Site-specific Deletions Involving the tal-1 and sil Genes Are Restricted to Cells of the T Cell Receptor α/β Lineage: T Cell Receptor δ Gene Deletion Mechanism Affects Multiple Genes

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Summary

Site-specific deletions in the tal-1 gene are reported to occur in 12–26% of T cell acute lymphoblastic leukemias (T-ALL). So far two main types of tal-1 deletions have been described. Upon analysis of 134 T-ALL we have found two new types of tal-1 deletions. These four types of deletions juxtapose the 5' part of the tal-1 gene to the sil gene promoter, thereby deleting all coding sil exons but leaving the coding tal-1 exons undamaged. The recombination signal sequences (RSS) and fusion regions of the tal-1 deletion breakpoints strongly resemble the RSS and junctional regions of immunoglobulin/T cell receptor (TCR) gene rearrangements, which implies that they are probably caused by the same V(D)J recombinase complex. Analysis of the 134 T-ALL suggested that the occurrence of tal-1 deletions is associated with the CD3 phenotype, because no tal-1 deletions were found in 25 TCR-3'/α + T-ALL, whereas 8 of the 69 CD3- T-ALL and 11 of the 40 TCR-β/δ + T-ALL contained such a deletion. Careful examination of all TCR genes revealed that tal-1 deletions exclusively occurred in CD3- or CD3+ T-ALL of the α/β lineage with a frequency of 18% in T-ALL with one deleted TCR-δ allele, and a frequency of 34% in T-ALL with TCR-δ gene deletions on both alleles. Therefore, we conclude that α/β lineage commitment of the T-ALL and especially the extent of TCR-δ gene deletions determines the chance of a tal-1 deletion. This suggests that tal-1 deletions are mediated via the same deletion mechanism as TCR-δ gene deletions.

Recurrent chromosomal aberrations, such as translocations and inversions involving the Ig and TCR loci, are nonrandomly associated with lymphoid malignancies. It is generally assumed that these chromosome aberrations are caused through “illegitimate” V(D)J recombinase activity by the enzyme system, which normally provides for the rearrangement processes in Ig and TCR gene complexes (1–5).

The reciprocal t(1;14)(p32;q11) is an example of a chromosome aberration that is probably caused by “illegitimate” V(D)J recombinase. This translocation is exclusively found in T cell acute lymphoblastic leukemia (T-ALL) and involves both the so-called tal-1 gene (also known as SCL or TCL5) and the TCR-δ gene complex (6–10). Approximately 3% of pediatric T-ALL have a t(1;14) (11), and six of the seven translocation breakpoints analyzed to date cluster in the Dδ-Jδ region of the TCR-δ locus on chromosome 14 and in a 1-kb region of the 5' part of the tal-1 locus on chromosome 1 (8–10).

The tal-1 gene is a transcriptionally complex locus in which the 5' noncoding region has two distinct transcription initiation sites and a variable pattern of alternative exon utilization (12–14). At least six different forms of mRNA are expressed, predominantly in early hematopoietic cells (13–15). The TAL-1 protein contains a so-called helix-loop-helix DNA binding motif (HLH motif) (12, 13, 16, 17), which is also found in several other proteins involved in control of cell proliferation or differentiation (18–21). Therefore, dysregulation of the tal-1 gene expression by chromosomal aberrations may contribute to the leukemic transformation in T-ALL.
The 5' part of the tal-1 locus can also be affected by a site-specific, submicroscopic deletion (tal-1 deletion) of \(~90\) kb, which occurs at high frequency in T-ALL only (14, 22, 23). As a result of this \(~90\)-kb deletion, the coding exons of the tal-1 gene are juxtaposed to the first noncoding exon of the recently described sil gene (24), which is therefore almost completely deleted (see Fig. 1 A). The expressed sil-tal-1 fusion mRNA produces a normal TAL-1 protein, but is transcriptionally controlled by the sil gene promoter (14, 25).

Also tal-1 deletions are assumed to be caused by the V(D)J recombination system, because the breakpoints seem to cluster at heptameric/nonsense recombination signal sequences (RSS) (14, 25), which are homologous to those used in the Ig and TCR rearrangement processes (4, 5, 26–28). Moreover, the fusion regions of the tal-1 deletion breakpoints show non-templated nucleotide addition (N region), P region nucleotides, and deletion of nucleotides by exonucleic nibbling from the flanking sequences, all of which are hallmarks of V(D)J recombination processes in Ig and TCR genes (5, 26, 29).

So far three types of tal-1 deletions are described. The two main types of tal-1 deletion use the same 5' heptamer RSS, located between the first and second sil exon, but different 3' heptamer-nonsense RSS in the 5' part of the tal-1 locus, 1.7 kb apart of each other (14, 25), whereas the third type (type C) has only been found in one patient and does not use any RSS (25). In our attempt to determine the occurrence of the tal-1 deletions in a series of 134 T-ALL, we identified two new types of tal-1 deletions that use the same 5' heptamer RSS as types 1 and 2, but different 3' heptamer-nonsense RSS. In contrast to suggestions in other publications (14, 25), the tal-1 deletions exclusively occurred in T-ALL of the \(\alpha/\beta\) T cell lineage and were especially correlated with TCR-\(\delta\) gene deletions, which are characteristic for the \(\alpha/\beta\) lineage.

