40 yrs/l</td>
<td></td>
<td>LPS, TPA</td>
<td></td>
<td>1 del(6)(q25)</td>
<td></td>
</tr>
<tr>
<td>12.90 KFJ</td>
<td></td>
<td>BM/</td>
<td>3/11</td>
<td>2 del(20)(q12.1)</td>
<td></td>
</tr>
<tr>
<td>73 yrs/U</td>
<td></td>
<td>LPS, TPA, CB</td>
<td></td>
<td>1 del(19)(p13.3q13.3)</td>
<td></td>
</tr>
<tr>
<td>11.91 LT</td>
<td></td>
<td>BM, B/</td>
<td>7/18</td>
<td>2 del(20)(q12.1)</td>
<td></td>
</tr>
<tr>
<td>58 yrs/U</td>
<td></td>
<td>MP6</td>
<td></td>
<td>1 del(19)(p13.3q13.3)</td>
<td></td>
</tr>
</tbody>
</table>

*Previous treatment: U, untreated; S, splenectomy; I, interferon; dCF, 2'-deoxycoformycin; C, chlorambucil; P, prednisolone.
† Evaluated cell sources and cultures: BM, bone marrow; B, blood; LN, lymph node. LPS, CB, TPA, MP6, PWM are mitogens, see text. The bold font indicates cultures in which clonal abnormalities were identified. Unmarked cultures include those with no metaphases.
‡ The number of abnormal metaphases includes clonal, nonclonal recurrent, and sporadic aberrations, but not constitutional abnormalities.
§ Patients previously reported in ref 3.
¶ Nonclonal aberrations that occur as clonal aberrations in other patients.
To obtain elongated chromosomes of normal T cells from patient 9.90, ethidium bromide (10 pg/mL) during the final 2 hours of a phytohemagglutinin (PHA)-stimulated lymphocyte culture was used.

The cells were harvested according to medium color change over a number of days. In general, cells were harvested on days 4 and/or 5, with additional harvests in some patients after up to 25 days in culture. Colchicine (Demecolcine, Sigma) to a final concentration of 1.25 or 0.125 μmol/L was added 1 hour before harvest. The cells were treated with a hypotonic (75 mmol/L) potassium chloride solution, and fixed in acetic acid/methanol. Slides were, in general, flame dried to improve the spreading of the metaphases.

Cyto genetic analysis. Conventional Q- and G-banding techniques were used.\textsuperscript{11,18} Cyto genetic analysis was performed with a Cytoscan Image analysis system (Applied Imaging, Sunderland, UK). Chromosome abnormalities were given, and clones were defined according to the International System for Human Cytogenetic Nomenclature.\textsuperscript{19,20} Two cells with identical structural abnormalities, two with identical supernumerary chromosomes, or three with the same missing chromosomes constituted a clone. Single cell abnormalities that appeared as clonal changes in other patients were referred to as "nonclonal recurrent" aberrations. We also regarded nonclonal abnormalities involving recurrent regions, but with breakpoints that did not exactly coincide with those of clonal aberrations, as in the case of the pericentric inversions of chromosome 2, as "nonclonal recurrent."

RESULTS

In samples from 6 of the 36 patients (17%) no metaphase was found. Twenty male patients of 30 evaluable (67%) showed clonal abnormalities and another two had nonclonal recurrent aberrations (Table 1). Eight patients showed no clonal abnormalities, but only sporadic chromosome and chromatid aberrations. No evaluable patient lacked chromosomal abnormalities entirely. One patient had a constitutional abnormality that was also found as a clonal change in other patients (see below). Most patients exhibited a large number of different chromosomal abnormalities. The abnormalities were mainly structural, and inversions and deletions were notably more common than translocations. Unstable aberrations, such as dicentrics, rings, and chromatid aberrations also appeared. There were few numerical abnormalities.

The chromosomes preferentially involved in clonal abnormalities were Nos. 1, 2, 5, 6, 11, 19, and 20 (Fig 1). Clonal changes involving chromosomes 4, 12, 13, 14, and 15 were

---

Fig 1. The location of clonal and nonclonal recurrent abnormalities in the most frequently affected chromosomes. *A sporadic chromosome aberration including at least a part of a region involved in clonal abnormalities, although not with exactly coincident breakpoints. **Constitutional inversion of chromosome 5.
CHROMOSOME ABNORMALITIES IN HCL

occasionally found. No correlation between the number of clonal abnormalities and the number of metaphases investigated was observed. Cells with clonal abnormalities were frequently seen in multiple cultures, if available (Table 1).

In our study, MP6 was the single most effective mitogen for hairy cells. No significant impact of previous treatment on the chromosome findings was found, but three of five patients with multiple previous treatment regimens had clonal abnormalities involving chromosome 5 (Table 1).

