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

By Timo M. Breit,* Ellen J. Mol,* Ingrid L. M. Wolvers-Tettero,* Wolf-Dieter Ludwig,‡ Elisabeth R. van Wering,§ and Jacques J. M. van Dongen*

*From the *Department of Immunology, University Hospital Dijkzigt/Erasmus University, 3000 DR Rotterdam, The Netherlands; the ‡Department of Hematology/Oncology, Universitätsklinikum Steglitz, D-1000 Berlin 45, Germany; and the §Dutch Childhood Leukemia Study Group, 2506 LP The Hague, The Netherlands*

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-γ/δ^+ T-ALL, whereas 8 of the 69 $CD3^-$ T-ALL and 11 of the 40 TCR-α/β^+ 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 recombination. This translocation is exclusively found in T cell acute lymphoblastic leukemia (T-ALL)[1] 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, disregulation of the *tal*-1 gene expression by chromosomal aberrations may contribute to the leukemic transformation in T-ALL.

[1] *Abbreviations used in this paper: hprt*, hypoxanthine-guanine phosphoribosyltransferase; MNC, mononuclear cells; RSS, recombination signal sequence; T-ALL, T cell acute lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase.

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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 recombinase system, because the breakpoints seem to cluster at heptamer-nonamer 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-nonamer 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-nonamer RSS. In contrast to suggestions in other publications (14, 25), the *tal*-1 deletions exclusively occurred in T-ALL of the α/β T cell lineage and were especially correlated with TCR-δ gene deletions, which are characteristic for the α/β 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-γ/δ^+ 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-α/β^+ T-ALL (30%), and 25 TCR-γ/δ^+ T-ALL (19%). In random series of T-ALL, this immunophenotype distribution probably is ~70, ~20, and ~10%, 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 cell samples were frozen and stored in liquid nitrogen. 12 T cell lines were included as positive or negative controls (CEM, HPB-ALL, MOLT16, JURKAT, H9, HUT78, DND41, PEER, RPMI-8402, MOLT4, HSB-2, GH1) (31).

Immunologic Marker Analysis. The MNC of the T-ALL patients were analyzed for nuclear expression of terminal deoxynucleotidyl transferase (TdT), for cytoplasmic expression of TCR-β (βF1), for cell membrane expression of T cell markers CD1 (66IIC7), CD2 (Leu-5b), CD3 (Leu-4), CD4 (Leu-3a), CD5 (Leu-1), CD6 (OKT17), CD7 (3A1), and CD8 (Leu-2a), for HLA-DR antigen, and for reactivity with the mAbs BMA031 (anti-TCR-α/β), 11F2 (anti-TCR-γ/δ), and TCR-δ1 (anti-TCR-δ). The rabbit anti-TdT antiserum was purchased from Supertechs (Bethesda, MD); the mAbs of the Leu series, anti-HLA-DR and 11F2, were obtained from Becton Dickinson & Co. (San Jose, CA); the CD1 antibody was obtained from Monosan/Sanbio (Nistelrode, The Netherlands); OKT17 was from Ortho Diagnostic Systems (Raritan, NJ); the 3A1 hybridoma was from the American Type Culture Collection (Rockville, MD); TCR-δ1 and βF1 were obtained from T Cell Diagnostics (Cambridge, MA). The mAb BMA031 was kindly provided by Dr. R. Kurrle (Behring, Marburg, Germany). Immunofluorescence stainings were evaluated with fluorescence microscopes (Carl Zeiss, Inc., Oberkochen, Germany) and/or a FACScan® flowcytometer (Becton Dickinson & Co.).

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 λ 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 subcloned in the pUC19 cloning vector.

Isolation of sil and tal-1 Gene DNA 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 (32). The oligonucleotide primer sets for the SILDB and TALDB2 probes, including the artificial tails containing restriction sites for cloning, are given in Table 1. All oligonucleotides were synthesized according to published *tal*-1 gene sequences (13, 14, 22) on a DNA synthesizer (392; Applied Biosystems, Inc., Foster City, CA) with the solid-phase phosphotriester method and used without further purification. Probe TALDB1 was isolated by cloning an ~800-bp MspI-MspI fragment, containing the 3′ side of the *tal*-1 deletion type 1 (Fig. 1 *A*), from the CML-0 genomic library (33).