**Materials and Methods**

**Cell Samples.** Cell samples were obtained from a nonrandom group of 134 T-ALL patients at initial diagnosis. To obtain sufficient numbers of CD3

\(^{+}\) T-ALL (especially TCR-\(\gamma/\delta^n\) T-ALL), we have selected T-ALL cell samples based on their CD3/TCR immunophenotype, resulting in 69 CD3

\(^{+}\) T-ALL (51% of the total series), 40 TCR-\(\alpha/\beta^n\) T-ALL (30%), and 25 TCR-\(\gamma/\delta^n\) T-ALL (19%). In random series of T-ALL, this immunophenotype distribution probably is \(~70\%, \sim20\%, \sim10\%\), respectively (30). Mononuclear cells (MNC) were isolated from peripheral blood, bone marrow, or pleural exudate by Ficoll-Paque (density, 1.077 g/ml; Pharmacia, Uppsala, Sweden) density centrifugation. The numbers of CD3

\(^{+}\) T-ALL (especially the group of 134 T-ALL patients at initial diagnosis. To obtain sufficient CD3

\(^{+}\) T-ALL, MOLT16, JURKAT, H9, HUT78, DND41, PEER, RPMI-8402, MOLT4, HSB-2, GH1) (31).

**Isolation of the tal-1 Locus from a Genomic Library.** Screening with the B2EE-2.0 (9) and TALDB2 probes of the CML-0 genomic library, constructed of MboI partial digested DNA from a chronic myeloid leukemia (CML) patient cloned in the EMBL3 \(\lambda\) replacement vector (32), yielded several phage inserts, which covered the whole tal-1 locus. The phage inserts were digested for restriction enzyme analysis and some genomic restriction fragments were sub-cloned in the pUC19 cloning vector.

**Isolation of sil and tal-1 Gene Probes.** DNA probes were obtained by cloning the purified PCR amplification products of granulocyte DNA from a healthy donor using specific oligonucleotide primer sets. pUC19 was used as cloning vector (52).

**Isolation of the tal-1 Locus from a Genomic Library.** Screening with the B2EE-2.0 (9) and TALDB2 probes of the CML-0 genomic library, constructed of MboI partial digested DNA from a chronic myeloid leukemia (CML) patient cloned in the EMBL3 \(\lambda\) replacement vector (32), yielded several phage inserts, which covered the whole tal-1 locus. The phage inserts were digested for restriction enzyme analysis and some genomic restriction fragments were sub-cloned in the pUC19 cloning vector.

**Southern Blot Analysis.** DNA was isolated from frozen MNC as described previously (32, 34). A 15-\(\mu\)g sample was digested with the appropriate restriction enzymes (Pharmacia), size-fractionated on 0.7% agarose gels, and transferred to Nytran-13N nylon membranes (Schleicher & Schuell, Inc., Dassel, Germany) as described (32, 34). tal-1 deletions were studied using \(^{32}\)P random oligonucleotide-labeled B2EE-2.0 (9), TALDB1, TALDB2, and silDB probes in EcoRI, HindIII, and BglII digests. TCR-\(\gamma^n\) gene rearrangements were detected with the J\(\gamma1\), J\(\gamma2\), and C\(\gamma^n\) probes (34, 35) in EcoRI and HindIII digests. TCR-\(\delta^n\) gene rearrangements were detected with the J\(\delta1\), J\(\delta2\), and C\(\delta^n\) probes (34, 36) in EcoRI and KpnI digests. The configuration of the TCR-\(\gamma^n\) genes was analyzed by use of the J\(\gamma1.2\), J\(\gamma1.3\), J\(\gamma2.1\), and C\(\gamma^n\) probes (34, 37) in EcoRI and HindIII digests. The configuration of the TCR-\(\delta^n\) genes was analyzed by use of the V\(\delta1\), V\(\delta2\), J\(\delta1\), J\(\delta2\), C\(\delta^n\), and \(\gamma/\delta^n\) probes (34, 37–40) in EcoRI, HindIII, and BglII digests.

**PCR Amplification Analysis.** PCR was essentially performed as described previously (32, 41). A 0.1-\(\mu\)g sample of DNA, 12 pmol of the 5' and the 3' oligonucleotide primer, and 1 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) were used in each PCR reaction of 100 \(\mu\)l. The oligonucleotide primers are listed in Table 1. These oligonucleotides were designed according to our own sequence data and published tal-1 (14) and sil gene sequences (22). The PCR reaction mixture was incubated at 94°C for 3 min, at 60°C for 2 min, and at 72°C for 3 min in a thermal cycler (Perkin-Elmer Cetus). After this initial cycle, denaturing, annealing, and extension steps were performed for another 39 cycles at 94°C for 1 min, at 60°C for 1 min, and at 72°C for 3 min, respectively. After the last cycle an additional extension step of 72°C for 7 min was executed. The PCR products of tal-1 deletion types 1 and 2 were size fractionated by 10% polyacrylamide gel-electro-
phoresis and visualized by ethidium bromide staining to demonstrate differences in size.

Sequence Analysis. 1 µl of the original PCR product, 12 pmol of the limiting primer, 600 pmol of the opposite primer, and 5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) were used in each asymmetric PCR reaction of 500 µl. The reaction mixture was incubated for a total of 25–30 cycles with the above-described regular temperature cycles. After the asymmetric amplification, the PCR product was precipitated twice in 50% ethanol plus 0.1 vol of 2 M NaAc, pH 5.6 (41). The dried pellet was resolved in 22 µl H2O, half of which was used in the sequence reaction. 50-pmol sequence primer was used in each reaction (sequence primers are indicated in Table 1). All sequence reactions were performed with the T7-sequencing kit (Pharmacia) following the manufacturer's instructions using 32P radiolabeling, and run on a normal, denaturing 8% polyacrylamide sequence gel. All germline sequences and fusion regions of tal-1 deletions were sequenced in both directions.

