Unusual Deletions Within the Immunoglobulin Heavy-Chain Locus in Acute Leukemias

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We have investigated the structure of the Ig heavy (IGH) chain locus in 309 cases of acute leukemia. Seventy-one cases of B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) were analyzed: in six cases deletion of joining (JH) segments in the presence of cytogenetically normal chromosome 14 was observed. Similar deletions were seen in 1 out of 8 cases of biphenotypic acute leukemia analyzed: this case exhibited t(9;22)(q34;q11) and expressed both myeloid and B cell differentiation antigens. Five of the 7 cases analyzed had deleted the JH segments from both chromosomes. Because these deletions may have contributed to the pathogenesis of the disease we have attempted to define their boundaries. Using probes that map both 5' and 3' of JH, the 3' (centromeric) boundary of the deletions was mapped to an approximately 30-kb central region of the 60 kb between C3 and C1 in 10 of the 12 deleted chromosomes. In the remaining two chromosomes, the 3' boundary mapped to SU. The 5' (telomeric) boundary could not be defined. However, three cases with biallelic deletion of JH showed biallelic deletion of the most proximal variable (VH) (VH6 and VH5-82) genes, indicating that the deletions spanned over 500 kb. VH6-51 and VH5-83 were retained in germline configuration and no gross deletions were observed using a VH3 subgroup-specific probe, indicating that the 5' boundary mapped within the VH locus. Unusual deletions of the portion of the IGH locus including JH segments and the Cβ and Cδ genes may occur in acute leukemias with immunophenotypic evidence of commitment to the B cell differentiation pathway. The possible consequences of the deletions remain to be determined. However, the clustering of the centromeric boundary of the deletions to SU and to a region between the Cβ-Cγ3 genes, a known “hot spot” for recombination, may indicate the operation of a distinct pathogenic mechanism.

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T HE GENES encoding the receptors for antigen (Ig in B cells and T-cell receptors [TCR] in T cells), are assembled during lymphocyte differentiation from widely dispersed DNA sequences, by somatic recombination.1 The human Ig heavy (IGH) chain locus maps to chromosome 14q32.3 and consists of variable (VH), diversity (DH), joining (JH), and constant (CH) region genes.2 Formation of a functional IGH gene results initially from apposition of VH, DH, and JH segments.3 The Vp-DH5-JH unit may be subsequently transposed to any of the further downstream, (centromeric) CH genes through homologous recombination of the switch regions, situated immediately 5' of the CH genes. The CH genes located between recombined switch regions are deleted. This process is referred to as class-switching.5 Errors during the processes of creation of a VDJ unit and in class-switching may be of central importance in the pathogenesis of some B-cell malignancies resulting in chromosomal translocations and deregulation of genes that control cell proliferation and differentiation.6,7

As part of our diagnostic assessment of acute leukemias, we have investigated the configuration of the IGH locus using JH and CH probes in 309 cases.6–10 We and others1,11–12 have noted that some cases of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and biphenotypic acute leukemias with immunophenotypic evidence for commitment to the B-cell differentiation pathway may have deletions of the JH segments of either one or both chromosomes. In cases in which adequate cytogenetic data were available it was apparent that these deletions could not be caused by simple chromosomal loss, because all cases had cytogenetically normal chromosome 14s. Therefore, we have sought to define the extent and the consequences of these deletions within 14q32.3 in acute leukemias. We report the preliminary data on the mapping of the extent of the deletions.

MATERIALS AND METHODS

Patient Material

Peripheral blood (PB) and bone marrow (BM) samples were obtained at diagnosis and in three cases at remission after obtaining informed patient consent. Mononuclear cell fractions were prepared by centrifugation over Ficoll-Hypaque (1.077 g/cm3) (Sigma, St Louis, MO). Cells were washed three times in ice-cold phosphate-buffered saline (PBS) before further processing. Unfractionated PB from normal donors was used as a source of control DNA.

