In this study five monoclonal antibodies (MoAbs) to T-cell receptor (TCR) proteins (WT31, αF1, βF1, TCR-β and δTCS-1) were used to identify discrete maturation stages in 40 cases of T-cell acute lymphoblastic leukemia (T-ALL). These MoAbs reacted exclusively with CD3+ T cells and did not label B-lineage and myeloid cells. In 17 of the 40 T-ALL cases studied the leukemic blasts lacked membrane and cytoplasmic TCR chains (group I). In 12 cases T-ALLs did not have membrane CD3/TCR but expressed cytoplasmic TCR proteins heterogeneously: nine cases had cytoplasmic TCRβ chains (βF1*; group II), one case had cytoplasmic TCRα chains (αF1*, βF1*; group III), and two cases were labeled by both αF1 and βF1 MoAbs (group IV). The remaining 11 cases were mCD3*: nine were TCRβ+ (group Vα) and two exhibited TCRαβ+ (TCRα±1*, δTC5±1*; group Vb). The analysis of the

T-ALLs showed that: (1) the lack of TCR protein expression was due to the lack of TCR gene rearrangements only in one of nine cases; (2) five of five TCRβ+, TCRα cases studied had germline TCRα genes (ie, no detectable TCRα gene deletions); (3) seven of eight cases with TCRδ gene deletions expressed TCRα proteins, whereas in 12 of 20 of the T-ALLs with TCRβ gene rearrangements the synthesis of the corresponding protein occurred; only 2 of 16 cases with rearranged TCRδ genes expressed TCRδ chains. The T-ALL categories identified with anti-TCR MoAbs did not have additional characteristic phenotypic patterns and may correspond to the normal stages of T-cell development more precisely than those defined by other differentiation antigens.

The rearrangement and expression of the genes encoding Ig and T-cell receptor (TCR) chains are crucial events during the differentiation of B- and T-lymphoid cells, respectively. In acute lymphoid leukemia (ALL), lineage-associated and inappropriate genes may rearrange without the corresponding protein being synthesized. For example, in B-lineage ALL both Ig and TCR genes might be rearranged but only B-lineage and myeloid cells. In 17 of the 40 T-ALL cases studied the leukemic blasts lacked membrane and cytoplasmic TCR chains (group I). In 12 cases T-ALLs did not have membrane CD3/TCR but expressed cytoplasmic TCR proteins heterogeneously: nine cases had cytoplasmic TCRβ chains (βF1*; group II), one case had cytoplasmic TCRα chains (αF1*, βF1*; group III), and two cases were labeled by both αF1 and βF1 MoAbs (group IV). The remaining 11 cases were mCD3*: nine were TCRβ+ (group Va) and two exhibited TCRαβ+ (TCRα±1*, δTC5±1*; group Vb). The analysis of the TCRα, TCRβ, TCRγ, and TCRδ gene configurations in 23 of the 40 T-ALLs showed that: (1) the lack of TCR protein expression was due to the lack of TCR gene rearrangements only in one of nine cases; (2) five of five TCRβ+, TCRα cases studied had germline TCRα genes (ie, no detectable TCRα gene deletions); (3) seven of eight cases with TCRδ gene deletions expressed TCRα proteins, whereas in 12 of 20 of the T-ALLs with TCRβ gene rearrangements the synthesis of the corresponding protein occurred; only 2 of 16 cases with rearranged TCRδ genes expressed TCRδ chains. The T-ALL categories identified with anti-TCR MoAbs did not have additional characteristic phenotypic patterns and may correspond to the normal stages of T-cell development more precisely than those defined by other differentiation antigens.

The investigation of TCR protein expression is now facilitated by the availability of monoclonal antibodies (MoAbs) to different TCR chains. Such reagents have been previously applied to study the appearance of TCR-related proteins during human T-cell ontogeny and normal thymic development. Blasts showing features of immaturity such as the lack of TCR structures and a high proliferative activity are a minor population in fetal and infant human thymus, and these cells’ commitment to the T-cell lineage is indicated by the cytoplasmic expression of CD3 chains (cCD3). The differentiation of these immature human T cells is a stepwise process that includes the synthesis and accumulation of TCRα and TCRβ chains followed by the membrane insertion of the fully assembled CD3/TCRαβ complex. Together with the major TCRαβ lineage, TCRγδ bearing cells are also found in the human thymus, but these are present in relatively low proportions throughout fetal and infant development.