Results

Two New Types of tal-1 Deletions. Screening of 134 T-ALL by Southern blot analysis revealed two new types of tal-1 deletions in addition to the already described types 1 (type A), 2 (type B), and C (14, 25). We designated these new tal-1 deletions types 3 and 4 (Figs. 1 A and 2). Based on the Southern blot data, it was concluded that the 3' breakpoints of these two new tal-1 deletions were located upstream of the tal-1 locus, whereas the 5' breakpoints were apparently identical to the 5' breakpoints of tal-1 deletions types 1 and 2 (Figs. 1 A and 2). To determine the exact location and the germline sequences of the new tal-1 deletion breakpoints, DNA of patient MB with tal-1 deletion type 3 was amplified by PCR using the sildb and taldb2 oligonucleotide primers (Table 1), which resulted in an ~3-kb PCR product. After cloning and sequencing of this PCR product, a tal-1 deletion type 3 sequence primer was made (Table 1). A 4.3-kb XbaI-XbaI fragment isolated from a genomic library was sequenced by use of the taldb3-seq primer, which provided the germline sequence of the tal-1 deletion type 3 breakpoint region (Fig. 1 B). Based on the sequencing data of the 3' side of a 1.9-kb Sall-XbaI subclone, the taldb4 primer was made (Table 1). This primer in combination with the sildb primer resulted in an ~0.6-kb PCR product when DNA from patient BD with tal-1 deletion type 4 was amplified. Based on the direct sequencing data of this PCR product, a tal-1 deletion type 4 sequence primer was made (Table 1). Sequencing with this primer of the 1.9-kb Sall-XbaI subclone provided the germline sequence of the tal-1 deletion type 4 breakpoint region (Fig. 1 B).

The sequence analysis showed that the tal-1 deletion types 3 and 4 both used the same 5' heptamer RSS as types 1 and

Figure 1. Restriction map and germline sequences of tal-1 deletion breakpoint regions. (A) Restriction map of the tal-1 locus and the 5' part of the sil gene involved in the ~90-kb tal-1 deletion. The various types of tal-1 deletion breakpoints are indicated with arrows: sildb, breakpoint in sil gene; taldb, breakpoints in tal-1 gene. Noncoding exons are indicated as dotted boxes; solid boxes represent protein-coding exons. The HLH motif is indicated in tal-1 exon 6 (12, 13). The relevant restriction sites are indicated: B, BamHI; Bg, BgII; E, EcoRI; H, HindIII; S, SacI; X, XbaI; * Polymorphic restriction site. Open boxes below the restriction map represent the probes used for Southern blot hybridization. (B) Germline sequences surrounding the breakpoints of the various types of tal-1 deletions. The heptamer sequence of the 5' RSS in the sil gene and heptamer-nonamer sequences of the 3' RSS in the tal-1 gene are underlined. The arrow indicates the location of the breakpoints.
Figure 2. Southern blot analysis of the various types of tal-1 deletions. HindlII digests of DNA from patients with different types of tal-1 deletion: type 1, patient SL; type 2, patient PV; type 3, patient MB; type 4, patient BD. The Southern blot filter was successively hybridized with the SLLDB and TALDB1 probes (Fig. 1 A). The sizes of the rearranged bands are indicated. G, germline band.

Table 1. Oligonucleotide Primers Used in PCR and Sequencing Analysis of tal-1 Deletions or Isolation of DNA Probes

| Code    | Position/size | Sequence | Reference |
|---------|---------------|----------|-----------|
| tal-1 deletion      |              | bp       |           |
| 5' all types       | sildb         | -155     | GGGGAGCTCGTGGGAGAAATTAAAG   | 22 |
|                   | sildb-seq     | -111     | GGTATCATCTGAGCTAAGGTATGTTG  | 22 |
| 3' type 1          | tal1d1        | +155     | GCCTCGAAGGGTCCACATCTAC      | 14 |
|                   | tal1db1-seq   | +111     | CACACTCGGACACAGAGCCTG       | 14 |
|                   | tal1db1-5'    | +28      | TCACATCCCCACCGATGCAC        | 14 |
| 3' type 2          | tal1db2       | +152     | TGTTAAAATGGGAGATAAGTGTCGAC  | This paper |
|                   | tal1db2-seq   | +110     | AACTTATAGCCTTAAAGGG         | This paper |
| 3' type 3          | tal1db3       | +58      | TGCATGACCTCTGATGGACAGCC     | This paper |
|                   | tal1db3-seq   | +15      | ATCTACACTGCAGTTACTGTGAC     | This paper |
| 3' type 4          | tal1db4       | +450     | GAGTTATAGGGGCTGTCACACCAC   | This paper |
|                   | tal1db4-seq   | +34      | TACATCTTATATGATGTTAAATTTAGC| This paper |
| DNA probes         | sildb5'       | 330      | CACAGAGATCTTGATCCTTGAGGCC   | 22 |
|                   | sildp3'       |          | CGGAGGCTTCCGCGAGGTAGTGGT   | 22 |
| TALDB2             | tal1d2p5'     | ~575     | TGTAAGCTTGGCTAAGTCTACACCTCTC| 14 |
|                   | tal1d2p3'     |          | CCTGTCATAGGGACATAATGGCC    | 13 |

*The position of the oligonucleotide primer is indicated upstream (-) or downstream (+) relative to the heptamer RSS. The sizes of the DNA probes are given. The position of the DNA probes are indicated in Fig. 1 A.