Immunophenotyping

Immunophenotyping was performed using a panel of monoclonal antibodies (MoAbs) to T-cell, B-cell, myeloid and non-lineage-specific differentiation antigens as previously described.10 Analysis was performed by flow cytometry and by immunocytochemistry to detect both cell-surface and cytoplasmic-antigen expression. In certain cases, immunoelectronmicroscopy was used to detect myeloperoxidase expression.11

Genotypic Analysis

High-molecular-weight DNA was prepared from mononuclear cells by conventional methods and digested to completion with restriction endonucleases for 2 hours at 37°C. Restriction endonucleases used in all samples were EcoRI, HindIII, and BamHI. Other restriction enzymes used in this study included BglII, SacI, PstI, and KpnI. DNA fragments were electrophoresed in 0.6% to 0.8% agarose and were transferred to positively-charged nylon membranes (Hybond N*; Amersham, UK) in 1.5 mol/L NaCl, 0.25 mol/L sNa, 1% sodium dodecyl sulfate, 100 mmol/L Tris (pH 8.3). The membranes were hybridized overnight at 42°C with a radiolabeled probe, and autoradiography was performed for 1–4 days. The probes used were a 2.6-kilobase pair (kb) fragment of human Ig heavy (IGH) chain locus. Unusual deletions of the portion of the IGH locus including JH segments and the Cβ and Cδ genes may occur in acute leukemias with immunophenotypic evidence of commitment to the B cell differentiation pathway. The possible consequences of the deletions remain to be determined. However, the clustering of the centromeric boundary of the deletions to SU and to a region between the Cβ-Cγ3 genes, a known “hot spot” for recombination, may indicate the operation of a distinct pathogenic mechanism.
mol/L NaOH by overnight capillary transfer. Hybridization to 32P-labeled probes was performed in 0.5 mol/L sodium phosphate, pH 7.2, 10% (wt/vol) sodium dodecyl sulfate (SDS), 10 mol/L EDTA in a hybridization incubator (Technne, Duxford, UK), and washed to a final stringency of 0.1 × SSC (SSC is 0.15 mol/L NaCl and 0.015 mol/L sodium citrate) at 65°C for 10 minutes. Autoradiography was performed with intensifying screens at -80°C for 16 to 72 hours. Reprobing of filters was performed after stripping filters of radioactive probe by 60-second exposure to 1.5 mol/L NaCl, 0.5 mol/L NaOH, and washing in distilled water.

To determine whether deletions involved one or both alleles, filters were hybridized simultaneously with a probe within the IGH locus and with D14S20, an anonymous polymorphic DNA probe that maps to the telomere of chromosome 14, telomeric of the IGH locus. The total number of counts in each radioactive band was calculated by placing the labeled filter in a y-particle wire-detection system ("Autograph"; Oxford Positron, UK). The ratio of counts (IGH/D14S20) in the test samples was then compared with the ratio observed in DNA extracted from the PB of a normal individual.

The majority of probes used in this study are shown in Fig 1. The derivation and full description of the genomic DNA probes used in this study may be found in the following references:

1. DQ52/5'JH clone, a BamHI-PstI 2.1-kb fragment.
2. JH clone (C76R51A) spanning JH3 to the distal HindIII site.
3. Cp exons 1 to 3 (C57R4), a 1.2-kb EcoRI fragment.
4. 5'6 (pCW35), a 2.0-kb PstI fragment.
5. YC6 (pMBW1), a 1.1-kb BamHI fragment.
6. 3'C6 (phage clone 706.1), a 17.6-kb HindIII fragment cloned into Charon 35.
7. Cy3 (pSy3h), a 0.6-kb Sac1 fragment derived from the four hinge regions of Cy3 that shows only a small amount of cross-hybridization with other Cy genes.
8. Probe A is a 7.6-kb BamHI fragment situated 5' of Cy3 subcloned into pBluescript from cos Ig6.
9. A BamHI-HindIII fragment (probe B) corresponding to the Iγ3 region has also been derived from this cosmid.
10. Other probes used in this study not shown in Fig 1 included: (9) A Vγ6-specific probe, the Vγ6 gene maps approximately 90 kb telomeric of Jγ2.
11. A Vγ5-subgroup-specific probe. Three Vγ5 genes have been identified that lie approximately 400, 900, and 1100 kb telomeric of Jγ. These correspond to 16.0-, 10.3-, and 5.6-kb fragments seen in HindIII digests on conventional DNA blotting.