In the present study we first assessed the fidelity of expression of TCR proteins to T-lineage cells by labeling normal T and B lymphoid, erythroid, and myeloid progenitors as well as leukemic blasts of the B and the myeloid lineages with αF1, βF1, WT31, TCRβ-1, and δTCS-1 MoAbs. Secondly, we tested 40 cases of T-ALL with these reagents and identified successive stages of leukemic TCR development characterized by the absence of TCR proteins, the accumulation of cytoplasmic TCRα and/ or TCRβ chains, and the surface expression of fully assembled TCR structures. Thirdly, we characterized the phenotypic features of these different stages of maturation. Finally, we investigated the configuration of the TCRβ, TCRγ, and TCRδ genes in 23 representative cases of these groups of patients and compared it with the expression of the encoded proteins.

MATERIALS AND METHODS

Handling of samples. Fetal samples were obtained after legal termination of pregnancy and gestational age determined on the basis of foot length (range 12 to 15 weeks). Fetal liver cell suspensions were prepared with surgical blades and by pressing small tissue fragments through a steel mesh into cold RPMI-1640 (GIBCO, Paisley, Scotland).

Normal peripheral blood (PB) was obtained from healthy volunteers (19 to 35 years old). Bone marrow (BM) samples from patients with acute myeloid leukemia (AML) in complete remission regenerating after chemotherapy were also studied. PB and BM samples of patients with acute leukemia at presentation were sent to the laboratory for immunologic diagnosis.
samples studied were from patients aged 6 to 65 years (median 20). Mononuclear cells from PB, BM, and cell suspensions were separated after centrifugation on Ficoll-Hypaque density gradient and washed three times in phosphate-buffered saline (PBS). The viability, assessed by trypan blue dye exclusion, was greater than 95% in the patients' samples and greater than 80% in the fetal samples studied.

Staining of cells. The anti-TCR MoAbs used in this study were as follows. MoAb BF1 (T Cell Sciences, Cambridge, MA) identifies a common determinant of the TCRβ chain, whereas a recently developed reagent, aF1 (T Cell Sciences), has been shown to specifically recognize TCRα chains. The epitopes to which BF1 and aF1 bind are not accessible on the surface of intact cells and these MoAbs are applied after treating the cells with fixatives. The membrane expression of TCRαβ was detected with the MoAb WT31 (gift of Dr W. Tax, Nijmegen, The Netherlands), which reacts with an epitope of the CD3ε chain, accessible only when the fully assembled, UK and CD3/TCRαβ complex is inserted into the cell membrane. Although WT31 also weakly reacts with TCRγδ cells, it can operationally be regarded as a TCRαβ reagent because such cells are labeled with a much higher intensity. 

Two washes in PBSA, 3 μL droplets from the wells were transferred onto a 12-spot PFTF-treated microscope slide (Hendley, Essex, UK), covered with a 1:1 mixture of glycerol:PBS and a coverslip, and observed in a Zeiss epifluorescence microscope (Carl Zeiss, Oberkochen, Germany). The remaining cells in the wells were fixed with 0.5% paraformaldehyde and analyzed with a FACScan (Becton Dickinson, Mountain View, CA).