1 The underlined sequences represent the aspecific nucleotides, which generate restriction sites.

5 Sequence information used to design the oligonucleotide primers was derived from the indicated literature references or from our own sequence data.
Table 2. Frequency of tal-1 Deletions in T-ALL.

| T-ALL       | No. of patients | tal-1 deletions |
|-------------|----------------|-----------------|
|             |                | Type 1 | Type 2 | Type 3 | Type 4 | Total     |
| CD3-        | 69             | 8.7% (6)* | 2.9% (2) | 0% (0) | 0% (0) | 11.6% (8) |
| TCR-γ/δ*    | 25             | 0% (0)     | 0% (0)     | 0% (0) | 0% (0) | 0% (0)    |
| TCR-α/β*    | 40             | 20% (8)    | 2.5% (1)  | 2.5% (1) | 2.5% (1) | 27.5% (11) |
| Total       | 134            | 10.4% (14) | 2.2% (3)  | 0.7% (1) | 0.7% (1) | 14.2% (19) |

* No. of deletions.

Occurrence of tal-1 Deletions Is Related to CD3 Phenotype and TCR Gene Configuration. The overall percentage of tal-1 deletions in our series of T-ALL was 14.2% (19/134), but these tal-1 deletions appeared to be restricted to CD3- T-ALL (8/69) and TCR-α/β* T-ALL (11/40), whereas no tal-1 deletions were found in TCR-γ/δ* T-ALL (0/25) (Table 2). The presence or absence of tal-1 deletions was not associated with other immunophenotypic characteristics (Table 3).

Since CD3- T-ALL theoretically represent precursor stages of both TCR-γ/δ* and TCR-α/β* T-ALL, we tried to use the configuration of the TCR-δ genes as an additional marker to determine whether the CD3- T-ALL group could be divided into γ/δ lineage or α/β lineage committed subgroups, and whether such a subdivision corresponded with the occurrence of tal-1 deletions. The configuration of the TCR-δ gene on each allele can potentially pass three consecutive stages: germline, rearranged, and deleted. Analysis of the TCR-δ gene configuration of the 19 T-ALL and 4 T cell lines with tal-1 deletions revealed that all but one contained at least one deleted TCR-δ allele (Table 3): only one T-ALL (1/19) with a tal-1 deletion had no deletion of the TCR-δ gene. 31.6% (6/19) had one deleted TCR-δ allele with a rearrangement on the other allele, and 63.2% (12/19) had deletions of both TCR-δ alleles. Also in the four T cell lines with a tal-1 deletion, a high frequency of TCR-δ gene deletions was found: seven of eight TCR-δ alleles were deleted (Table 3).

Further analysis of the eight TCR-δ gene rearrangements in the T-ALL with a tal-1 deletion showed that seven were complete Vδ-Jδ rearrangements, and one TCR-α/β* T-ALL (patient MG) contained a Vα-Jα1 rearrangement (Table 4). Sequence analysis of the seven Vδ-Jδ junctional regions revealed that all these TCR-δ rearrangements in both CD3- T-ALL and TCR-α/β* T-ALL were out of frame and therefore nonfunctional (Fig. 4). The rearranged TCR-δ gene in cell line RPMI 8402 is caused by a t(11;14)(p15;q11) and therefore represents a nonfunctional TCR-δ gene as well (39). This implies that all T-ALL and T cell lines with a tal-1 deletion have deleted their TCR-δ genes (37/46 alleles) and/or contain nonfunctional TCR-δ gene rearrangements (9/46 alleles).

TCR-γ gene analysis of the T-ALL and T cell lines with a tal-1 deletion revealed that 21.7% (10/46) and 78.3% (36/46) of the rearranged TCR-γ alleles involved the TCR-γ1 and TCR-γ2 locus, respectively (Table 3), which represents a normal rearrangement pattern, as found in the total group of 134 analyzed T-ALL. Analysis of the TCR-β configuration of the 19 T-ALL and 4 T cell lines revealed rearrangements in all cases, but without any preferential pattern.

tal-1 Deletions Coincide with TCR-δ Gene Deletions. Based on the above-described results, we decided to determine the incidence of TCR-δ gene rearrangements and deletions in the total group of 134 T-ALL. The results allowed us to divide the 134 T-ALL in subgroups on the basis of their TCR-δ gene configuration in addition to their CD3 phenotype, as presented in Table 5. It became clear that in the CD3- T-ALL group almost all tal-1 deletions (7/8) cluster in a small subgroup (39% of all CD3- T-ALL), which is defined by containing at least one deleted TCR-δ allele. Since TCR-α/β* T-ALL also contain at least one deleted TCR-δ allele, virtually all tal-1 deletions coincide with TCR-δ gene deletions. In addition, it is remarkable that the far majority of tal-1 deletions in TCR-α/β* T-ALL (9/11) cluster in the major subgroup, which is defined by deletion of both TCR-δ alleles (63% of all TCR-α/β* T-ALL). Only two TCR-γ/δ* T-ALL (2/25) had one deleted TCR-δ gene, and no tal-1 deletions were found in this CD3- subgroup. The overall results show that the frequency of tal-1 deletions in T-ALL with both TCR-δ alleles deleted (34.3%) is about twice that of tal-1 deletions in T-ALL with one TCR-δ allele deleted (17.6%) (Table 5). This suggests that not the CD3 immunophenotype, but the extend of TCR-δ gene deletion determines the chance of having a tal-1 deletion. This is further supported by the finding that only one tal-1 deletion was found in the 65 T-ALL without a TCR-δ gene deletion, in contrast to 18 of the 69 T-ALL with one or two deleted TCR-δ genes.