Southern Blot Analysis. DNA was isolated from frozen MNC as described previously (32, 34). A 15-μg 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-β gene rearrangements were detected with the Jβ1, Jβ2, and Cβ probes (34, 35) in EcoRI and HindIII digests. The configuration of the TCR-γ genes was analyzed by use of the Jγ1.2, Jγ1.3, Jγ2.1, and Cγ probes (34, 36) in EcoRI and KpnI digests. The configuration of the TCR-δ genes was analyzed by use of the Vδ1, Vδ3, Jδ1, Jδ2, Cδ, δ_{REC}, and ψJα 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-μg 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 μl. 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 H_2O, 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 ^{35}S 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 tal1db2 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 tal1db3-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 SalI-XbaI subclone, the tal1db4 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 SalI-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

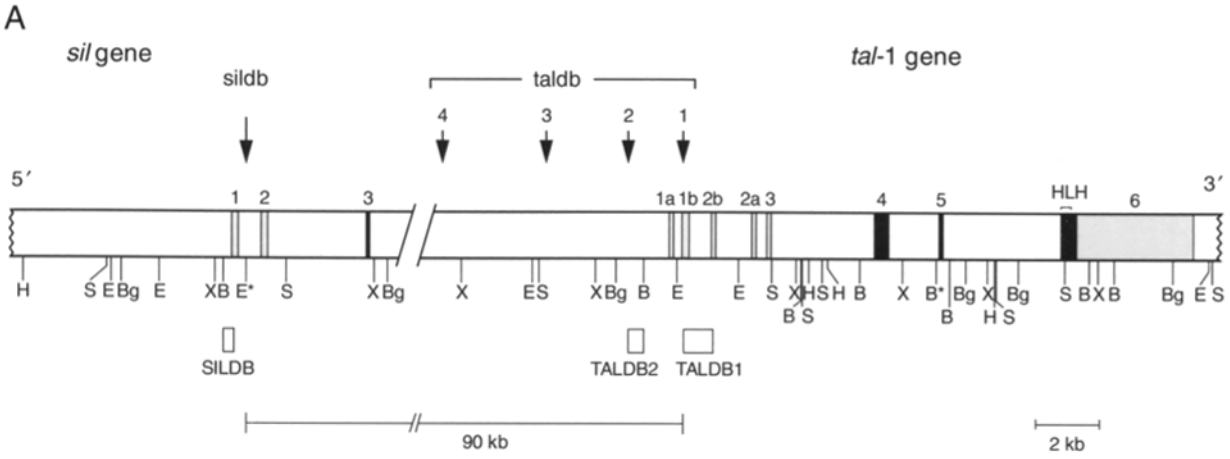


Figure 1. Restriction map and germline sequences of *tal*-1 deletion breakpoint regions. (*A*) Restriction map of the *tal*-1 locus and the 5′ part of the *sil* gene involved in the ~90-kb *tal*-1 deletion. The various types of *tal*-1 deletion breakpoints are indicated with arrows: *sildb*, breakpoint 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*, BglII; *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.

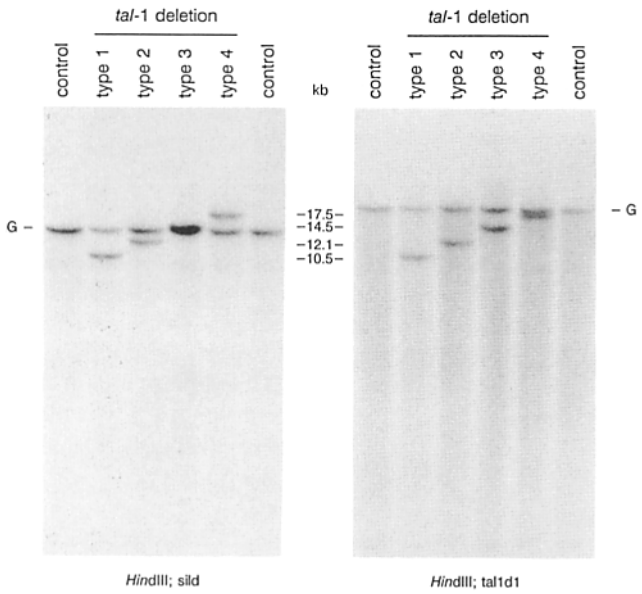


Figure 2. Southern blot analysis of the various types of *tal*-1 deletions. HindIII digests of DNA from patients with different types of *tal*-1 deletion: type 1, patient SL; type 2, patient PV; type 3, patient MB; type 4, patient BD. The Southern blot filter was successively hybridized with the SILDB and TALDB1 probes (Fig. 1 *A*). The sizes of the rearranged bands are indicated. *G*, germline band.

