Immunophenotypic and Cytogenetic Analysis of Molt-3 and Molt-4: Human T-Lymphoid Cell Lines With Rearrangement of Chromosome 7

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Molt-3 and Molt-4 are T-cell lines originally derived in 1971 from a patient with T-cell acute lymphoblastic leukemia. An unusual T-cell antigen receptor γ-chain gene (T-gamma) rearrangement detected by Southern blot analysis of Molt-4 prompted an in-depth study of the immunophenotype and karyotype of both cell lines. Molt-3 and Molt-4 had immunophenotypic characteristics of thymocytes with expression of CD1 and CD5. Both cell lines had a hyper-tetraploid karyotype with two rearranged no. 7 chromosomes: 2der(7)t(7;7)(p15;q11). The presence of a break in chromosome band 7p15 suggested the involvement of T gamma. We cloned the rearranged BamHI fragments spanning the known T-gamma constant and joining regions. Comparison with germline clones of T gamma did not suggest any of the clones included a breakpoint region. Thus the 7p15 chromosomal abnormality in Molt-3 and Molt-4 is not associated with the currently described joining and constant regions of T gamma.

In this report, we describe a chromosomal rearrangement involving chromosome 7, band p15, in the T-lymphoid leukemic cell lines Molt-3 and Molt-4. The presence of the rearrangement in two independent cell lines established from the same patient suggests that it played a role in the pathogenesis of the leukemia that produced these cell lines.

An unusual rearrangement of T gamma observed in our Molt-4 cell line led us to analyze clones containing joining and constant regions of the gene in a search for the breakpoint region of the chromosomal translocation. No breakpoint was identified, suggesting that the rearrangement was due to a point mutation in our Molt-4 cells.

MATERIALS AND METHODS

Cell culture. The Molt-3 and Molt-4 cell lines were kindly provided by J. Minowada (Fujisaki Cell Center, Fujisaki, Japan). Both cell lines were originally derived from the leukemic cells of the same individual.27 Cells were maintained in RPMI 1640 cell culture medium supplemented with 10% fetal calf serum (Gibco Laboratories, Lawrence, MA), penicillin (100 IU/mL), streptomycin (100 mg/mL), and L-glutamine (20 mmol/L). Cultures were maintained at 37°C in 5% CO2 and 95% humidified ambient air.

Immunophenotypic analysis. Each cell line was analyzed for the presence of cell surface antigens associated with lymphoid development and differentiation. The antibodies used are listed in Table 1. Those antibodies with cluster designations28 are noted. Immunophenotypic analysis was performed by indirect immunofluorescence with either an Ortho Spectrum III or a FACS IV (Beckton-Dickinson, Sunnyvale, CA) as previously described.29

Cytogenetic analysis. Metaphase chromosome spreads were obtained from Molt-3 and Molt-4 cultures using previously described methods.30 Briefly, cells were received at a concentration of 10^6/mL. Direct preparations were performed using 1 mL of the cell suspension, and the remaining cells were diluted 1:10 (and also 1:20 for Molt-3) in RPMI 1640 medium supplemented with 20% fetal bovine serum and L-glutamine (29 mg/mL). Chromosomes were harvested from the diluted specimens directly and after 24 additional hours in culture by exposing them to Colcemid (0.7 μg/mL) for 20 minutes and the 0.075 M KCl for 12 minutes at 37°C. The preparations were fixed in 3:1 methanol to glacial acetic acid, and slides were made in the usual fashion. Chromosomes were G-banded using the Wright’s technique of Sanchez et al.31 A total of ten metaphase cells from the 24-hour cultures of Molt-3 and ten from the diluted direct preparations of Molt-4 were fully analyzed, as these were the processes that yielded the best quality chromo-
clones. Restriction sites: EcoRI joining regions of T gamma: J1.3 and J2.3.2933 T-gamma J1.3 beneath its restriction map. The constant and joining regions. The size of each clone is constant region DNA probes are shown beneath the germline Molt-4. indicates the site of the new EcoRI and CD8 antigens. CD4 and CD5. Molt-3 was positive for the sheep erythrocyte cytes (Table 1). Both cell lines had strong expression of CD1 and CD5. Molt-3 was positive for the sheep erythrocyte receptor (CD2) as detected by the monoclonal antibody 35.1, while Molt-4 failed to express this marker. Both cell lines were originally reported to form rosettes with sheep erythrocytes. Both cell lines expressed low to moderate levels of the CD4 and CD8 antigens. The expression of the CD7 antigen was much stronger in Molt-3 cells than in Molt-4 cells. Neither cell line had immunophenotypic characteristics of a B-lymphoid or myeloid lineage.

