Development of T3/T Cell Receptor Gene Expression in Human Pre-T Neoplasms

By Stefania Pittaluga, Michael Uppenkamp, and Jeffrey Cossman

Acquisition of mature T cell function and the T cell antigen receptor repertoire occur in the thymus. In an effort to delineate the cascade of events leading to T cell maturation, we analyzed a series of clonal human precursor T cell neoplasms representing early, middle, and late stages of intrathymic differentiation. Rearrangements of the T cell receptor β and γ genes appear concurrently and are preceded by surface expression of the 3A1 (CD7) molecule. Subsequent transcription of the β gene is coordinated with surface expression of T1 (CD5) and T11 (CD2), transcription of T3 mRNA, and the appearance of intracellular T3 (CD3) protein. As late events, Tα gene transcripts appear and, finally, T3, the multichain complex linked to the T cell receptor, is presented on the cell surface. Findings reported here provide a model of the developmental orchestration of genes encoding antigen recognition in human T cells.

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MATERIALS AND METHODS

Cells. T lymphoblastic neoplasms were classified by conventional microscopy as either acute lymphoblastic leukemia or lymphoblastic lymphoma and immunotyped as T cell lineage as described.25 Viable cell suspensions were used for cell surface marker study and DNA/RNA extraction. T acute lymphoblastic leukemia cell lines, provided by the American Type Culture Collection (Rockville, MD) included CEM, 8402, Molt-4, and Jurkat.

Cell surface phenotyping. Viable cells were treated with monoclonal antibodies and analyzed by indirect immunofluorescence in a fluorescence-activated cell sorter (FACS II, Becton Dickinson, Sunnyvale, CA) as described.30 Cytoplastic staining of T3 was performed on acetone-fixed cytospin preparations with an avidin–biotin complex immunoperoxidase detection technique as previously outlined.31

DNA/RNA analysis. High-molecular-weight DNA was extracted from lymphoid cells and digested to completion with the appropriate restriction endonuclease (BamHI, EcoRI, HindIII, and Bcl-1 from Biolab, New England Nuclear, Beverly, MA), size-fractionated by electrophoresis in agarose gels, and transferred to nylon paper (Gene Screen Plus, DuPont, New England Nuclear). The blots were hybridized with random-primed 32P-labeled DNA probes at specific activities of 300 to 600 cpm/pg. The following probes were used: a subcloned constant region of the β chain (4w1-Par1 fragment of cDNA YT35 provided by T. W. Mak), a full-length cDNA of Tα (PY14 provided by T. W. Mak), and a genomic fragment including the Jγ region of the human Tα gene (HindIII-EcoRI fragment of M13H60 provided by T. Rabbitts). Blots were autoradiographed following high-stringency washes. Total cellular RNA was isolated by ultracentrifugation on a guanidine isothiocyanate/CsCl gradient.40 Twenty micrograms
of total RNA was subjected to electrophoresis through 1% agarose/ formaldehyde denaturing gels and was transferred to nylon filters. Hybridization was carried out with probes described above and also with a human T3 cDNA provided by C. Terhorst, Harvard Medical School. High-stringency washes were carried out at 50°C in 0.1X SSC for 15 minutes, and blots were autoradiographed by standard techniques.

RESULTS

Phenotyping. All samples were unreactive with all B cell markers and expressed one or more T cell markers (Table 1). Each strongly reacted with 3A1 (CD7), but other T cell markers were selectively expressed in these early T cell neoplasms. For example, T11 (CD2) was negative in 2 cases (1 and 3) which alternatively expressed T1 (CD5); T6 (CD1), a marker expressed by cortical thymocytes, was unreactive in 5 of 10 samples (1, 2, CEM, 8402, HSB); and surface T3 (CD3) was positive in only 2 of 10 samples. In contrast, intracellular T3 was detectable in all but 3 cases (1, 2, and 3) and displayed a combined nuclear membrane and cytoplasmic distribution (Fig 1).