2, but different 3′ heptamer-nonamer RSS (Fig. 1 *B*). The heptamer-nonamer RSS for *tal*-1 deletion type 2 is located ~1.7 kb upstream of the type 1 RSS (14, 25), whereas the RSS of types 3 and 4 are located ~4.5 and ~7.7 kb upstream of the type 1 RSS, respectively (Fig. 1 *A*). The RSS of all *tal*-1 deletions display a strong homology with the heptamer-nonamer RSS sequences of Ig and TCR genes.

Fusion Regions of tal-1 Deletion Breakpoints. In total, 19 *tal*-1 deletions were found in the 134 T-ALL analyzed: 14 type 1, three type 2, one type 3, and one type 4 (Table 2). In addition, four T cell lines (RPMI 8402, HSB-2, CEM, MOLT 16) contained a type 1 *tal*-1 deletion. PCR products containing the fusion regions of the *tal*-1 deletion breakpoints were obtained by amplification of the different *tal*-1 deletions from the 19 T-ALL and 4 T cell lines (Fig. 3 *A*). Subsequent sequencing of the *tal*-1 deletion PCR products revealed the sequences of the breakpoint fusion regions (Fig. 3 *B*). All fusion regions contained randomly inserted nucleotides (N region) except for patient MD, who had a fusion region consisting of two P region nucleotides only. The average N region insertion was 6.4 nucleotides, and a total of 14 P region nucleotides were observed in the 23 fusion regions analyzed (19 T-ALL and 4 T cell lines). Trimming occurred in the far majority of the *tal*-1 deletions. Thus, the fusion regions are highly homologous to the junctional regions of rearranged Ig and TCR genes.

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′ 3′	
5′ all types	sildb	−155	GGGGAGCTCGTGGGAGAAATTAAG	22
	sildb-seq	−111	GGTATCATCTGAGCTAAGGTATGTG	22
3′ type 1	tal1d1	+155	GCCTCGAAGGGTCCACATCTAC	14
	tal1db1-seq	+111	CACACTCGGACACAGAGCCTG	14
	tal1db1-5′	+28	TCACAATCCCACCGCATGCACA	14
3′ type 2	tal1db2	+152	TTGTAAAATGGGGAGATAATGTCGAC	This paper
	tal1db2-seq	+110	AACTTATATGACCTTTAAAAGG	This paper
3′ type 3	tal1db3	+58	TGCATGCACTCTGATGAGCAGCC	This paper
	tal1db3-seq	+15	ATCTACACTGCAGTTACTGTGTGAC	This paper
3′ type 4	tal1db4	+450	GGATTATAGGTGCCTGTCACCAC	This paper
	tal1db4-seq	+34	TACATCTTATAGTATGTAAATTATGCC	This paper
DNA probes				
SILDB	sildp5′	330	CACAGGATCCTTGATCCTGGAGCGC	22
	sildp3′		CCGAAGCTTCCGCGGAGCTGAGGTCTG	22
TALDB2	tal1d2p5′	~575	TGTAAGCTTTGGCTAAGTCACATCTCTC	14
	tal1d2p3′		CCTGTCAATAGGGACATAAATGCC	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*.

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

§ 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).

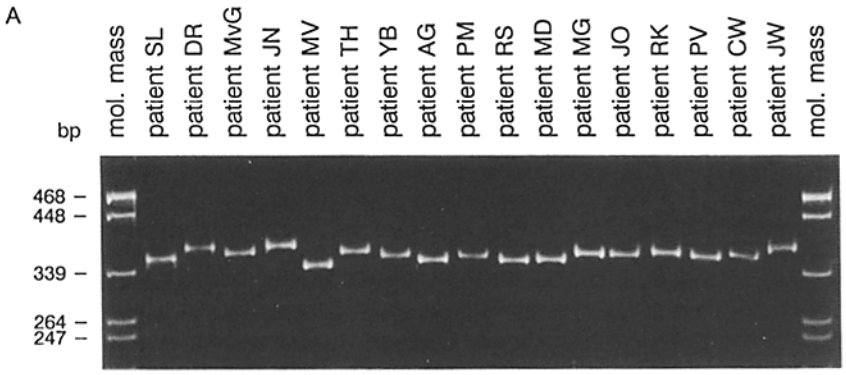
Further analysis of the eight TCR-δ gene rearrangements in the T-ALL with a *tal*-1 deletion showed that seven were complete Vδ-Jδ rearrangements, and one TCR-α/β^+ T-ALL (patient MG) contained a Vα-Jδ1 rearrangement (Table 4). Sequence analysis of the seven Vδ-Jδ junctional regions revealed that all 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 ψJα 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 ψJα (40, 42). The specific δREC-ψJα 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 ψJα alleles were deleted in the T-ALL and T cell lines with a *tal*-1 deletion. Only two TCR-α/δ