Southern blot analysis. High molecular weight (mol wt) DNA was size-fractionated by agarose electrophoresis, blotted to nylon membranes, and hybridized with radioactive DNA probes as described previously. DNA from placenta was used as a germline control. Restriction fragment size was determined by comparison with HindIII digested λ-phage DNA.

Molecular cloning. Molt-4 or placental DNA was digested to completion with BamHI and ligated to the arms of the replacement vector EMBL-3. After packaging and titering the subgenomic library, ~150,000 plaques were screened with a genomic DNA probe to the constant region of T gamma (see below) according to the method of Maniatis et al. Positive plaques were purified by rescreening. Phage DNA was extracted and the inserts mapped as described in the Results section.

Probes. T gamma was analyzed with three genomic DNA probes initially provided by Drs J.G. Seidman and Thomas Quertermous, Department of Genetics, Harvard Medical School. T-gamma C recognizes constant region sequences common to both constant regions of T gamma.29 T-gamma J1.3 detects the two downstream joining regions of T gamma: J1.3 and J2.3.30,31 T-gamma J1.1 recognizes the two upstream joining regions of T-gamma: J1.1 and J2.1.31 The specific sites recognized by these probes are shown in Fig 1. T-gamma V1 is a genomic variable region probe that hybridizes to all members of the V-gamma 1 subgroup of variable regions.32,33 This probe was used to map the location of the variable regions shown in Fig 1.

RESULTS

Immunophenotypic analysis. Molt-3 and Molt-4 had immunophenotypic characteristics corresponding to thymocytes (Table 1). Both cell lines had strong expression of CD1 and CD5. Molt-3 was positive for the sheep erythrocyte receptor (CD2) as detected by the monoclonal antibody 35.1, while Molt-4 failed to express this marker. Both cell lines were originally reported to form rosettes with sheep erythrocytes. Both cell lines expressed low to moderate levels of the CD4 and CD8 antigens. The expression of the CD7 antigen was much stronger in Molt-3 cells than in Molt-4 cells. Neither cell line had immunophenotypic characteristics of a B-lymphoid or myeloid lineage.

Cytogenetics. A representative G-banded karyotype of one metaphase cell from the Molt-4 line is shown in Fig 2. This cell line is hypertetraploid with a modal number of 96 chromosomes, including two X and two Y chromosomes. It has structural as well as numerical abnormalities. All cells have one extra no. 4, 8, 11, and 15 chromosomes, two extra no. 20 chromosomes, and loss of two no. 18 chromosomes. There are interstitial deletions of part of the long arms of one no. 1 and one no. 6 chromosome, an isochromosome composed of two copies of the long arm of 17 joined at a single centromere, and rearrangements of two of the no. 7 chromosomes. The latter consist of extra copies of the long arm of chromosome 7 (breaks in band 7q11) attached to the short arms of the two no. 7 chromosomes (breaks in bands 7pl5:2der(7)(7;7)(p15;q11)).