RESULTS

The reactivity of anti-TCR MoAbs with lymphohematopoietic cells. In suspensions of viable PB mononuclear cells (five samples), 71% to 83% of lymphocytes were labeled by WT31. In the same samples TCRβ-1 cells ranged from 0.2% to 19% while less than 0.1% to 3% were δTCS-1. No positive cells were seen with aF1 and BF1 MoAbs in these tests because the epitopes recognized by these reagents are not accessible on viable cells. The anti-TCR MoAbs were then applied onto acetone-fixed cytocentrifuge preparations of the same samples and cells labeled by aF1 and BF1 MoAbs were 68% to 80% of lymphocytes. In these fixed cytosin TCRβ-1 cells were also brightly stained and represented a proportion of cells identical to that seen in cell suspension. In contrast, the staining with MoAbs WT31 and δTCS-1 was weak and could not be improved by using other fixatives such as cold methanol or acetone-methanol. Therefore, in the following experiments WT31 and δTCS-1 MoAbs were used in cell suspension while aF1 and BF1 were applied to cytocentrifuge preparations. The reactivity of TCRβ-1 was investigated on both fixed and unfixed cells. The association of the epitopes detected by anti-TCR MoAbs with T-lineage cells was assessed by investigating their expression in fetal liver (five samples) and BM lymphohematopoietic precursors (five samples). It was
shown that βF1, WT31, TCRδ-1, and δTCS-1 reacted exclusively with CD3+ T cells. In particular, no reactivity was observed with normoblasts, myeloblasts, and maturing myeloid cells, as identified by phase-contrast microscopy. Similarly, αF1 was negative on these cells but a weak, spotted reactivity was occasionally seen in the nucleus of cells with myeloid-like morphology; this weak labeling was easily distinguishable from the cytoplasmic and membrane reactivity with T cells seen in cytocentrifuge preparations. All anti-TCR MoAbs were negative on normal TdT+ and cμ+ B-cell precursors of fetal and postnatal origin.

Similar observations were made when these MoAbs were applied to non-T leukemic blasts in 10 CD19c, cCD22c, TdT+ B-lineage ALLs as well as in 10 CD13+, CD33+, cCD22+, CD19+, cCD3+ AMLs. The latter group included cases that expressed, in addition to myeloid-associated antigens, CD7 (two cases), TdT (two cases), and both (one case). MoAbs βF1, WT31, TCRδ-1, and δTCS-1 were invariably negative whereas MoAb αF1 produced a weak spotted nuclear staining, similar to that occasionally observed in normal cells, in two B-lineage ALL and three AML cases: in the remaining non-T leukemias, no staining of blasts was seen, except for a few residual normal T lymphocytes.

The expression of TCR chains in T-cell acute leukemias. The expression of TCR proteins was investigated in 40 cases of T-ALL at diagnosis. All these cases had greater than 80% blasts and expressed CD3 molecules in the cytoplasm and membrane. In T-ALL with cCD3 but no detectable mCD3/TCR (29 cases), four groups could be distinguished with anti-TCR MoAbs (Table 1; Fig 1). In 17 cases the blasts had no detectable TCR chains (group I: αF1−, βF1+, WT31+, TCRδ-1, δTCS-1), despite their cytoplasmic CD3ε chains (UCHT1+). In nine cases cytoplasmic TCRβ and CD3ε chains were seen without positivity for αF1 and no membrane TCRαβ or TCRγδ (group II: αF1−, βF1−, WT31+, TCRδ-1, δTCS-1). In these blasts the TCRβ chains were distributed along the perinuclear area. One cCD3+ case had only αF1 positivity, which was dotlike and strikingly different from the labeling seen with the same antibody in cells also expressing TCRβ chains (see below). However, in the αF1−, βF1− blasts CD3ε chains were distributed along the perinuclear area. This αF1−, βF1− T-ALL was WT31+, TCRδ-1, and δTCS-1 (group III). Finally, two cases showed positivity with both αF1 and βF1 MoAbs but lacked membrane TCR expression (group IV: αF1−, βF1−, WT31+, TCRδ-1, δTCS-1). In one of these cases low levels of mCD3 were detectable on 10% to 20% of blasts whereas the other case had less than 1% mCD3+ blasts.

The remaining 11 cases exhibited mCD3 in 36% to 95% of blasts: nine of these cases were also WT31+ and showed staining with αF1 and βF1 in cytocentrifuge preparations as expected (group Va; Table 1; Fig 1). The other two cases were TCRδ-1+ and did not express cytoplasmic or membrane TCRαβ chains (group Vb; Table 1; Fig 1). The usage of V61-J61 in these two cases was indicated by δTCS-1 positivity and confirmed by gene rearrangement analysis (see below; Table 2).