A



B

	5′ *tal*-1 deletion	fusion region	3′ *tal*-1 deletion type 1
	TCCTCACAATTTCTGGCTCA		GTTGGTTTTCATTTCTTCTT
Patients			
SL	TCCTCACAATTTC	CGGCGGCCc	GTTGGTTTTCATTTCTTCTT
DR	TCCTCACAATTTCTGGCTCA	CACGGGGGG	TGGTTTTCATTTCTTCTT
MvG	TCCTCACAATTTCTGGCTCA	tgGAAGACAAA	GTTGGTTTTCATTTCTTCTT
JN	TCCTCACAATTTCTGGCTCA	GGCGATTGATTGCAATc	GTTGGTTTTCATTTCTTCTT
MV	TCCT	ACCC	GGTTTTCATTTCTTCTT
TH	TCCTCACAATTTCTGGCTC	GAGCCCCGT	TTGGTTTTCATTTCTTCTT
YB	TCCTCACAATTT	GGGG	GTTGGTTTTCATTTCTTCTT
AG	TCCTCACAATTTCTGGCT	ACc	GTTGGTTTTCATTTCTTCTT
PM	TCCTCACAATTTCTGGC	CC	TTGGTTTTCATTTCTTCTT
RS	TCCTCACAATTTCTGGC	CCAGTG	TGGTTTTCATTTCTTCTT
MD	TCCTCACAATTTCTGGCTCA	ac	GTTGGTTTTCATTTCTTCTT
MG	TCCTCACAATTTC	CGCAGAGGATACAGTc	GTTGGTTTTCATTTCTTCTT
JO	TCCTCACAATTTCTGGCTCA	CTCGGTG	GGTTTTCATTTCTTCTT
RK	TCCTCACAATTTCTGGC	CTTCCCCc	GTTGGTTTTCATTTCTTCTT
Cell lines			
RPMI 8402	TCCTCACAATTTC	CGGATCAAA	TCATTTCTTCTT
HSB-2	TCCTCACAATTTCTGGCTCA	tCT	GTTGGTTTTCATTTCTTCTT
CEM	TCCTCACAATTTCTGGC	AAGTGGA	TTGGTTTTCATTTCTTCTT
Molt-16	TCCTCACAATTTCTGGCTCA	tTAGGGGTTC	GGTTTTCATTTCTTCTT
			3′ *tal*-1 deletion type 2
			TCTTAATTTCTTTCTCTATG
Patients			
PV	TCCTCACAATTTCTGGCT	GCC	ATTTCTTTCTCTATG
CW	TCCTCACAATTTCTGGCTC	GTA	CTTAATTTCTTTCTCTATG
JW	TCCTCACAATTTCTGGCTCA	tCTTGGGTA	CTTAATTTCTTTCTCTATG
			3′ *tal*-1 deletion type 3
			CCTAGGACAGTACTTGTCAC
MB	TCCTCACAATTTCTGGCT	GATCCTTg	CCTAGGACAGTACTTGTCAC
			3′ *tal*-1 deletion type 4
			GGGCCACAAAGAACGTTTAT
BD	TCCTCACAATTTCTGGCTCA	tTA	GGGCCACAAAGAACGTTTAT