Configuration of δREC and ψαx Gene Segments in T-ALL with a tal-1 Deletion. The TCR-δ deletion mechanism, which is responsible for the deletion of nonfunctional TCR-δ rearrangements, is assumed to use two so-called deleting elements, δREC and ψαx (40, 42). The specific δREC-ψαx rearrangement was found in three T-ALL and one T cell line with a tal-1 deletion (Table 4). In one T-ALL (patient BD) this rearrangement was present only in a small subpopulation of the leukemic cells (Table 4). 80.4% (37/46) of the δREC alleles and 78.3% (36/46) of the ψαx alleles were deleted in the T-ALL and T cell lines with a tal-1 deletion. Only two TCR-α/δ
Figure 3. PCR and sequence analysis of tal-1 deletion fusion regions. (A) PCR products obtained via amplification of the DNA from patients with tal-1 deletion types 1 or 2 were size fractionated in an ethidium bromide-stained 10% polyacrylamide gel. The PCR reaction was performed using the sildb primer and either the talldbl primer (type 1) or talldb2 primer (type 2). (B) Sequences of the fusion regions of all tal-1 deletions are aligned with the known (underlined) sil and tal germline sequences. Lower-case characters at the end of a fusion region represent P region nucleotides (29). All other nucleotides of the fusion region represent N region nucleotides.
Table 3. Characteristics of 19 T-ALL and 4 T Cell Lines with a tal-1 Deletion

| Immunologic markers | TdT | HLA-DR | CD1 | CD2 | CD3 | CD4 | CD5 | CD6 | CD7 | CD8 | TCR-α/β | Cyβ | Southern blot analysis |
|---------------------|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|----------|-----|-----------------------|
|                     | %   |        |     |     |     |     |     |     |     |     |          |     | G, allele in germline configuration; R, rearrangement of the involved allele; T, translocation of involved allele; D, deletion of the involved allele. |
| TdT                 | +   | +      | +   | +   | +   | +   | +   | +   | +   | +   | +        | +   | +        |
| HLA-DR (L243)       | -   | -      | -   | -   | -   | NT  | -   | NT  | -   | -   | -        | -   | —NT—    |
| CD1 (66IIC7)        | 65  | -      | -   | -   | -   | -   | -   | -   | -   | -   | -        | -   | —NT—    |
| CD2 (Leu-3b)        | +   | +      | +   | +   | +   | +   | +   | +   | +   | +   | +        | +   | —NT—    |
| CD3 (Leu-4)         | -   | -      | -   | -   | -   | -   | -   | -   | -   | -   | -        | -   | —NT—    |
| CD4 (Leu-3a)        | +   | -      | -   | -   | -   | -   | -   | -   | -   | -   | -        | -   | —NT—    |
| CD5 (Leu-1)         | +   | +      | +   | +   | +   | +   | +   | +   | +   | +   | +        | +   | —NT—    |
| CD6 (OKT17)         | 73  | NT     | +   | 71  | -   | 63  | +   | 43  | +   | NT  | 28       | 35  | —NT—    |
| CD7 (3A1)           | +   | +      | +   | +   | +   | +   | +   | +   | +   | +   | +        | +   | —NT—    |
| CD8 (Leu-2a)        | 73  | -      | -   | -   | -   | 73  | 61  | -   | 34  | 53  | 17       | —   | —NT—    |
| TCR-α/β (BMA031)    | —   | -      | -   | -   | -   | -   | -   | -   | -   | -   | —        | —   | —NT—    |
| TCR-γ/δ (11F2)      | NT  | —      | —   | —   | —   | NT  | —   | NT  | —   | —   | —        | —   | —NT—    |
| Cyβ (8F1)           | NT  | +      | +   | NT  | +   | 60  | +   | NT  | +   | +   | NT + 20  | 73  | +        |

* Immunofluorescence data of patient JO were difficult to interpret due to high background. ±, positivity between 15 and 75%.

† T cell lines: Tc1, RPMI 8402; Tc2, HSB-2; Tc3, CEM; Tc4, MOLT 16 (31).

§ Immunologic marker analysis: +, >75% of the cells are positive; –, <15% of the cells are positive; positivity between 15 and 75% is indicated. NT, not tested.

‡ Southern blot analysis: interpretation of the results using the described TCR probes. G, allele in germline configuration; R, rearrangement of the involved allele; T, translocation of involved allele; D, deletion of the involved allele.
Table 4. TCR-δ Rearrangements and Configuration of Deleting Elements in 19 T-ALL and 4 T Cell Lines with a tal-1 Deletion

| T-ALL patients and cell lines* | CD3 phenotype | TCR-δ rearrangements | Deleting elements |
|-------------------------------|---------------|----------------------|-------------------|
| PV                            | CD3-          | Vδ1-Jδ1/Vδ1-Jδ1     | D/D               |
| MV                            | CD3-          | D/δREC-ψJα/Vδ3-Jδ2   | R/G               |
| DR                            | CD3-          | D/Vδ3-Jδ1           | D/G               |
| SL                            | CD3-          | D/Vδ1-Jδ1           | D/D               |
| CW                            | CD3-          | D/Vδ1-Jδ1           | D/D               |
| HSBMI 8402                    | CD3-          | D/ΔREC-ψJα          | R/R               |
| MVP                           | CD3-          | D/t(11;14)          | D/G               |
| JN                            | CD3-          | D/D                 | D/D               |
| TH                            | CD3-          | D/D                 | D/D               |
| CEM                           | CD3-          | D/D                 | D/D               |
| MD                            | α/β+          | D/Vδ1-Jδ1           | D/D               |
| MG                            | α/β+          | D/Vα-Jδ1            | D/D               |
| RK                            | α/β+          | D/ΔREC-ψJα          | D/R               |
| BD                            | α/β+          | D/D/ΔREC-ψJα        | D/D/R*            |
| YB                            | α/β+          | D/D                 | D/D               |
| AG                            | α/β+          | D/D                 | D/D               |
| PM                            | α/β+          | D/D                 | D/D               |
| RS                            | α/β+          | D/D                 | D/D               |
| JO                            | α/β+          | D/D                 | D/D               |
| JW                            | α/β+          | D/D                 | D/D               |
| MB                            | α/β+          | D/D                 | D/D               |
| Molt 16                       | α/β+          | D/D                 | D/D               |

Gene configuration: G, allele in germline configuration; R, rearranged allele; D, deleted allele.