TCRδ-1 MoAb was also applied to acetone-fixed cytocentrifuge preparations of all 40 cases to assess the expression of cytoplasmic TCRδ chains. No cases were exclusively labeled on cytocentrifuge preparations, although it was shown that this reagent labeled air dried acetone-fixed cytoospins of the two mTCRγδ+ T-ALLs as well as of normal PB TCRδ+ cells.

Phenotypic features of T-ALL with different stages of TCR development. The expression of TCR proteins was then compared with that of other T-cell differentiation antigens. Of 40 cases studied, 38 were strongly CD7+ on 60% to more

### Table 1. TCR Expression and Phenotypic Features of T-ALL

<table>
<thead>
<tr>
<th>mCD3</th>
<th>No. of Cases</th>
<th>CD7</th>
<th>cCD3</th>
<th>TdT</th>
<th>CD10</th>
<th>CD1</th>
<th>CD4</th>
<th>CD8</th>
<th>CD21*</th>
</tr>
</thead>
<tbody>
<tr>
<td>βF1−,αF1−</td>
<td>17</td>
<td>17/17#</td>
<td>17/17</td>
<td>15/17</td>
<td>5/16</td>
<td>8/17</td>
<td>13/16</td>
<td>5/16</td>
<td>7/16†</td>
</tr>
<tr>
<td>βF1−,αF1−</td>
<td>9†</td>
<td>9/9</td>
<td>9/9</td>
<td>9/9</td>
<td>3/9</td>
<td>9/9</td>
<td>8/9</td>
<td>8/9</td>
<td>6/9</td>
</tr>
<tr>
<td>βF1−,αF1−</td>
<td>1†</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>βF1−,αF1−</td>
<td>25</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

*Cases with membrane CD21 expression are indicated. Two additional βF1−, αF1− cases and one additional αF1− case had cytoplasmic but not membrane CD21 expression.

†60% to 91% of blasts were βF1− and the labeling was localized in the perinuclear area (see text).

‡80% of blasts were αF1+ with a dotlike staining probably corresponding to the Golgi area (see test and Fig 1).

§In one case 20% of blasts were βF1− and 36% reacted with αF1. In the other case βF1 and αF1 positivity were seen in 76% and 80% of blasts, respectively (see text and Fig 1). In the latter case, 10% to 30% of blasts had low levels of mCD3.

¶WT31 positivity ranged from 21% to 70%. All the cases were also βF1+ and αF1+ and did not react with TCRδ-1 and δTCS-1.

||In one case 82% and 63% of blasts were labeled by TCRδ-1 and δTCS-1, respectively. In the other case, 90% of blasts were stained by both MoAbs. These cases were βF1− and αF1−.

#Number of cases with >20% positive blasts over number of cases studied.

**In two cases CD7 was weakly expressed on 55% and 91% of blasts.

††One case had 10% CD1+ cells. The remaining positive cases had >20% positive cells.
Fig 1. Representative cases of the stages of TCR protein expression in T-ALL. Cells were labeled in suspension with UCHT1 (CD3; ---), WT31 (●●●●), and TCRβ-1 (-----) MoAbs and analyzed with a FACScan (a, d, g, j, m, and p). Cytocentrifuge preparations of the same cases were stained with βF1 (b, e, h, k, n, and q) and αF1 (c, f, i, l, o, and r) MoAbs, followed by goat antisera to mouse IgG1 and mouse IgG2a conjugated to TRITC and FITC, respectively. In each case the same microscopic field was photographed twice using selective filters for TRITC and FITC. All microscopic fields illustrated contained an approximately equal number of leukemic blasts. Of the mCD3- T-ALLs studied (29 cases), 17 cases lacked membrane and cytoplasmic TCR expression (group I; a through c), nine cases expressed cytoplasmic TCRβ chains (group II; d through f), one case was αF1', βF1' (group III; g through i), and two cases were αF1', βF1' (group IV; j through l). The remaining cases were mCD3' and expressed TCRβ (nine cases; group Va; m through o) or TCRγδ (two cases; group Vb; p through r). The labeling with UCHT1, βF1, and αF1 MoAbs was localized in the perinuclear area of the mCD3- leukemic blasts (e, k, l, n, and o), but in the αF1', βF1' case αF1' staining was dot-like (l). Identical dot-like staining was seen in occasional βF1' blasts (large arrow in n and o) in the mTCRγδ' cases. Small arrows point to residual βF1', αF1' T lymphocytes.