The karyotype of the Molt-3 cell line is very similar to that of Molt-4. It is hypertetraploid with a modal number of 95 chromosomes, including two X and two Y chromosomes. There are one extra no. 6 and no. 20 and two extra no. 8 chromosomes, and there is loss of one no. 9 chromosome. Structural rearrangements include an unbalanced translocation involving the long arm of one no. 2 and an extra copy of the short arm of chromosome 2 (der(2)t(2;2)(p15;q11)), interstitial deletions of part of the long arms of two no. 6 chromosomes (2del(6)(q13q21)), and rearrangements of two no. 7 chromosomes that are identical to those seen in Molt-4 (2der(7)(7;7)(p15;q11)).

The rearrangements of chromosome 7 in these two cell lines are of particular interest because they involve a break in band 7p15. The T-gamma locus has previously been mapped to this chromosomal band.14

Southern analysis. Because the chromosomal band to which the T-gamma locus has been mapped, 7p15, is involved in the rearrangement of two of the no. 7 chromosomes in Molt-3 and Molt-4 cells, we performed Southern

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Table 1. Immunophenotypic Characteristics of the Molt-3 and Molt-4 Cell Lines

<table>
<thead>
<tr>
<th>Cluster Designation/Antibody</th>
<th>Molt-3</th>
<th>Molt-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1/OKT6</td>
<td>92.7*</td>
<td>96.0</td>
</tr>
<tr>
<td>CD2/35.1</td>
<td>72.9</td>
<td>1.5</td>
</tr>
<tr>
<td>CD3/OKT3</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>CD4/Leu3</td>
<td>23.9</td>
<td>51.8</td>
</tr>
<tr>
<td>CD5/T101</td>
<td>99.2</td>
<td>99.6</td>
</tr>
<tr>
<td>CD7/3A1</td>
<td>98.9</td>
<td>18.2</td>
</tr>
<tr>
<td>CD8/OKT8</td>
<td>13.5</td>
<td>56.8</td>
</tr>
<tr>
<td>CD9/BA-2</td>
<td>23.6</td>
<td>78.9</td>
</tr>
<tr>
<td>CD10/BA-3</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>CD11a/TA-1</td>
<td>88.6</td>
<td>9.3</td>
</tr>
<tr>
<td>CD11b/OKM-1</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CD13/MCS-2</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td>CD24/BA-1</td>
<td>0.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Surface Ig (polyvalent)</td>
<td>2.2</td>
<td>0.2</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Leu7</td>
<td>10.1</td>
<td>0.8</td>
</tr>
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</table>

*Percentage positive.
**T-LYMPHOID CELLS**

**Fig 2.** Representative G-banded karyotype of one Molt-4 metaphase cell showing hypertetraploidy with rearrangements of two of the no. 7 chromosomes, 2der(7t7;7)p15q11, as well as interstitial deletions of part of the long arms of one no. 1 and one no. 6 chromosomes, and an isochromosome composed of two copies of the long arm of no. 17 joined at a single centromere. The loss of one no. 14 is random in this cell.

**Interpretation:** 95,XXYY,+4,+7t7,+8,+11,+14,+15t18,+18,+20,+20,del(11)(q21q42), del(6)(q13q21), i(17q), +2der(7)t(7;7)(p15;q11)

(loss of chromosome 14 is random in this cell)

 blot analysis with probes to constant and joining regions of T gamma (Fig 3). The germline restriction map of T gamma and probes used are shown in Fig 1.