* The T-ALL and T cell lines are ordered according to their immunophenotype (first CD3-, followed by TCR-αβ and the configuration of their TCR-δ genes, i.e., from Vδ3-Jδ rearrangement and ΔREC-ψJα rearrangement to deletion).

ΔREC-ψJα rearrangement present in a small subpopulation.

Figure 4. Junctional region sequences of nonfunctional TCR-δ rearrangements in T-ALL with a tal-1 deletion. Sequences of the junctional regions of the TCR-δ rearrangements are aligned with the known (underlined) Vδ and Jδ germ-line sequences (37). All rearrangements are out of frame. Underlined sequences in the junctional regions represent Dδ nucleotides. Lowercase characters represent P region nucleotides, and all other junctional region nucleotides represent N region nucleotides. Overlined sequences are stop codons when read in the correct reading frame.
| tal-1 deletions in: | G/G | R/G | R/R | D/R | D/D |
|-------------------|-----|-----|-----|-----|-----|
| CD3- T-ALL (8/69) | 0   | (0/7) | 3.3 | 23.5 | 30.0 |
| TCR-γ/δ+ T-ALL (0/25) | - | - | 0 | 0 | - |
| TCR-α/β+ T-ALL (11/40) | - | - | - | 13.3 | 36.0 |
| Total T-ALL (19/134) | 0 | (0/7) | 1.9 | 17.6 | 34.3 |

* TCR-δ configuration: G, allele in germline configuration; R, rearranged allele; D, deleted allele.

loci were left with both deleting elements in germline configuration on the same allele (Table 4). It is noteworthy that both these two TCR-α/δ loci contained a TCR-δ gene rearrangement of the Vδ3 gene segment, which can only be obtained via inversion of the TCR-δ locus (Table 4). This inversion might inhibit δREC-ψJα rearrangements. This would imply that further TCR-δ gene deletions by specific δREC-ψJα rearrangements are impossible in the T-ALL with a tal-1 deletion.

### Discussion

**Five Types of tal-1 Deletions.** So far two main types of tal-1 deletions have been reported, types 1 and 2 (14, 25). Here we describe two new types of tal-1 deletions, designated types 3 and 4. Whereas types 1 and 2 were found in relatively high frequencies (10.4 and 2.2%, respectively) in our series of T-ALL, types 3 and 4 were each observed only once (0.7%). The rare type C tal-1 deletion described by Aplan et al. (25) is an unusual deletion because it does not use any RSS (Aplan’s types A and B are types 1 and 2, respectively).

All types of tal-1 deletions result in a complete deletion of the coding exons of the sil locus, but leave the tal-1 coding exons undamaged. Thus, the oncogenic effect of the tal-1 deletions is not the result of an alteration of the TAL-1 protein, but an aberrant expression of the normal TAL-1 protein, which may contribute to the leukemic transformation of immature T cells into T-ALL.

**RSS Used in tal-1 Deletions.** The tal-1 deletion types 1, 2, 3, and 4 use the same 5’ RSS, which consists of a heptamer sequence only and is located between the first and second noncoding sil exons. The 3’ RSS used in these tal-1 deletions consist of different heptamer-nonamer sequences, with spacers of 24 nucleotides (types 1 and 3) or 12 nucleotides (types 2 and 4). All four 3’ RSS are located in the noncoding 5’ part of the tal-1 locus. These 3’ RSS are highly homologous to the consensus RSS used in regular Ig and TCR gene rearrangement processes (Fig. 5) (27, 28).

Based on several remarkable observations, there has been a lot of speculation on the exact mechanism causing the tal-1 deletions: for instance, the fact that the 3’ RSS consists of a heptamer-nonamer sequence, whereas the 5’ RSS consists only of a heptamer with homology to the consensus heptamer of ~70% (5/7 nucleotides). It can be anticipated that such a small heptamer sequence with no demand for absolute homology will be present at various locations in the 5’ region of the sil locus. Nevertheless only one “specific” heptamer is used in all types of tal-1 deletions. In addition, the 3’ RSS of the tal-1 deletion type 2 displays the highest homology with the consensus RSS of Ig and TCR genes, but this type of tal-1 deletion represents only a minority of the total number of tal-1 deletions. Hence, there are other (sequence) factors that contribute to the development of a tal-1 recombination event.

Sequences homologous to the RSS of Ig and TCR genes do not only occur in the sil and tal-1 genes, but also in other genes, and may lead to recombination and thereby deletion. An example of such site-specific deletions is observed in blood

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**Figure 5.** RSS used in rearrangement processes of Ig, TCR, tal-1, and hprt genes. The RSS used in the various types of tal-1 deletions or hprt deletions are aligned with the consensus heptamer-nonamer sequence of Ig and TCR genes (25, 27, 43). (-) Nucleotide homologous to the RSS consensus sequence.