RESULTS

Frequency of JH Deletions in Acute Leukemias

As part of our routine diagnostic assessment we have analyzed the configuration of the IGH locus in 309 cases of
acutel. Diagnosis was made on cytologic and cytochemical appearances, supplemented by detailed immunophenotypic analysis of both cell surface and cytoplasmic antigen expression as previously described.  

Bi-phenotypic leukemias were defined by the concurrent expression of multiple antigens of more than one hematopoietic lineage on individual blasts: a weighted scoring system was used to differentiate reproducibly possible biph- enotypic cases from leukemias which expressed "unexpected" differentiation antigens, such as CD7+ acute myeloid leukemia (AML) and ALL that expressed single myeloid anti- gens.

Using these criteria, the cases analysed comprised 210 cases of AML, 71 cases of BCP-ALL, and 20 cases of T-cell precursor ALL (TCP-ALL): eight cases were considered to be bipheno- typic acute leukemia. Of the 71 cases of BCP-ALL, 8 exhibited obvious deletions of one or both JH segments. Four cases had deleted both alleles of JH (Table 1: cases 1 through 4), whereas 4 had deleted a single JH allele: of this latter group in only 2 cases were cytogenetic data available and therefore only 2 of these 4 were studied in further detail (Table 1: cases 6 and 7).

One of the 8 bipheno- typic leukemias studied exhibited biallelic deletion of JH (Table 1: case 5). This case, which had the cytologic appearances of an undifferentiated, Sudan Black-positive AML, and coexpressed abundant myeloid antigens (CD13, CD33, and myeloperoxidase), was considered bipheno- typic by the simultaneous expression of both CD10 and CD19 indicative of commitment to both myeloid and B cell differentiation pathways. Otherwise neither monoallelic nor biallelic JH deletions were observed in any of the cases of AML or TCP-ALL studied. Because these deletions may have contributed to the pathogenesis of the disease, we therefore attempted to map their extent within the IGH locus in the seven cases in which cytogenetic data indicated the presence of cytogenetically intact chromosome 14.

### Characteristics of Patients With JH Deletions

Patient characteristics are summarized in Table 1. The following features are of note. Firstly, JH deletions were observed both in pediatric and adult cases. All patients attained complete remission. Although all the adult patients subsequently relapsed and died, both pediatric patients (patients 2 and 6 in Table 1) remain in remission 30 and 20 months after diagnosis, respectively. Therefore, it is unlikely that JH deletions are associated with a specific prognostic subgroup. This is reflected in the cytogenetic diversity observed in these cases. All cases retained two copies of cytogenetically normal chromosome 14. The case of bipheno- typic acute leukemia had the t(9;22)(q34;q11) translocation with rearrangement of the BCR gene detected on conventional DNA blot; however, there was no docu- mented antecedent chronic myeloid leukemia (CML). Trisomy 21 was seen in three cases: in one case this was associated with Down's syndrome. In our series of 71 cases of BCP-ALL of 30 cases examined, 10 had rearrangement and/or deletion of Cx genes whereas five had rearrangement of Ca genes. However, 6 of 7 cases with JH deletions had rearrangement and/or deletion of the Ig light-chain genes. The somatic origin of these deletions was shown by the presence of normal-sized JH fragments in DNA from remis- sion BM samples in cases 1, 2, and 7. Also, in case 1 it was possible to derive phenotypically normal polyclonal B-cells by Epstein-Barr viral transformation of a remission sample (data not shown).