The constant region probe (T-gamma C) detects two germline EcoRI restriction fragments (7.2 and 5.0 Kb) or BamHI restriction fragments (20 and 12 Kb) corresponding to the two known constant regions of T gamma (C-gamma 1 and C-gamma 2). Southern analysis of Molt-3 DNA digested with EcoRI and hybridized with the constant region probe did not reveal any rearrangement from germline. The 5.0-Kb band was consistently less intense than the 7-Kb band, suggesting the loss of one or more alleles of C-gamma 1. The same analysis of Molt-4 DNA was similar to Molt-3 with the addition of a rearranged band of 4.5 Kb (Fig 3A). Molt-3 and Molt-4 had identical rearranged bands of 17 and 14 Kb detected with T-gamma C following BamHI digestion (Fig 3B). A joining region probe (T-gamma J1.3) used for Southern analysis recognizes J-gamma 1.3 and J-gamma 2.3 yielding 3.4- and 1.8-Kb germline EcoRI fragments and 20- and 12-Kb germline BamHI fragments (data not shown). T-gamma J1.3 failed to detect rearrangements of T gamma following EcoRI digestion. Diminished intensity of the 1.8-Kb band was consistently observed in both cell lines. T-gamma J1.3 detected two rearranged BamHI fragments of the same size as those detected by the constant region probe (data not shown). No rearranged bands were detected on Southern analysis when Sstl-digested Molt-3 or Molt-4 DNA was probed with T-gamma C. In each case, the 9-kb band corresponding to C-gamma 1 diminished intensity when compared with the 10-Kb band corresponding to C-gamma 2 (see below).

Both cell lines had identical rearrangement of the β-chain...
of the T-cell receptor as reported previously. No immunoglobulin heavy chain gene rearrangements were detected in either cell line (data not shown).

**Molecular cloning of rearranged T-gamma fragments.** In order to investigate the possibility that the breakpoint of the 7;7 rearrangement included the T-gamma locus, we cloned and mapped the rearranged BamHI fragments identified by Southern blot analysis in Fig 3. The results, shown in Fig 1, indicate that neither of the rearranged fragments included a putative breakpoint region. Instead, each corresponded to a rearrangement involving the same variable region (V-gamma 1.3) with an upstream joining region (J-gamma 1.1 and J-gamma 2.1). This was determined by comparing the restriction map of each rearranged clone with the germline restriction map. In each case, the maps were identical from 3' to 5' until a variable region was encountered. Thus, the rearranged clones appear to be the product of rearrangements of V-gamma 1.3 with either J-gamma 1.1 or J-gamma 2.1. In the latter case, all of the upstream constant and joining regions have been deleted. This provides an explanation for the diminished intensity of bands corresponding to those regions seen on Southern analysis (Fig 3).

The presence of EcoRI sites in the intervening sequences between the constant and joining regions of T gamma prevent the detection of variable-joining rearrangements of EcoRI-digested genomic DNA with the constant region probe. Since the rearranged BamHI fragments did not appear to include the chromosomal breakpoint, the 4.5-Kb rearranged EcoRI fragment may have resulted from a point mutation that created a new EcoRI site. Southern analysis of SstI-digested Molt-3 and Molt-4 DNA was performed using the T-gamma C probe (Fig 3C). Both cell lines had the same germline band, indicating that the 4.5-Kb EcoRI fragment in Molt-4 cells was due to a point mutation. The location of the new EcoRI site within the C-gamma 1 region (Fig 1) is suggested by the decreased intensity of the 5-Kb Molt-4 band in Fig 3A.

**DISCUSSION**

The Molt-3 and Molt-4 cell lines were established by Minowada et al in 1971 from the peripheral blood of a 19-year-old man with acute lymphoblastic leukemia. Both cell lines were originally described as having lymphoid morphology with phenotypic characteristics of T cells. The immunophenotypic data presented in Table I confirm that both cell lines have maintained an unambiguous T-lymphoid immunophenotype during 16 years in culture. Their immunophenotypic profiles correspond closest to thymocytes with strong expression of CD1 and variable expression of CD4 and CD8. The discrepancy between CD2 expression in Molt-3 and Molt-4 is interesting in that both cell lines were originally reported to form E rosettes. The lack of detectable CD2 expression in Molt-4 that we describe here is probably the result of changes during in vitro culture. In 1984, the same cell line was found to be 56% positive for CD2 expression as detected by the monoclonal antibody 35.1 in our laboratory. Thus, prolonged culture may have resulted in progressive loss of the CD2 antigen as recognized by 35.1.