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Table 6. Junctional Diversity of TCR-δ and TCR-γ Gene Rearrangements and Fusion Region Diversity of tal-1 and hprt Deletions

| Rearrangement/deletion (no. of alleles analyzed) | No. of inserted nucleotides | No. of deleted nucleotides |
|------------------------------------------------|-----------------------------|---------------------------|
|                                                 | Mean | Range   | Mean | Range   |
| TCR-δ gene rearrangements*                       |      |         |      |         |
| VS-Jδ (n = 45)                                  | 28.3 | 5-47    | 5.0  | 0-20    |
| TCR-γ gene rearrangements*                       |      |         |      |         |
| Vγ-Jγ (n = 30)                                  | 7.3  | 0-25    | 9.1  | 1-27    |
| tal-1 deletion fusion regions†                   |      |         |      |         |
| Type 1 (n = 46)                                 | 7.2  | 0-17    | 5.6  | 0-24    |
| Type 2 (n = 10)                                 | 7.5  | 3-15    | 5.6  | 1-14    |
| Type 3 (n = 1)                                  | 8    |         | 2    |         |
| Type 4 (n = 1)                                  | 3    |         | 0    |         |
| hprt deletion fusion regions‡                    |      |         |      |         |
| Class 1 (n = 15)                                | 5.3  | 0-10    | 5.2  | 0-27    |
| Class 2 (n = 2)                                 | 10.0 | 8-12    | 9.5  | 3-16    |
| Class III (n = 1)                               | 3    |         | 8    |         |

* Data from reference 46.
† Combined results of this paper and references 14, 22, and 25.
‡ Data from reference 43.

T lymphocytes and involves the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (hprt) on chromosome Xq26 (43). Three types of deletions have been observed in the hprt gene, designated classes I–III, and all three damage the gene. The three types of hprt deletions use the same 5' KSS, which is located in intron 1 and consists of a sole heptamer, but different 3' KSS, which are located in intron 3 and consist of heptamer-nonamer sequences with spacers of different sizes (Fig. 5). The fusion regions of these hprt deletions show N regions, P region nucleotide insertion, and deletion of nucleotides by trimming of the flanking sequences, and are therefore homologous to the Ig and TCK junctional regions and tal-1 breakpoint fusion regions (43). The hprt deletions occur at a low frequency of ~10^-7 and are not oncogenic (44, 45).

Fusion Regions of tal-1 Deletion Breakpoints. Because fusion regions of tal-1 and hprt deletions strongly resemble junctional regions of normal Ig and TCR gene rearrangements, the fusion region nucleotide insertion and deletion of all tal-1 and hprt deletions described to date were compared to the junctional regions of TCR-δ and TCR-γ gene rearrangements (Table 6). Remarkably, the average insertions observed in the tal-1 breakpoint fusion regions (7.2 nucleotides) and the hprt breakpoint fusion regions (5.7 nucleotides) were comparable to the average insertion of the TCR-γ gene rearrangement (7.3 nucleotides), but lower than that of the TCR-δ gene rearrangements (28.3 nucleotides), due to the use of Dδ gene segments in the latter rearrangement (Table 6) (46). However, average nucleotide deletion of the tal-1 deletions (5.4 nucleotides) and the hprt deletions (5.8 nucleotides) were less extensive than in TCR-γ gene rearrangements (9.1 nucleotides), but were comparable to TCR-δ gene rearrangements (5.0 nucleotides) (Table 6) (46). Although the average nucleotide deletions of tal-1 deletion types 1 and 2 were identical (5.6 nucleotides), the nucleotide deletions at the 5' and 3' flanking sides differed markedly between the two types of tal-1 deletions. In tal-1 deletion type 1, the average nucleotide deletion of the 5' flanking side (3.8 nucleotides) was more than twice that of the 3' flanking side (1.8 nucleotides), whereas in tal-1 deletion type 2 the reversed situation was observed with the average nucleotide deletion at the 5' flanking side (2.0 nucleotides), being approximately half of that at the 3' flanking side (3.6 nucleotides). This difference is most probably related to the different sizes of the spacers in the 3' RSS of these two types of tal-1 deletions. The type 1 heptamer-nonamer contains a 24-bp spacer and therefore resembles the RSS of a TCR V gene segment, whereas the type 2 heptamer-nonamer with a 12-bp spacer resembles the RSS of a TCR J gene segment (4, 27). So the “J gene–alike” side is trimmed twice as much as the “V gene–alike” side in both types of tal-1 deletions. This resembles the TCR-γ rearrangements where deletion by trimming of the Jγ gene segments is about twice that of the Vγ gene segments (46). Also, in hprt deletions this heptamer-spacer-nonamer-related nucleotide deletion is observed, where the “J gene–alike” side of the hprt deletion class I is trimmed over twice as much as the other side. These combined data suggest that the size of the spacer induces direction to the activity of the recombination enzyme complex.

tal-1 Deletions Are Restricted to the TCR-α/β Lineage. tal-1 deletions are restricted to malignancies of the T cell lineage since they have not been discovered in any other hematopo-
etiologic malignancy tested (8, 22, 25). However, the frequencies of the tal-1 deletions in T-ALL differ markedly between the reported studies, from 12% (14) to 26% (22). This difference may be caused by the compilation of the analyzed series of T-ALL. The high frequency of 26% tal-1 deletions may be an overestimation caused by an overrepresentation of TCR-α/β+ T-ALL (22), because in our series such a high frequency of tal-1 deletions (27.5%) was only found in the group of TCR-α/β+ T-ALL. However, in the study by Aplan et al. (25), almost all tal-1 deletions (10/11) were found in CD3−− T-ALL, whereas only half of our tal-1 deletions (8/19) were found in CD3− T-ALL.

