A new transcript in the TCRB locus unveils the human ortholog of the mouse pre-D\(\beta\)1 promoter

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Introduction: While most transcripts arising from the human T Cell Receptor locus reflect fully rearranged genes, several germline transcripts have been identified. We describe a new germline transcript arising from the human TCRB locus.

Methods: cDNA sequencing, promoter, and gene expression analyses were used to characterize the new transcript.

Results: The new germline transcript encoded by the human TCRB locus consists of a new exon of 103 bp, which we named TRBX1 (X1), spliced with the first exon of gene segments C\(\beta\)1 or C\(\beta\)2. X1 is located upstream of gene segment D\(\beta\)1 and is therefore deleted from a V-DJ rearranged TCRB locus. The X1-C\(\beta\) transcripts do not appear to code for a protein. We define their transcription start and minimal promoter. These transcripts are found in populations of mature T lymphocytes from blood or tissues and in T cell clones with a monoallelic TCRB rearrangement. In immature thymocytes, they are already detectable in CD1a\(^+\)CD34\(^+\)CD4\(^-\)CD8\(^-\) cells, therefore before completion of the TCRB rearrangements.

Conclusions: The X1 promoter appears to be the ortholog of the mouse pre-D\(\beta\)1 promoter (PD\(\beta\)1). Like PD\(\beta\)1, its activation is regulated by E\(\beta\) in T cells and might facilitate the TCRB rearrangement process by contributing to the accessibility of the D\(\beta\)1 locus.

Introduction
T cell receptor (TCR) gene rearrangements are complex multistep processes occurring at different stages of thymocyte maturation. They involve double-strand DNA breaks at the recombination signal sequences that border the V(D)J gene segments and that are recognized by the RAG1/RAG2 recombinases [1]. The process is tightly controlled, with the TCRB locus being rearranged before TCR\(\alpha\). For TCRB it occurs in two steps. First, at the CD4\(^-\)CD8\(^-\) double negative stage a D\(\beta\) gene segment recombines with a J\(\beta\) segment yielding a D\(\beta\)J\(\beta\) partially rearranged genomic DNA. These early rearrangements occur at the CD1\(^-\)CD34\(^+\) stage. In a second step, ending before the CD4\(^+\)CD8\(^-\) double positive (DP) stage, a V\(\beta\) gene segment recombines with D\(\beta\)J\(\beta\) [2]. If this rearrangement leads to the production of a complete TCRB chain, the latter dimerizes with the pre-TCRA chain and initiates \(\beta\)-selection. During \(\beta\)-selection, the pre-TCR signaling suppresses RAG1/2 expression, induces several rounds of division and differentiation toward CD4\(^+\)CD8\(^-\) DP

Abstract
thymocytes. At the DP stage, reexpression of RAG1/2 enables TCRα rearrangements to produce TCR-positive DP cells [3]. Our previous work on T lymphocytes infiltrating human melanoma tumors led us to construct TCRB cDNA libraries [4]. Starting from tumoral RNA, we first used a SMART-PCR on cDNA extended from a Cβ primer, and cloned the amplified products. Sequencing these products provided information on frequencies of tumor-specific cytolytic T cell clones present in the tumor [4]. A significant proportion of the sequences corresponded to a new TCRB germline transcript that we describe here.

**Materials and Methods**

**Construction of TCRB-targeted cDNA libraries**

These libraries [4] were built up from RNA reverse-transcribed with an antisense Cβ primer [nt 63–47 of exon 1] in the presence of SMART II (Clontech®, Mountain View, CA, USA) [5], an oligonucleotide engineered to be copied at the 3’-end of the growing cDNA during reversion, ought to an intrinsic Terminal deoxynucleotidyl Transferase (TdT) activity of the RT. A RNaseH- RT-enzyme in an appropriate buffer is needed for this 3’-extension of the cDNA. With a primer consisting in the core of the SMART primer (5’-gagctggtaacaacgcagagta) and a Cβ primer (primer 2 of Table 1) located near the 5’-end of Cβ, the cDNA was amplified by PCR for a limited number of cycles with an annealing step at 60°C. Products shorter than 150 bp were removed by a Sepharose CL-6B size-exclusion column (Pharmacia-Amer sham). Under these conditions, the products derived from full length TCRB (TRB-LVDJC) transcripts are in the range of 480 bp with 33 nucleotides coming from the primed region of Cβ. Using a small fraction of the extracted RNA, in order to obtain the “smarted” cDNA from 0.5–1% of the TCRB transcripts, allows to estimate the frequency of the most prevalent clonotypes present in that sample, assuming a copy number of 200 productive TRB-LVDJC transcripts/T cell. The method also allows to readily define the 5’-ends of the TCRB transcripts, as an alternate 5’-RACE approach, since many of the isolated clones represent full length cDNA.

