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 hypotetraploid 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.  

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

By James M. Greenberg, Rogelio Gonzalez-Sarmiento, Diane C. Arthur, Christopher W. Wilkowski, Barbara J. Streifel, and John H. Kersey

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 hypotetraploid 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.
clones. Restriction sites: EcoRI beneath its restriction map. The 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 cytoces (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 7p15:2der(7)(t(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)t(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

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>
</tbody>
</table>

*Percentage positive.

**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
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 SstI-digested Molt-3 or Molt-4 DNA was probed with T-gamma C. In each case, the 9-kb band corresponding to C-gamma 2 diminished intensity when compared with the 10-Kb band corresponding to C-gamma 1 (see below).

Both cell lines had identical rearrangement of the β-chain...
of the T-cell receptor as reported previously.\textsuperscript{79} 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.\textsuperscript{77} 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 closely to thymocytes with strong expression of CD1, CD4, and variable expression of 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.\textsuperscript{80} 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\textsuperscript{27} 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.\textsuperscript{76} Banded chromosome analysis of the Molt-3 and Molt-4 cell lines published in 1975 revealed karyotypes very similar to ours.\textsuperscript{8} 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.\textsuperscript{11} 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.\textsuperscript{39} 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...
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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Immunophenotypic and cytogenetic analysis of Molt-3 and Molt-4: human T-lymphoid cell lines with rearrangement of chromosome 7

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