In our study, tal-1 deletions were detected only in TCR-α/β+ (27.5%) and CD3− T-ALL (11.6%), but not in TCR-γ/δ+ T-ALL. This suggested that the occurrence of tal-1 deletions is restricted to T-ALL of the α/β differentiation lineage. Therefore, we wished to investigate whether the CD3− T-ALL with a tal-1 deletion represented precursors of TCR-α/β+ T-ALL or precursors of TCR-γ/δ+ T-ALL. One of the present theories concerning the separation of the α/β and γ/δ differentiation pathways assumes that all T cells that do not productively rearrange their TCR-γ and/or TCR-δ genes are capable of differentiation into TCR-α/β-committed T cells by deletion of the TCR-α locus, which is embedded in the TCR-α locus (30, 40, 42). If so, the configuration of the TCR-δ genes in CD3− T-ALL can be used cautiously as an α/β-γ/δ lineage marker. Therefore, we divided the CD3− T-ALL into two subgroups on basis of their TCR-δ gene configuration. The first subgroup consisted of CD3− T-ALL (n = 42) without deletion of the TCR-δ locus but with TCR-δ gene rearrangement in most of them, and therefore resembled T-ALL of the γ/δ lineage (Table 5). The other CD3− T-ALL subgroup (n = 27) had one or both TCR-δ alleles deleted and therefore may represent an early stage of the α/β lineage. Almost all tal-1 deletions in CD3− T-ALL (7/8) were found in this putative α/β lineage CD3− subgroup, and only one was found in the putative γ/δ lineage CD3− subgroup. However, sequencing of the junctional regions revealed that all TCR-δ rearrangements in CD3− T-ALL with a tal-1 deletion were nonfunctional. Therefore, these T-ALL could never express a TCR-δ chain and consequently belonged to the α/β lineage. Thus, all tal-1 deletions appeared to be restricted to T-ALL of the α/β lineage.

Interestingly, the reported hprt deletions, which are comparable to the tal-1 deletions, were found in T cell clones derived from mature blood T lymphocytes (45). Although the precise TCR-α/β-TCR-γ/δ phenotype of these T cell clones was not reported, their CD4/CD8 phenotype strongly suggests that they belonged to the α/β lineage (45). This would be in line with the restriction of tal-1 deletions to the α/β lineage.

The finding that all tal-1 deletions were detected in the TCR-α/β+ T-ALL or CD3− T-ALL of the α/β lineage may be caused by a combination of two mechanisms. The first mechanism is based on the theory that in Ig or TCR gene recombination both recombining elements must be transcriptionally active (2, 4). If tal-1 deletions are indeed caused by "illegitimate" V(D)J recombination, the simultaneous expression of the sil and tal-1 genes may be a prerequisite for recombination and thus deletion. This is supported by the findings that tal-1 expression in the few cases tested was restricted to TCR-α/β+ T-ALL and CD3− T-ALL of α/β lineage, whereas sil expression was not restricted to a particular subgroup of T-ALL (24; Breit et al., unpublished results). The second mechanism is based on the theory that once both TCR-δ alleles are rearranged, but no TCR-γ/δ expression occurs due to nonfunctional rearrangements of TCR-γ and/or TCR-δ genes, the rearranged TCR-δ genes will be deleted via a special deletion mechanism involving the δREC and ψα recombination elements (40, 42). TCR-δ gene deletion prepares the allele for subsequent rearrangement of TCR-α gene segments and thereby forces the T cells to differentiate into the α/β lineage (30). One might speculate that a special TCR-δ gene-deleting recombinase complex is present only in T cells of the α/β lineage and is also responsible for the tal-1 deletions and hprt deletions. This is supported by the finding that the frequency of tal-1 deletions increases with the number of deleted TCR-δ alleles. Where in T-ALL without TCR-δ gene deletions the frequency of tal-1 deletions was just 1.5% (1/65), in T-ALL with one deleted TCR-δ allele this frequency was 17.6% (6/34) and in T-ALL with TCR-δ gene deletions on both alleles the frequency of tal-1 deletions was substantially higher, 34.3% (12/35). Interestingly, further deletion of the remaining (nonfunctional) TCR-δ genes in the T-ALL with a tal-1 deletion was not possible via δREC-ψα rearrangements in most of them because of deletion of the δREC and/or ψα gene segments (Table 4). Additional support for our speculation is found in the observation that the hprt deletions probably exclusively occur in TCR-α/β+ T lymphocytes, which generally have TCR-δ gene deletions on both alleles.

Rearrangement studies in which lymphoid cell lines of different lineages are transfected with extrachromosomal vectors containing the TCR-δ gene-deleting elements and/or sil-tal-1 gene constructs might prove whether indeed a special TCR-δ gene-deleting recombinase complex exists and whether this enzyme complex is involved in tal-1 deletions. Based on our data, we hypothesize that the multiple enzymes of the Ig/TCR gene recombination complex are differentially expressed, related to the differentiation lineage and differentiation stage of the lymphoid cells. The mechanisms regulating such putative differential expression could also determine the occurrence of oncogenic and nononcogenic rearrangements and deletions in other genes with RSS homologous to the Ig and TCR genes. This would explain the restriction of particular chromosome aberrations to specific types of lymphoid leukemias, such as tal-1 deletions in T-ALL of the α/β lineage.
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Note added in proof: After submission of this manuscript, MacIntyre et al. (47) published a study on 39 T-ALL patients and concluded also that the occurrence of tal-1 deletions correlated with commitment to the α/β lineage.

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