Cytogenetic studies of Molt-4 performed by Huang et al shortly after establishment of the cell line in 1971 revealed a diploid karyotype that converted to a tetraploid karyotype after 11 months in culture. Thus, the tetraploidy that we describe now has been a feature of Molt-4 (and presumably Molt-3) for many years, but it was probably not a feature of these cells in vivo. Tetraploid karyotypes have been described in acute leukemia, presumably secondary to DNA replication without cell division. Banded chromosome analysis of the Molt-3 and Molt-4 cell lines published in 1975 revealed karyotypes very similar to ours. The differences in numerical abnormalities observed by the previous investigators and us are most likely due to in vitro nondysjunction events that have occurred over time. Likewise, the fact that we have found an unbalanced rearrangement of one chromosome 2 in Molt-3 and an interstitial deletion of part of the long arm of one chromosome 1 in Molt-4 that were not present in the 1975 study suggests that these also represent cultural changes. More important is our finding of structural rearrangements that appear to be the same as those reported previously. These include an interstitial deletion of part of 6q (two in Molt-3 and one in Molt-4), an unbalanced translocation involving the short arm of chromosome 7, an extra copy of the long arm of chromosome 7 (two each in Molt-3 and Molt-4), and an isochromosome 17q (one in Molt-4 only). Although the previous investigators have interpreted the abnormalities of chromosomes 6 and 67 differently, the banding pattern of their chromosomes is also consistent with our interpretation. Because the structural rearrangements of chromosomes 6 and 7 are present in both the Molt-3 and Molt-4 cell lines, and because they have been identified by two different laboratories 13 years apart, it is highly likely that these rearrangements were present in the leukemic cells in vivo.

The molecular genetics data presented in this study demonstrate evidence of a point mutation or a very small deletion/insertion, resulting in the creation of an EcoRI site detected by the constant region probe, but no evidence of a chromosomal rearrangement in the DNA spanning the most 5' variable regions to the most 3' constant regions of T gamma described to date. The constant region probe does not detect rearrangements of genomic DNA digested with EcoRI. This is because EcoRI sites located in the intervening sequences between the constant and joining regions of T gamma prevent the detection of the restriction fragment-linked polymorphism produced by variable-joining region recombination. Thus, the identification of a rearranged EcoRI fragment detected by the constant region probe suggested that an alternative rearrangement had taken place; one that did not involve V-J recombination but possibly a chromosomal translocation. Clones of the rearranged BamHI fragments were obtained in order to cover as much of the T-gamma locus as possible. Mapping of these clones confirmed that none was involved in a chromosomal translocation. Similar findings have been reported by Tighe et al. In addition, Southern blots of SstI-digested DNA from Molt-3 and Molt-4 probed with the constant region probe were identical, supporting our conclusion that the rearranged EcoRI fragment is due to a point mutation (or a very small
T-lymphoid cells

insertion/deletion) that has created a new EcoRI site in our Molt-4 cells 400 bp upstream from the original 3' EcoRI site. As both the 4.5- and 5.0-Kb EcoRI fragments can be detected by Southern blot analysis, this new EcoRI site is most likely found in only one of the four copies of chromosome no. 7. We cannot describe the exact nature of the alteration to the genomic DNA that resulted in the creation of the new EcoRI site because we have not directly cloned and mapped this region. Our conclusion is based on Southern analysis (Fig 3) and detailed restriction mapping of the BamHI clones covering the T-gamma locus in Molt-4 (Fig 1).

Although the breakpoint of the 7;7 rearrangement was not identified in these studies, it is still possible that the T-gamma locus is involved. Our studies did not analyze regions downstream of C-gamma 2 or regions upstream of V-gamma 1. In addition, other yet to be described regions of T gamma might be associated with the breakpoint region. Techniques such as field inversion gel electrophoresis, which can resolve extremely large DNA restriction fragments, should help address these possibilities. No genes encoding growth factors or oncogenes have been mapped to chromosome 7, band q11. Cloning and sequencing the breakpoint region will provide the opportunity to identify transcriptional units on chromosome 7, band q11, that are possibly involved in the neoplastic transformation process.

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