### Mapping the Extent of the Deletions Within the IGH Locus

The extent of the deletions within the IGH locus on 14q32.3 was mapped using the probes shown in Fig 1. Representative conventional Southern DNA blots are shown in Figs 2 and 3.

**Definition of the 3'centromeric) boundary.** Of the 7 cases examined, 10 of the 14 IGH loci showed deletion of

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### Table 1. Summary of Data on Patients With Deletions Within the IGH Locus

<table>
<thead>
<tr>
<th>Case/ Material</th>
<th>Age/ Sex</th>
<th>WCC</th>
<th>Cytogenetics</th>
<th>5'IgH Locus</th>
<th>IgL Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 PB/BM</td>
<td>17/M</td>
<td>155</td>
<td>Trisomy 21</td>
<td>D/D</td>
<td>G/R</td>
</tr>
<tr>
<td>2 PB/BM</td>
<td>13/F</td>
<td>75</td>
<td>Hyperdiploid</td>
<td>D/D</td>
<td>G/R</td>
</tr>
<tr>
<td>3 BM</td>
<td>42/M</td>
<td>2.5</td>
<td>Hyperdiploid</td>
<td>D/D</td>
<td>G/R</td>
</tr>
<tr>
<td>4 PB</td>
<td>35/M</td>
<td>259</td>
<td>Trisomy 21</td>
<td>D/D</td>
<td>G/R</td>
</tr>
<tr>
<td>5 PB</td>
<td>46/F</td>
<td>87</td>
<td>t(9;22)(q34;q11)</td>
<td>D/D</td>
<td>G/R</td>
</tr>
<tr>
<td>6 PB</td>
<td>7/F</td>
<td>35</td>
<td>t(7;9)(q34;q12)</td>
<td>N/T</td>
<td>G/R</td>
</tr>
<tr>
<td>7 PB</td>
<td>37/M</td>
<td>160</td>
<td>46,XY</td>
<td>N/T</td>
<td>G/R</td>
</tr>
</tbody>
</table>

All cases with the exception of case 5 had the immunophenotype of BCP-ALL, ie, CD19+ CD10+ TdT+ clq+. Case 5 coexpressed both myeloid and B cell differentiation antigens as well as t(9;22)(q34;q11) and was therefore classified as a bipheno- typic acute leukemia. Case 5 was studied as indicated: identical results were obtained from both sources in cases 1 and 2 but in case 5 a clear rearranged Cx fragment was observed in BM but not in the corresponding blood sample. Remission samples from patients 1, 2, and 6 showed normal configuration of JH and Ca sequences.

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...both JH and Cμ. However, two loci (cases 1 and 5, Table 1) retained Cμ in a rearranged configuration. These rearrangements were detected in both HindIII and XbaI restriction digests, indicating that rearrangement to Sμ had occurred. The biphenotypic leukemia had different configuration of Cμ in PB and BM samples. In the BM sample, a clear rearranged Cμ fragment was observed, whereas the same fragment was not detected in the PB. The reasons for this difference are obscure but may reflect continuing rearrangement. Variation in the configuration of the IGH locus between blood and BM samples has been reported previously.29

All cases with deletion of Cμ exhibited deletion of all Cδ sequences. No rearrangement of three probes that map to Cδ and its immediate 3' region was observed in any case. Therefore, the configuration of the closest 3' (centromeric) Cδ gene was examined using a probe specific for Cγ3.20 From pulsed-field data it has been estimated that Cγ3 maps about 60 kb 3' of Cδ (Fig 1). No rearrangements of Cγ3 were detected in any of the cases with a wide range of enzyme digests. Using simultaneous hybridization with the Cγ3 and a probe outside the IGH locus, quantitative imaging showed that both alleles of Cγ3 were retained (Fig 3). Therefore, all deletions that involved both JH and Cμ mapped between Cδ and Cγ3. This region has not been completely cloned in man. Probes to the Iγ3 region (the region from which transcription of Cγ3 initiates in the absence of class-switching22) and to a region further 5' were derived from a previously isolated cosmid clone, coslg6.21 Again both probes retained germline configuration in a variety of enzyme digests. Isolation of further informative 5' probes has been hampered by the presence of highly repetitive DNA.