Chromosome 5. Thirteen of the 30 patients (43%) with evaluable metaphases showed clonal (n = 12) and/or nonclonal recurrent (n = 1) abnormalities of chromosome 5 (Table 1). One patient showed three, and three patients two different clones involving chromosome 5. The most commonly occurring abnormalities were inversions (n = 7 including the constitutional inversion, see below) (Fig 2a and b) and interstitial deletions (n = 4) (Fig 2c), all except one involving band 5q13. Supernumerary normal (n = 4) and derivative (n = 4) chromosomes 5 were also common. An additional patient with a del(5)(q11q13) clone had single cells with trisomy 5 and t(5;13)(q13;q?), respectively, among 89 studied cells. One patient with trisomy 5 had two additional clones with extra chromosome 5 derivatives from a t(1;5) (q42;p15.1) and an inv(5)(p13q13), respectively. Five of the six pericentric inversions included band 5q13 and three of them were found in supernumerary chromosomes (Fig 2b).

Sporadic aberrations also involved the 5q13 region: one terminal deletion in 5q13, the t(5;13)(q13;q?) mentioned above, and one insertion of the segment 5q11-13q22 into a chromosome 8. On the other hand, sporadic translocations involved bands p1, p15, and q35, similar to the sites of clonal translocations (see below).

One additional patient had a constitutional pericentric inversion, with the breakpoints p13.1 and q13.3, as defined by the use of high-resolution banding.16 The inversion was present in all metaphases studied from blood and BM during active disease and in complete remission, as well as from skin fibroblasts, whereas no other clonal aberration was seen in the eleven metaphases achieved from BM when involved with HCL.

Clonal translocations (n = 3) affected the telomeric parts of either arm, with breakpoints in 5p15, 5q31, and 5q33-35.

Chromosome 2. Clonal and/or nonclonal recurrent interstitial deletions (Fig 2d) and inversions involving the pericentric region of chromosome 2 were found in 10 patients (33%). The region common to all these abnormalities was band 2q11 (Fig 1). Because the banding pattern in this region is not very distinct, the exact location of the breakpoints is somewhat uncertain. The true incidence of these aberrations may be higher because of a conservative assessment of the abnormalities.

Chromosome 1. Terminal deletions of chromosome 1 at band q42 were found as clonal and nonclonal aberrations in three and two patients, respectively. A translocation between 1q42 and band p15.1 of a supernumerary chromosome 5 (see above) was also found.

Chromosome 6. Clonal terminal deletions involving band 6q25 occurred in four patients, and as non-clonal abnormalities in another five. One patient exhibited deletions of the long arm with different break points in different cells: q21-23 in one metaphase, q23-25 in two, and q25 in one cell. This patient also had a clonal pericentric inversion comprising most of the chromosome, with breakpoints at p25 and q25.

Chromosome 11. Chromosome 11 was, together with chromosome 5, most frequently involved in clonal abnormalities, such as terminal deletions in band 11q23 and interstitial deletions with the breakpoints q13q21 or q21q23 (Fig 2e). Pericentric inversions, one clonal and one nonclonal recurrent, involving the break points p13-15 and q21-23, occurred in two patients. In addition, eight patients showed additional material, the origin of which could not be identified, on the short arm.
Chromosome 19. Clonal pericentric inversions were found in cells from three patients, and in a further two as nonclonal aberrations (Fig 2f). One patient exhibited a ring chromosome with undefined breakpoints.

Chromosome 20. Clonal terminal deletions of the long arm of chromosome 20, all including the region q13.1 to qter, were found in two patients, and as nonclonal recurrent changes in another two.

Numerical abnormalities. Only a few numerical abnormalities were present, except for the additional chromosomes 5 (see above). Two patients showed an additional del(12)(p11) derivative chromosome, and one patient showed clonally missing chromosomes 7 and 21 together with a marker chromosome.

Clonal break points. Clonal break points, ie, identical breakpoints involved in different aberrations, clonal or nonclonal, in the same patient, were identified in ten patients. Band 5q13 was involved in different abnormalities in two patients. Also chromosomes 11 and 19 showed terminal and interstitial deletions, inversions, and translocations with coincident break points. One patient showed a deletion in 6q25 and a chromatid break in the same band.

Multiple clones. The frequency of cells with clonal abnormalities varied greatly, from 20 of 23 cells (87%) in one patient to zero in others. Fifteen patients had more than one clonal aberration (Fig 3) and 14 of these exhibited distinct multiple clones. Multiple clonal abnormalities often coexisted in different combinations in different metaphases. This is exemplified by one patient with 17 aberrant metaphases of 43 studied; 10 of these contained six different clonal abnormalities in various combinations with other abnormalities, so that no two metaphases had identical karyotype.