DNA rearrangements. Ta gene rearrangements were demonstrated in three of five primary cases and in all cell lines. Cases 1 and 2 displayed only germline configurations of the Ta genes when analyzed with three different restriction enzymes (BanH1, EcoRI, and HindIII), as previously reported. Ta gene rearrangements involved a single allele (cases 3, 5, and Jurkat) or both alleles (in all cell lines) and, in one exceptional situation, three nongermline Ta bands and deletion of the germline band (case 4). This latter feature was confirmed by analysis with all three restriction endonucleases. By cytogenetic analysis, case 4 exhibited trisomy of chromosome 7, which carries the T locus at band 7q3538#. This appears to be a Ta rearrangement in Jurkat and a deletion of a Ta band in Molt-4.

mRNA transcription. Tα mRNA was detectable in each sample (Fig 4) except cases 1 and 2, which contained germline Ta genes. Case 3, with an early phenotype (3A1 +, T11 -), harbored a trisomy of chromosome 7, showed three different rearrangements of the Ta gene, as it did for Ta. Presumably, the three nongermline bands are due to three copies of the Ta gene, located at chromosome 7p15.23

Ta gene rearrangements were undetected in many cases, including cases 1 and 2, despite digestion with several restriction enzymes (Fig 3). Lack of nongermline bands might be due to either unrearranged Ta genes or to the unusually large size of the Ta genome.42,28,29 We were able to identify a Ta rearrangement in Jurkat and a deletion of a Ta band in Molt-4.

Table 1. Phenotyping of Samples

<table>
<thead>
<tr>
<th>Cells</th>
<th>3A1 (CD7)</th>
<th>T1 (CD5)</th>
<th>T11 (CD2)</th>
<th>T6 (CD1)</th>
<th>cT3 (CD3)</th>
<th>sT3 (CD3)</th>
<th>Ta DNA</th>
<th>Ta RNA</th>
<th>Tα DNA</th>
<th>Tα RNA</th>
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<tr>
<td>Case 1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>G</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>G</td>
<td>-</td>
<td>G</td>
<td>-</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R</td>
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<td>G</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>RRR</td>
<td>1.3</td>
<td>G</td>
<td>RRR</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>1.0, 1.3</td>
<td>G</td>
<td>1.3, 1.5</td>
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<tr>
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<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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<td>1.3</td>
<td>G</td>
<td>-</td>
<td>RR</td>
</tr>
<tr>
<td>Molt-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>RR</td>
<td>1.0, 1.3</td>
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</tr>
</tbody>
</table>

+, >50% of cells stained; -, <20% of cells stained; cT3, intracellular T3; sT3, surface T3; G, germline; R, rearranged; D, deleted; --, none detected; mRNA sizes are given in kilobases.
Fig 2. Tα gene rearrangements. Genomic DNA was digested with the indicated enzyme, size separated in agarose gels, and analyzed on Southern blots with the Jα probe. Lanes are numbered according to the case number. C represents a germline (unrearranged) control, and the germline bands are indicated with dashes adjacent to the gel. No rearrangements were detected in either case 1 or 2 when digested with both enzymes BamH1 or EcoRI. Rearrangements were detected in all other cases; for example, two rearrangements were seen in an EcoRI digest of case 3, and three rearrangements were present in a BamH1 digest of case 4.

Fig 3. Southern blot analysis of the Tα gene. Despite digestion with two different restriction enzymes, BamH1 and HindIII, both cases 1 and 2 showed the same restriction fragment as the germline control (C).