than 99% of cells, whereas 2 mCD3'+TCRαβ' cases expressed CD7 weakly and heterogeneously on 55% and 81% of blasts (Table 1). TdT positivity was seen in 37 of 40 cases on 50% to 97% of cells; the TdT- cases were mCD3', βF1', αF1' (two cases) and mCD3', WT31', βF1', αF1' (one case; Table 1). The other markers were more frequently expressed in cases with at least one chain of the TCR (TCR+) as compared to cases without any TCR expression (TCR-). In particular, 22 of 23 TCR+ versus 8 of 17 TCR- cases were CD2' (chi-squared test: \( P < .005 \)). In a proportion of T-ALL cases studied blasts expressed CD21, as previously reported\(^2\); CD21' leukemic blasts were observed in both TCR+ and TCR- cases (Table 1). Both TCRγδ+ T-ALL were mCD3+ and blasts showed nuclear TdT. Both cases expressed CD2, CD5, and CD1a heterogeneously and were negative with CD6 and CD8 MoAbs. One case was CD4+ and CD21+ (Table 1).

The configuration of TCR genes in T-ALL subgroups with different TCR protein expression. In 23 of the 40 T-ALL cases included in this study sufficient material was available for DNA extraction and subsequent Southern blot analysis of the TCRβ, γ, and δ genes. Nine of the 23 cases studied were mCD3+ T-ALLs with no detectable cytoplasmic TCR chains (Table 2). In three of these cases no rearranged TCRβ genes were documented (patients 2, 6, and 9 in Table 2). The immaturity of these cases was also confirmed.
Table 2. TCR Gene Rearrangements in T-ALL Cases With Different TCR Protein Expression

<table>
<thead>
<tr>
<th>Patients</th>
<th>TCRβ1</th>
<th>TCRβ2</th>
<th>TCRγ1</th>
<th>TCRγ2</th>
<th>TCRδ1</th>
<th>TCRδ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>βF1+, αF1 -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>D/D</td>
<td>R/R</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>2</td>
<td>G/G</td>
<td>G/G</td>
<td>G/G</td>
<td>G/G</td>
<td>G/G</td>
<td>G/G</td>
</tr>
<tr>
<td>3</td>
<td>R/G</td>
<td>R/G</td>
<td>R/R</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>4</td>
<td>D/D</td>
<td>R/R</td>
<td>D/D</td>
<td>R/R</td>
<td>D/R</td>
<td>(G)/G</td>
</tr>
<tr>
<td>5</td>
<td>G/G</td>
<td>G/G</td>
<td>R/G</td>
<td>G/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>6</td>
<td>G/G</td>
<td>G/G</td>
<td>R/G</td>
<td>G/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>7</td>
<td>D/G</td>
<td>R/G</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>8</td>
<td>R/G</td>
<td>G/G</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>9</td>
<td>G/G</td>
<td>G/G</td>
<td>R/R</td>
<td>G/D</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>βF1+, αF1 -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>R/R</td>
<td>R/R</td>
<td>D/D</td>
<td>R/R</td>
<td>G/G</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>12</td>
<td>D(R)/G</td>
<td>R/G</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>13</td>
<td>D(R)/G</td>
<td>R/G</td>
<td>R/R</td>
<td>G/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>14</td>
<td>D(R)/G</td>
<td>R/G</td>
<td>D/D</td>
<td>R/R</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>mCD3+, WT31+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>R/R</td>
<td>G/G</td>
<td>D/D</td>
<td>R/R</td>
<td>D/R</td>
<td>D/G</td>
</tr>
<tr>
<td>16</td>
<td>R/R</td>
<td>R/R</td>
<td>D/D</td>
<td>R/R</td>
<td>D/D</td>
<td>D/D</td>
</tr>
<tr>
<td>17</td>
<td>D/R</td>
<td>R/R</td>
<td>D/D</td>
<td>R/R</td>
<td>D/R</td>
<td>D/D</td>
</tr>
<tr>
<td>18</td>
<td>G(D)/G</td>
<td>R/G</td>
<td>D/D</td>
<td>R/R</td>
<td>D/R</td>
<td>D/D</td>
</tr>
<tr>
<td>19</td>
<td>R/G</td>
<td>G/R</td>
<td>D/D</td>
<td>R/R</td>
<td>D/R</td>
<td>D/D</td>
</tr>
<tr>
<td>20</td>
<td>R/G</td>
<td>G/G</td>
<td>D/D</td>
<td>R/R</td>
<td>D/D</td>
<td>G(D)/D</td>
</tr>
<tr>
<td>21</td>
<td>G(D)/G</td>
<td>R/G</td>
<td>D/D</td>
<td>R/R</td>
<td>D/D</td>
<td>D/D</td>
</tr>
<tr>
<td>mCD3+, TCRδ1+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>D/R</td>
<td>G/G</td>
<td>D/R</td>
<td>R/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
<tr>
<td>23</td>
<td>R/G</td>
<td>G/G</td>
<td>R/G</td>
<td>G/G</td>
<td>R/R</td>
<td>G/G</td>
</tr>
</tbody>
</table>