Constitutional abnormalities. Two patients were found to have constitutional abnormalities, both documented through karyotyping of skin fibroblasts. One patient had an inv(5)(p13.1q13.3), without other clonal changes (see above). The second patient had a Robertsonian translocation between chromosomes 13 and 14, and additionally a clonal pericentric inversion of chromosome 2 in leukemic cells.

DISCUSSION

Our investigation presented a high frequency of clonal abnormalities in HCL. These were in part abnormalities that are common in different hematologic tumors, and previously described in HCL. However, some of the most frequent abnormalities in our study are rare in other types of leukemias. In contrast to the case in most other hematologic tumors, the structural abnormalities were mostly caused by inversions and deletions, but rarely translocations. Multiple clones and different types of abnormalities involving the same chromosome region in individual patients were other characteristic features.

In previously published investigations comprising more than 150 HCL patients, one fourth of the patients that were evaluated, had clonal abnormalities, as compared with two thirds in our study. Recently, fluorescence in situ hybridization techniques, permitting sensitive detection of numerical chromosome abnormalities in interphase cells, have been applied to 24 cases of HCL, without significant findings. However, no probe for the detection of chromosome 5 abnormalities was used. In our study, abnormalities involving chromosomes 1, 2, and 5 were the most significant findings. These were typically trisomy 5, structural abnormalities involving band 5q13, pericentric inversions and deletions of chromosome 2, and structural abnormalities of band 1q42.

Structural abnormalities of chromosome 5 are previously reported in three HCL cases; in two of them band p1 was involved. A clonal 5q13.3 breakpoint is reported in one patient with a variant form of HCL as part of a three-way translocation, t(1;5;10)(p34.3;q13.3;q22.1), with an additional t(5;14)(q31.1;q11) sub-clone. Trisomy 5, pericentric inversions of chromosome 5, and other structural abnormalities involving band 5q13 are rare in malignant lymphoma and in chronic lymphocytic leukemia (CLL). The malignancy most closely related to HCL. Of 512 CLL patients with clonal abnormalities included in the database of the International Working Party on Chromosomes in CLL, 14 (2.7%) involved chromosome 5, mostly as t(1;5) translocations. No single CLL patient had 5p13 abnormalities or pericentric inversions of chromosome 5. Trisomy 5 was found in conjunction with other trisomies in 1 CLL patient, and clonal breaks in band 5q13 were found in 3 (0.6%) (G. Juliusson, personal observation, 1993). In similar, int del(2), inv(2)(p11q13), and del(1)(q42) are reported in single cases of CLL only. Deletions of 5q, on the other hand, are characteristic features of the myelodysplastic syndromes and secondary myeloid leukemias. However, in these tumors the abnormalities always involve the distal part of the long arm, from q22 to q33, a region encoding several cytokines and cytokine receptors.

Among numerical abnormalities in HCL, trisomy 3 and 21, and monosomy 7, 10, and 12, and 17 have been reported. In our study, trisomy 5 was the only recurrent numerical abnormality; in addition, one patient showed monosomy 7 and 21.

Trisomy 12 is the most common abnormality in CLL, whereas structural abnormalities involving this chromosome are less frequent. In HCL, trisomy 12 was the
first identified chromosome abnormality,6,4 but thereafter it has been found in single patients only.11,21 Structural abnormalities on the short arm of chromosome 12, with breakpoint points in 12p13 and p11 were reported by Brito-Babapulle et al,10 and + del(12)(p11) was also found twice in our study. Of specific interest was that both these patients with partial trisomy 12 also had chromosome 5 and 14q abnormalities (patients GP and JF), and their HCL showed CLL-like features as regards the phenotype with lack of CD25 expression and/or CD5-positivity, high blood lymphoid cell counts, and poor response to IFN and deoxycoformycin.5

Previous studies have indicated recurrent 14q32 translocations and terminal deletions of chromosome 14 with breakpoints in the central part of the long arm.4,10,22 We found two patients with clonal chromosome 14 abnormalities (above). Such abnormalities are found in all B-cell malignancies, and are likely to be unspecific changes in HCL as well as in CLL.11 Deletions of the long arms of chromosome 6,10,22 and chromosome 1110,22 in HCL are also characteristic findings in CLL11,15 and lymphomas.29 Deletions at 11q14 and of 20q occur in myeloid disorders.29 19q13, a recurrent breakpoint, is the site of the bcl-3 oncogene,30 which was cloned from a rare but recurrent translocation t(14;19)(q32;q13) in CLL.31 A few other previously reported clonal abnormalities10 were found in single cells from our patients, ie, del(2)(q33-35), del(9)(q22), and del(18)(q21). The nonclonal recurrent abnormalities in our material might well indicate clones that were not detected because of a limited number of metaphases analyzed.