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% polyacryl amide gel. The PCR reaction was performed using the sildb primer and either the tal1db1 primer (type 1) or tal1db2 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*

		tal-1 deletion type 1																		type 2			type 3	type 4
		SL	DR	MvG	JN	MV	TH	YB	AG	PM	RS	MD	MG	JO*	RK	Tcl1‡	Tcl2‡	Tcl3‡	Tcl4‡	PV	CW	JW	MB	BD
Immunologic markers§										%														
TdT		+	+	+	+	70	32	71	+	67	+	59	+	59	+	+	−	−	+	45	+	+	+	+
HLA-DR	(L243)	−	−	−	−	−	−	NT	−	NT	−	−	−	NT	−	−	−	−	−	−	NT	NT	−	−
CD1	(66IIC7)	65	−	−	−	−	55	−	−	−	21	−	−	±	−	−	−	−	−	21	−	−	−	50
CD2	(Leu-5b)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	20	+	+	+	+	+
CD3	(Leu-4)	−	−	−	−	−	−	35	45	46	64	72	+	+	+	−	−	−	+	−	−	59	+	+
CD4	(Leu-3a)	+	−	−	−	73	68	−	−	+	34	18	−	±	+	−	−	+	−	39	+	34	48	37
CD5	(Leu-1)	+	+	+	+	+	71	+	+	+	+	+	+	+	+	+	+	64	+	+	+	+	+	+
CD6	(OKT17)	73	NT	+	71	−	63	+	43	+	NT	28	35	±	NT	25	72	20	+	+	+	36	NT	35
CD7	(3A1)	+	+	+	+	+	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CD8	(Leu-2a)	73	−	−	−	73	61	−	34	+	53	17	−	−	−	−	−	−	−	71	42	36	32	+
TCR-α/β	(BMA031)	−	−	−	−	−	−	23	49	23	NT	59	+	+	61	−	−	−	+	−	−	42	NT	35
TCR-γ/δ	(11F2)	NT	−	−	−	−	−	−	NT	−	NT	−	−	−	−	−	−	−	−	−	−	−	NT	−
Cyβ	(βF1)	NT	+	+	NT	+	60	+	NT	+	NT	+	+	+	NT	+	20	73	+	32	+	+	NT	+
Southern blot analysis¶																								
TCR-δ	genes	D/R	D/R	D/D	D/D	D/R	D/D	D/D	D/D	D/D	D/D	D/R	D/R	D/D	D/D	D/T	D/D	D/D	D/D	R/R	D/R	D/D	D/D	D/D
TCR-γ1	genes	D/D	D/D	D/D	D/D	D/R	D/D	D/R	D/D	D/D	D/D	D/D	D/R	D/D	D/D	D/D	D/D	D/D	D/D	D/R	R/R	R/R	D/D	R/R
TCR-γ2	genes	R/R	R/R	R/R	R/R	R/G	R/R	R/G	R/R	R/R	R/R	R/R	R/G	R/R	R/R	R/R	R/R	R/R	R/R	R/G	G/G	G/G	R/R	G/G
TCR-β1	genes	G/G	R/R	D/R	R/R	R/R	D/D	D/R	D/R	D/R	R/R	D/R	D/G	D/D	R/G	D/R	R/R	D/R	D/R	R/G	D/R	R/R	D/R	D/D
TCR-β2	genes	R/R	R/G	R/G	R/G	G/G	R/R	R/R	R/G	R/R	R/G	R/G	R/G	R/R	R/G	R/G	G/G	R/R	R/G	R/R	R/G	R/G	R/G	R/R

* Immunofluorescence data of patient JO were difficult to interpret due to high background. ±, positivity between 15 and 75%.
‡ T cell lines: Tcl1, RPMI 8402; Tcl2, HSB-2; Tcl3, CEM; Tcl4, 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	
			δ_{REC}	$\psi J\alpha$
PV	CD3⁻	Vδ1-Jδ1/Vδ1 – Jδ1	D/D	G/G
MV	CD3⁻	δ_{REC}-$\psi J\alpha$/Vδ3-Jδ2	R/G	R/G
DR	CD3⁻	D/Vδ3-Jδ1	D/G	D/G
SL	CD3⁻	D/Vδ1-Jδ1	D/D	D/G
CW	CD3⁻	D/Vδ1-Jδ1	D/D	D/G
HSB-2	CD3⁻	D/δ_{REC}-$\psi J\alpha$	R/R	D/R
RPMI 8402	CD3⁻	D/t(11;14)	D/G	D/D
MvG	CD3⁻	D/D	D/D	D/D
JN	CD3⁻	D/D	D/D	D/D
TH	CD3⁻	D/D	D/D	D/D
CEM	CD3⁻	D/D	D/D	D/D
MD	α/β^+	D/Vδ1-Jδ1	D/D	D/G
MG	α/β^+	D/Vα-Jδ1	D/D	D/D
RK	α/β^+	D/δ_{REC}-$\psi J\alpha$	D/R	D/R
BD	α/β^+	D/D/δ_{REC}-$\psi J\alpha$‡	D/D/R‡	D/D/R‡
YB	α/β^+	D/D	D/D	D/D
AG	α/β^+	D/D	D/D	D/D
PM	α/β^+	D/D	D/G	D/D
RS	α/β^+	D/D	D/G	D/D
JO	α/β^+	D/D	D/D	D/D
JW	α/β^+	D/D	D/D	D/D
MB	α/β^+	D/D	D/D	D/D
Molt 16	α/β^+	D/D	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-α/β^+) and the configuration of their TCR-δ genes (i.e., from Vδ-Jδ rearrangement and δ_{REC}-$\psi J\alpha$ rearrangement to deletion).
‡ δ_{REC}-$\psi J\alpha$ rearrangement present in a small subpopulation.