Abbreviation: NT, not tested.

*In these patients one TCRγ allele was rearranged to Jγ2.1 and the other to Jγ2.3.
†In these patients a polymorphic EcoRI site was present in the Jγ2.3 region (see also reference 55).
‡No TCRγ gene rearrangements could be detected in KpnI digests using the Jγ1.3 probe.
§One allele was V61-J61 and the other was D62-J61.
¶One allele was V61-J61 and the other was V62-J61.

by the blasts’ phenotypic features: CD7, CD5, cCD3, and TdT positivity together with absence of CD2, CD1a, CD4, or CD8 expression. The remaining six of these TCR- cases had at least one TCRβ1 or TCRβ2 allele rearranged. The study of the TCRγ and TCRδ genes in the same group of patients showed rearrangements in all but one case, which showed a germline configuration in all alleles: this case was also TCRβ germine (patient 2 in Table 2 and Fig 2). Thus, in only one of nine cases the lack of TCR proteins could be attributed to the absence of gene rearrangement.

The second group of cases studied included 5 mCD3- T-ALLs with βF1 positivity but no reactivity with αF1 and TCRδ-1 MoAbs. As expected five cases showed rearranged TCRβ genes. In addition, TCRγ and TCRδ genes were also rearranged in all cases but no deletion of the TCRδ locus was observed, implying the germline configuration of all TCRδ loci (Table 2; Fig 2).

Seven cases of mCD3+ T-ALL expressing mTCRαβ chains (WT31+, βF1+, αF1+) were also studied. In line with the observations at the protein level, all cases showed TCRβ gene rearrangements together with TCRδ deletions on at least one allele, suggesting the rearrangement of the TCRα locus (Table 2; Fig 2). All seven cases showed a deletion of the TCRγ1 locus on both alleles and rearrangement of both the TCRγ2 loci was seen in all seven cases (Table 2; Fig 2), confirming previous observations.

Finally, the two cases of mCD3+, TCRδ-1+, TCSα+ had TCRδ genes rearranged. As expected from the δTCS-1 reactivity, a V61-J81 rearrangement was found on one allele in both patients (Table 2).

DISCUSSION

In this report we first investigated the TCR protein status of normal lymphohematopoietic cells and observed that the expression of the epitopes detected by αF1, βF1, WT31, TCRδ-1, and δTCS-1 MoAbs was restricted to the T lineage: no membrane or cytoplasmic TCR protein expression was found even in the earliest identifiable B-lymphoid, myeloid, or erythroid progenitors in the samples studied. The fidelity of these molecules' expression to the T lineage contrasts with the frequent occurrence of inappropriate TCR gene rearrangements seen in B-lineage ALL. 2,35