Therefore, these data indicate that in all cases studied the 3' boundary of the deletions mapped to the central and thus far uncloned region of about 30 kb of the 60-kb region between Cδ and Cγ3 or more rarely to Sμ. The Vμ region was then investigated using probes specific for Vμ3, 4, 5, and 6 gene subgroups: these sequences are dispersed over at least 2,500 kb on chromosome 14q32.23 The Vμ6 gene is located 90 kb 3' of JH and is the single member of the subgroup: all five cases with biallelic JH deletion showed biallelic deletion of both the 5' region of JH and the most proximal Dμ region.24 A variety of probes were used in an attempt to determine the telomeric boundary. Firstly, a probe outside the JH locus that has been mapped close to the telomere of chromosome 14 within the 14q32.3 band (D14S20) was used: both copies of this were retained in all cases (Fig 3).
specific probe, V\textsubscript{H} 26.8, showed no gross deletions of this region (data not shown).

Therefore, these data indicate that the telomeric boundary of the deletions in cases with biallelic J\textsubscript{H} deletions resides within the V\textsubscript{H} locus, between V\textsubscript{H}5-B2 and V\textsubscript{H}5-B3, i.e., between 400 and 900 kb distant from J\textsubscript{H}.

**DISCUSSION**

We have shown that a subgroup BCP-ALL (6 of 7 cases analyzed in this study) and some cases of biphenotypic acute leukemia with cytogenetically normal chromosome 14 may have deleted a crucial segment of the IGH locus at 14q32.3. In about half the cases, the deletions involved both alleles. Similar results have been reported recently by Beisshuen et al.\textsuperscript{11} From our survey of acute and chronic leukemias it appears that such deletions occur only in acute leukemias of precursor cells that have made some commitment to the B-cell lineage; similar deletions in the absence of structural cytogenetic abnormalities of chromosome 14 were not observed in TCP-ALL, AML, or in a range of chronic leukemias of mature B-cells\textsuperscript{8-10} (and M.J.S. Dyer, H. Kayano, D. Jadayel, unpublished observations, 1993). The restricted distribution and the clustering of deletion breakpoints to either S\textsubscript{\mu} or to the central 30-kb region between C\textsubscript{\delta} and C\gamma\textsubscript{3} suggest that the deletions arose as a consequence of a specific mechanism operating on a subset of B-cell precursors.
The precise extent, nature, and any possible biologic significance of such deletions await molecular cloning experiments. These experiments have so far been hampered by the lack of probes spanning the area between Cα and Cy3 and by the highly repetitive nature of the DNA in this region. The corresponding region in the mouse (which is also 60-kb long) has been cloned in bacteriophage32, neither coding nor switch regions were identified and thus the functional significance, if any, of the region remains to be determined. Using single-copy probes derived from the mouse bacteriophage clones, we have failed to identify any homologous sequences within the human Cα-Cy3 region (J.M.H., M.J.S.D.; unpublished observations, 1992). Interestingly, genetic linkage analysis has shown that the region between Cα and Cy3 is a “hot spot” for recombination in humans.33

Unlike the B cell non-Hodgkin lymphomas, the IGH locus is not commonly a target for chromosomal translocations in BCP-ALL.32 Translocations involving 14q32.3 in pediatric leukemias have been associated with mixed lineage cases and have been shown not to involve the JH sequences directly, leading to the suggestion that an unidentified gene of importance in leukemogenesis may reside on 14q32.3.33 Whether the deletions described here result in the aberrant expression of other genes within the IGH locus in the 14q32.3 chromosomal band, and whether this expression is of importance in the pathogenesis of BCP-ALL, remains to be determined.

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