Patients	Vδ1	Junctional region	Jδ1
	CTCTTGGGGAACT		ACACCGATAAACT
SL	CTCTTGGGGA	CCCCCGGAAATAGTaGGACGGA	ACCGATAAACT
MD	CTCTTGG	CTTTCTCTCCGGGtACTGGGGGATTTgt	ACACCGATAAACT
PV	CTCTTGGG	CAATTTGGTTCAGTCATTTATGACCCCAAGGGGATAATT	CACCGATAAACT
PV	CTC	GACGCCGC	ACACCGATAAACT
CW	CTCTTGGGGA	GCTGAGATCCCCGTCCTCCCAGGGAGCGCGGGGACCTAt	ACACCGATAAACT
	Vδ3		
	ACTGTGCCTTTAG		
DR	ACTGTGCCTTTA	AGGACTGGGCCGGGGCGGGATGGGGG	T
			Jδ2
			CTTTGACAGCACA
MV	ACTGTGCCTTT	CTTACTGCCGGGACTTAGGAGGGGAG	GACAGCACA

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 germline 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.

Table 5. *Frequency of tal-1 Deletions in T-ALL Subgroups Defined by CD3 Phenotype and TCR-δ Gene Configuration*

tal-1 deletions in:	TCR-δ gene configuration				
	G/G	R/G	R/R	D/R	D/D
			%		
$CD3^-$ T-ALL	0	0	3.3	23.5	30.0
(8/69)	(0/7)	(0/5)	(1/30)	(4/17)	(3/10)
TCR-γ/δ^+ T-ALL	–	–	0	0	–
(0/25)	(0/0)	(0/0)	(0/23)	(0/2)	(0/0)
TCR-α/β^+ T-ALL	–	–	–	13.3	36.0
(11/40)	(0/0)	(0/0)	(0/0)	(2/15)	(9/25)
Total T-ALL	0	0	1.9	17.6	34.3
(19/134)	(0/7)	(0/5)	(1/53)	(6/34)	(12/35)

* 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 δ_{REC}-$\psi J\alpha$ rearrangements. This would imply that further TCR-δ gene deletions by specific δ_{REC}-$\psi J\alpha$ 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

	heptamer	nonamer	(spacer)	heptamer	
Ig/TcR consensus	CACAGTG	GGTTTTTGT	(12/23)	CACTGTG	
tal-1 deletion	---TC--	CC----CC-	(24)	GG-----	type 1
		A-----G-C	(12)	-T-----	type 2
		----C---C	(24)	--AA---	type 3
		-T--CC--G	(12)	TGA----	type 4
hprt gene deletion	---T--A	T--A-----	(12)	GTG----	class I
		CT----AT-	(23)	-T-----	class II
		T-----G--	(23)	TTTA---	class III

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.

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*				
Vδ-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 I (n = 15)	5.3	0–10	5.2	0–27
Class II (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′ RSS, which is located in intron 1 and consists of a sole hepatamer, but different 3′ RSS, 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 TCR junctional regions and *tal*-1 breakpoint fusion regions (43). The *hprt* deletions occur at a low frequency of $\sim 10^{-7}$ 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 hematopoi-

etic 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-δ 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 δ_{REC} and $\psi J\alpha$ 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 δ_{REC}-$\psi J\alpha$ rearrangements in most of them because of deletion of the δ_{REC} and/or $\psi J\alpha$ 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 recombinase 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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Address correspondence to J. J. M. van Dongen, Department of Immunology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

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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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