Fig 4. Northern blot analysis of β, α, and T3δ mRNA. Lane numbers refer to the individual case numbers, and mRNA sizes are given in kilobases (kb) on left. β Transcripts were detected in all samples except cases 1 and 2. Both the 1.3-kb and 1.0-kb message sizes could be detected in several cases. Tα transcripts were detected in normal thymus, case 5, and in the Jurkat cell line. No transcripts were seen in either cases 3 or 4, and the extremely faint band in case 2 appearing after prolonged exposure is most likely attributable to a small percentage of normal T cells (~7%) contaminating this sample. T3δ mRNA was found in varying abundance in most cases but was not detected in cases 1 or 2.

sequences. Both mature (1.3 kb) and immature (1.0 kb) Tα messages were simultaneously present in case 5 and in Jurkat. Tα mRNA was detectable only in case 5 and in Jurkat and, in each of these leukemias, both the incomplete (1.3 kb) and mature (1.5 kb) messages were transcribed (Fig 4). These two T cell neoplasms containing Tα transcripts, case 5 and Jurkat, were also the only cells with surface T3 expression. Tα mRNA was not detected in any samples tested.

T3δ transcripts 700 base pairs (bp) in length were detected in all samples except cases 1 and 2, which were also the same leukemias devoid of detectable intracellular and surface T3 protein (Fig 4). Intracellular T3 protein and T3 mRNA were simultaneously present in all other samples.
genes, and the 3AI show no evidence of B cell commitment, however, since they lack B cell markers, have germline immunoglobulin to T cell differentiation. These lymphoid cells are not committed and the absence of intracellular as well as surface T3. the lack of expression of T6, the cortical thymocyte marker, and 2. Further indication of immaturity in cases 1 and 2 are the lack of expression of T6, the cortical thymocyte marker, and the absence of intracellular as well as surface T3. Alternatively, it could be argued that such primitive cells are not committed to T cell differentiation. These lymphoid cells show no evidence of B cell commitment, however, since they lack B cell markers, have germline immunoglobulin genes, and the 3AI +, T1 +, and 3AI +, T11 + phenotypes are not found in B lineage neoplasms. Tp rearrangement occurs early in thymocyte differentiation and most precursor T cell neoplasms have similarly undergone such rearrangements. Rearrangements may involve one or both alleles and, in one exceptional case reported here, trisomy of chromosome 7 apparently provided one or both alleles and, in one exceptional case, we have begun to piece together the coordination of each of these critical features in individual precursor T cell development.

DISCUSSION

T lymphoblastic leukemias and lymphomas are relatively uniform clones considered to be arrested at intrathymic stages of differentiation; they generally reflect the phenotypically characterized compartments of early T cell ontogeny. Together, lymphoblastic malignancies of T and B cell lineage represent neoplasms of lymphocyte precursors and thereby serve as models for the evaluation of lymphocyte development. The understanding of the development of immature thymocytes into mature, functional T cells has been expanded by analysis of precursor T cell neoplasms obtained from either patient tissue or cell lines. Direct comparisons of neoplastic cells to normal cell differentiation, however, must take into consideration properties of the malignant cells, such as aberrant gene expression and potential instability of phenotype during cell culture. In the present study, we analyzed primary cases and well-characterized cell lines which, collectively, span the phenotypic range of early T cell development.

Precursor T cell neoplasms presented here encompass a spectrum of intrathymic T cell development ranging from primitive cells, whose antigen receptor genes were retained in the germline configuration, to cells with rearranged and transcribed antigen receptor genes and a nearly mature T cell phenotype. Associated with developments in antigen receptor gene recombination and expression was the sequential appearance of T cell-associated markers. By analyzing each of these critical features in individual precursor T cell clones, we have begun to piece together the coordination of events in the T cell developmental process.