A characteristic feature of HCL cells was the presence of distinct multiple clones with mostly unrelated abnormalities that occurred in different combinations in different cells (Table 1), without evidence for a karyotypic evolution. In our material, nearly half (14 of 30) of the patients showed unrelated and partially related clones. Cyogenetically unrelated clones have been reported in a number of hematologic neoplasms.32-35 They have been estimated to be most common in chronic lymphoproliferative disorders,35 ie, found in approximately 7% of the cases. The theory put forward by Heim and Mitelman30 that submicroscopic abnormalities link the different clones together could explain some, but probably not all, of the clonal patterns seen in our patients.

Specific chromosome regions were also often involved in more than one kind of aberration. As an example, a segment of chromosome 1, ie, q12q21, was involved in three different aberrations in the same patient, namely inserted at another location in chromosome 1 in one metaphase, inserted into chromosome 2 at the critical region q11-13 in another metaphase, and interstitially deleted in a third metaphase. "Jumping translocations" have been described in a few cases of leukemias and lymphomas, mainly of the B-cell type.46-38

The probability of an identical translocation, ie, an abnormality with two breakpoints, to occur independently in two different cells has been estimated to be $5 \times 10^{-11}$ at the resolution of the banding pattern used in the present investigation.39 These data may point to the presence of a common genetically unstable progenitor cell.

Tumorigenesis is considered to be a multistep process. It has been estimated that five to six independent steps are necessary for tumor formation,40 exemplified by the consistent, progressive accumulation of certain abnormalities in colon carcinoma: a RAS gene mutation, del(5q), del(17p), and del(18q).41 The recurring chromosomal abnormalities found in this study of HCL may enable the identification of some important gene loci.

The 5q13.3 band is likely to harbor candidate genes involved in some transforming event of HCL. This is suggested by the frequent occurrence of structural abnormalities involving this region, and the rare constitutional inv(5) (p13.1q13.3) abnormality found in our patient TÖ. The incidence of constitutional abnormalities is reported to be about 0.6% among almost twelve thousand newborn babies.42 Most aberrations were numerical, and balanced structural abnormalities affecting autosomal chromosomes were found in 0.2%,42 most commonly as Robertsonian translocations involving two of the chromosomes 13, 14, 21, and 22, as well as in our patient AW. Constitutional inversions have been found in 0.02% of 67,000 newborns,42 and in 0.03% of more than 3,000 adult patients with hematologic diseases.43 None of these reported inversions involved chromosome 5, and thus the constitutional inversion of our patient TÖ is extremely rare.

The RASA gene45 and the dihydrofolate reductase gene (DHFR),46 both located to 5q13, might be involved in the process leading to chromosome instability and neoplasia. RASA encodes the GAP protein, a GTPase activating protein that binds to RAS p21 proteins, and is implicated as a recessive oncogene.47 Mutant RAS genes may also induce structural chromosome instability.46 The RAS genes are involved in the control of cell proliferation, and can be activated in most types of human tumors at varying frequencies.48 More than 30% of human tumors contain a mutation in one of the RAS genes, N-ras, H-ras, and K-ras,49 located at 1p13, 11p15.5, and 12p12.1, respectively. Another RAS gene, RAB4, is located at 1q42.1-4.50 Seventeen of our 30 patients (57%) showed abnormalities, clonal or nonclonal, in 1q42, 5q13, the 11p, or 12p regions. Therefore, studies of RAS or RASA genes in HCL would be justified.

An inhibition of DHFR, also located to 5q13, could inhibit the production of thymidine monophosphate, and the resulting imbalance of the nucleotide pool in the cell may cause an increase of chromosome abnormalities and aneuploidy,51 and also affect the induction of folate-deficient fragile sites.52 Folate-induced fragile sites coinciding with recurrent HCL breakpoints are located at 2q11.2 and q13, 11q13.3 and q23.3, and 19p13.53 Twenty-four of our patients (80%) had clonal or nonclonal abnormalities involving at least one of these sites or 5q13.

In conclusion, our study has identified certain chromosome regions not randomly associated with HCL. Some of them seem common to a variety of hematologic tumors, whereas others, involving bands 1q42, 2q11, and 5q13, seem characteristic for HCL. Molecular studies of genes localized to these regions and their corresponding proteins might enable the identification of tumorigenic mechanisms. HCL is probably the systemic malignancy with the best treatment options, and it would be worthwhile to elucidate genetic mechanisms that distinguish HCL from other tumors.
REFERENCES


Hairy cell leukemia is characterized by clonal chromosome abnormalities clustered to specific regions

U Haglund, G Juliusson, B Stellan and G Gahrton