By applying the same MoAbs to a large series of T-ALL cases we have shown discrete stages of TCR development in
TCR EXPRESSION IN T-ALL

T-cell leukemia that reflect the maturation stages seen in normal thymus. The largest group of T-ALLs in our series lacked cytoplasmic and membrane TCR expression: CD7⁺, cCD3⁺, βF1⁺, αF1⁺, WT31⁺, TCRδ⁻ (group I). The phenotypic features of these T-ALL blasts correspond to those seen in a subset of immature fetal and infant thymocytes that proliferate rapidly. The presence of cCD3ε chains in the blasts' perinuclear area in these cases indicates that the synthesis of the CD3/TCR complex had been initiated. Clearly the lack of TCR protein expression in these T-ALL cases is only rarely attributable to the germline configuration of the corresponding genes: in our study one of nine such cases had all TCR genes in a germline configuration. RNA expression of TCR genes was not investigated in the present series but several previous studies have demonstrated the presence of such RNA transcripts in most T-ALL cases analyzed. We speculate that some of these may be nonfunctional, having derived from incomplete or out-of-frame rearrangements. However, the possibility that in some cases individual TCR proteins are synthesized and rapidly degraded cannot be ruled out (see below).

We have previously observed subpopulations of normal thymocytes expressing either TCRα or TCRβ chains. Such cells are seen from the 10th week of gestation in the fetal thymus and may represent intermediate maturation stages during thymic development. In this study we investigated the malignant counterparts of these immature cells and showed that in T-ALL blasts the synthesis and accumulation of an incomplete CD3/TCR complex is not infrequent. A similar phenomenon has been previously documented in B-precursor ALL where cytoplasmic μ heavy chains are seen in pre-B ALL cases. In contrast to pre-B ALL, where μ heavy chains are found and isolated Ig light chains are not expressed, the equivalent developmental stages in T-ALL are more complex. In nine cases we found only TCRβ chains, but in one case αF1 reactivity was seen in the absence of βF1 positivity and in two cases both TCRβ and TCRα chains were present without surface TCR expression. In one of these cases low levels of mCD3 were detectable in a minority of blasts, which were not labeled by WT31 MoAb. This is in line with previous observations indicating that the membrane expression of CD3 chains unlinked to TCR proteins can be induced on some T-cell lines.

In the αF1⁺, βF1⁻ T-ALL cases studied TCRβ chains were distributed along the perinuclear area, probably corresponding to the rough endoplasmic reticulum. When five such cases were investigated at DNA level no deletion of the TCRδ locus was seen, indicating a germline configuration of TCRα genes. In four of these five cases, cytogenetics studies have shown translocations involving the q11 region of the chromosome 14 (L.M. Secker-Walker and D. Campana, et al, manuscript in preparation) where the TCRα locus has been mapped. It appears that in these cases the lack of TCRα gene rearrangement is probably the most important limiting factor in the expression of a full TCRβ receptor.

Individual CD3/TCR chains and partial complexes may be stably expressed in the endoplasmic reticulum or transported to the Golgi apparatus where they are either stored or degraded. The labeling with αF1 MoAb in the αF1⁺,
betaF1 case studied was dotlike, suggesting a localization of the TCR alpha chains in the Golgi area (Fig 1i). This pattern of staining is in contrast to that seen with the same MoAb in normal T cells and in the remaining TCR alpha cases studied (Fig 1, c, f, i, o, r). Interestingly, in the same case CD3 epsilon molecules had a different sub-cellular localization and were seen around the nuclear membrane. In the two mCD3+ T-ALLs, UCHT1, alphaF1, and betaF1 labeling was localized around the perinuclear area. We hypothesize that the lack of one of the CD3 components, perhaps CD3 epsilon, may represent the limiting factor to the membrane expression of TCR molecules in these latter cases. Alternatively, TCR chains may be incomplete protein products that are unable to form a stable TCR/CD3 complex.