The earliest identifiable stage, analogous to the murine "prothymocyte," was surface expression of 3A1 (CD7), in association with either T1 or T11, in cells whose TCR genes were retained in the germline form. Neither Tp nor Tg gene rearrangements were demonstrable at this stage (cases 1 and 2) despite digestion with several restriction enzymes, and neither gene was apparently transcribed. Lack of nongermline Tg genes in these cases does not preclude the presence of Tg rearrangements because of the difficulty inherent in detection of gene rearrangements at this locus. Tg gene rearrangement, however, in the absence of rearrangement of Tg and Tg, genes, is unprecedented, and absence of Tg mRNA suggests that Tg genes might not yet be rearranged in cases 1 and 2. Further indication of immaturity in cases 1 and 2 are the lack of expression of T6, the cortical thymocyte marker, and the absence of intracellular as well as surface T3. Alternatively, it could be argued that such primitive cells are not committed to T cell differentiation. These lymphoid cells show no evidence of B cell commitment, however, since they lack B cell markers, have germline immunoglobulin genes, and the 3A1 +, T1 +, and 3A1 +, T11 + phenotypes are not found in B lineage neoplasms.

Tp rearrangement occurs early in thymocyte differentiation and most precursor T cell neoplasms have similarly undergone such rearrangements. Rearrangements may involve one or both alleles and, in one exceptional case reported here, trisomy of chromosome 7 apparently provided for rearrangement of three Tp and three Tg alleles. In early stages, Tp transcripts are incomplete, and their 1.0-kb size results from a D-J-C transcript following D-J joining. Case 3 in the current study is characterized by transcription of the 1.0-kb Tp mRNA without detectable complete (1.3 kb) transcripts. Its early thymocyte phenotype, 3A1 +, T11 −, T1 +, T6 +, cT3 −, sT3 −, and lack of Tg transcripts are indicative of an early differentiation stage. As with Tp, it is in this cell that we see the first evidence of Tg gene rearrangement. Whether Tp and Tg genes rearrange concurrently in human cells cannot be ascertained by this analysis but, based on findings reported here, rearrangements of both genes apparently occur as early events in T cell differentiation. It is noteworthy that Tp and Tg genes were always simultaneously rearranged.

As a subsequent step, full-sized 1.3-kb Tp mRNA was detected. The "mature" Tp message was seen in all five cell lines and in cases 4 and 5. Provocatively, intracellular T3 was identified only when the 1.3-kb Tp mRNA was detected, a finding that suggests the possible existence of a common factor(s) that might regulate the coordinated expression of Tp and Tg genes. Intracellular T3 is known to appear early in thymocyte differentiation, prior to localization on the outer plasma membrane.

Surface expression of T3 was identified only in cases in which Tp mRNA was detected. Two sizes of Tp mRNA, 1.5 and 1.3 kb, which likely represent complete and incomplete messages, respectively, were detectable in both case 5 and Jurkat. Lack of expression of surface T3 in the absence of Tp transcription is consistent with obligatory production of a complete α-β heterodimer, which is noncovalently linked to the T3 complex for surface membrane T3 to appear. Although Tg mRNA was not observed in any of the leukemias analyzed, low abundance message might not be demonstrable by use of a genomic J probe. Further analysis with cDNA probes will be necessary to determine whether the Tg...
gene is transcriptionally active in these human pre-T neoplasms.

The picture that has emerged from the current analysis of precursor T cell neoplasms is a cascade beginning with 3A1 expression, followed by T1 and T11 expression, and Tg and Tc gene rearrangement; Tg gene transcription, T36 gene transcription, and intracellular T3 accumulation; and, finally, Tc transcription and the appearance of surface T3 (Fig 5). In all, the elaboration of the antigen recognition function of T cells is shaped by regulated events within the thymus. It is tempting to speculate that regulation of antigen receptor development is mediated by surface molecules, such as 3A1, T1, and T11, which might serve as receptors for humoral or cellular factors present in the thymic microenvironment. In this regard, it is interesting to note that 3A1 appears on precursor cells, “prothymocytes,” prior to entry into the thymus, and perithymic mesenchyme expresses a moiety detectable with antibody 3A1.44 Analysis of the coordination of gene expression during T cell differentiation should provide leads into the identification of regulatory molecules.

ADDITIONAL

The ordered hierarchy of rearrangement and expression of genes of the T cell receptor–T3 complex outlined in the present investigation is consistent with recently reported studies of murine fetal thymocytes45 and human T cell leukemias.46

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