Previous studies have demonstrated that the genes encoding TCR gamma, TCR beta, TCR delta, and/or TCR alpha chains are rearranged in the majority of T-ALL cases. The comparative analysis of TCR genes and corresponding proteins in the same cases of T-ALL has not been performed before, and on the basis of our findings the TCR beta analysis showed that seven of eight T-ALL cases with at least one TCR alpha allele rearranged, i.e., TCR alpha allele deletion, had expressed TCR proteins, whereas only 12 of 20 cases with at least one TCR beta allele rearranged expressed TCR beta. The chances for a successful expression of the rearranged TCR alpha alleles appeared to be even lower: only 2 of 16 cases with at least one TCR alpha allele rearranged (representing a total of 25 rearranged TCR alpha alleles) showed expression of the corresponding protein. The low rate of TCR alpha expression may be due to a higher occurrence of incomplete or out of frame rearrangement in the TCR gamma genes and/or to putative regulation mechanisms for TCR gene expression. Alternatively, TCR delta chains may be synthesized and, in the absence of TCR gamma chains, rapidly degraded.

In our study we could not find evidence of cytoplasmic TCR delta chains in T-ALL blasts, confirming observations in normal human thymocytes (D. Campana, unpublished observations, June 1989). The experiments in normal and leukemic T cells have shown that the epitope recognized by TCR delta-1 MoAb is not destroyed during cytocentrifuge preparation, air drying, or acetone-fixation, but we cannot rule out the possibility that this epitope is not accessible in uncoupled cytoplasmic TCR delta chains. Nevertheless, it is relevant that the lack of cytoplasmic TCR delta chains has also been reported in studies on murine and avian thymocytes. The re-investigation of T-ALL cases with MoAbs specifically reacting with the TCR gamma chains will clarify this issue and enable the precise assessment of the rate of expression of this gene.

Recently, Gouttefangeas et al. observed differences in mTCR expression between T-ALL and T-lymphoblastic lymphoma (T-LL), suggesting that mTCR gamma is more frequently seen than mTCR alpha in T-ALL. In our study and in other series such TCR gamma predominance in mCD3+ T-ALL was not found. It is unlikely that this is due to the inclusion of T-LL cases in our series because all patients presented with high white blood cell count and/or massive BM involvement. Taking together the data so far reported (references 48, 51, and this study), it appears that blasts expressing TCR gamma are expected to be found in approximately one third of mCD3+ T-ALL cases. Considering that TCR gamma-bearing cells normally represent less than 1% of fetal and infant thymocytes, the relatively high proportion of TCR gamma T-ALLs may reflect a susceptibility to leukemogenesis of the TCR gamma lineage.

Previous investigators have categorized T-ALL in stages of development based on the expression of several differentiation antigens. These categories might not reflect the stages of normal differentiation exactly and are not directly related to the cells’ TCR status. In the present study we showed that most T-lineage associated markers are expressed in high frequency amongst the T-ALLs that synthesize TCR chains (TCR beta+), but otherwise no clear correlation pattern was seen with the different stages of TCR expression. Our view is that the T-ALL categories seen by TCR protein analysis describe the maturation stages of malignant cells more precisely than the other differentiation markers. The investigation of the correlations between TCR expression and clinical features may highlight the prognostic relevance of the new T-ALL classification proposed in this study.

ACKNOWLEDGMENT

We thank the clinicians participating in the UKALL Xa trial for sending samples. We also thank Prof P.C.L. Beverley (Middlesex Hospital, London, UK), Prof A.J. McMichael (John Radcliffe Infirmary, Oxford, UK), and Dr W. Tax (Nijmegen, The Netherlands) for the gift of antibodies; Prof R. Benner for his continuous encouragement; and T.M. van Os for assistance in producing Fig 2.

REFERENCES

8. Henry L, Tian WT, Rittershaus C, Ko JL, Marsh HC, Ip SH:
Two distinct immunogenic epitopes on the a chain of human T cell antigen receptor. Hybridoma 8:577, 1989


sion of T3 on human immature T cell lines with and without concomitant expression of the T cell antigen receptor complex. Eur J Immunol 17:1079, 1987


Stages of T-cell receptor protein expression in T-cell acute lymphoblastic leukemia

D Campana, JJ van Dongen, A Mehta, E Coustan-Smith, IL Wolvers-Tettero, K Ganeshaguru and G Janossy