| Table 1. Primers used for X1-Cβ transcripts quantification. |
|-------------------------------------------------------------|
| Sequence | Target | Position |
| 5’-GCTCAAACCATCTCTGAGGACA (#1 in Fig. 2A) | X1 sense | 73–93a |
| 5’-CGACCTCCGGGTGGG (#2 in Fig. 2A) | Cβ antisense | 33–16b |
| 5’-TGCTCCCTGAGGCGGTCGC (#3 in Fig. 2A) | Cβ sense | 196–178b |
| 5’-FAM-TTCAAGTCCTCCTCCAGGACCTG-TAMRA (P in Fig. 2A) | X1-Cβ probe, antisense | straddling X1 and Cβ |
| 5’-ATTGCCGACAGGTACGCAA | ACTB sense | 998–1017c |
| 5’-GCTTAGGATTTGGAACGCGA | ACTB antisense | 1133–1115c |
| 5’-TGCTCCCTGAGGCGGTCGC | ACTB probe | 1053–1078c |
| 5’-GGAGGCTATCCAGCCTACT | B2M sense | 114–132c |
| 5’-GACCGTCTCCTGCTAAAGAACA | B2M antisense | 302–281c |
| 5’-CGGATGGATGAAACCCAGACACATA | B2M probe | 220–194c |
| 5’-GGCTTACTGCTAGGCTGAT | EEF1A1 sense | 1079–1097c |
| 5’-CGGCGTGCGCAATCCAAAT | EEF1A1 antisense | 1160–1143c |
| 5’-AAATAGGCGGCCGCTATGCCCCTG | EEF1A1 probe | 1118–1141c |
| 5’-GGTGGAACCTGAGAATGATGA | GAPDH sense | 502–524a |
| 5’-GATGGCTGAGAAGGTGTTCA | GAPDH antisense | 645–626c |
| 5’-CCTCAAGATCATCCAGAATGCTCCCTG | GAPDH probe | 531–557c |

To exclude genomic signals, in each amplicon either one primer or the probe straddles two exons. Double dye probes obtained from Eurogentec (Liège, Belgium) are 6-FAM marked in 5’ and quenched in 3’ with TAMRA. All qPCR amplifications were performed on the same dT-primed cDNA templates with sense primers located at similar distances from the poly-A tail (785, 796, 874, 669, and 809 nt for X1-Cβ, ACTB, B2M, EEF1A1, and GAPDH, respectively).

*Positions are relative to the TSS of exon X1.
*Positions given from the 5’-end of exon 1 of Cβ1 and Cβ2
*Positions given according to the reference mRNAs: NM_001101.2 for ACTB; NM_004048 for B2M; NM_001402 for EEF1A1; NM_002046.3 for GAPDH.
primers 1 (X1 sense), and 3 (Cβ antisense, Fig. 2A and C and Table 1) and 0.625U of conventional Taq DNA polymerase (Takara) in a final volume of 25 μl, with 35 cycles (annealing at 60°C). These products were analyzed by gel electrophoresis and sequenced. To quantify the expression levels of X1-Cβ, amplified products of 64bp were obtained from the same amounts of cDNA with primers 1 and 2 (Table 1 and Fig. 2A), Hot Start Taq DNA Polymerase (Eurorgenetec, Liège, Belgium), annealing and extension at 62°C on a StepOnePlus thermocycler (ABI), and quantified with a FAM-TAMRA Double Dye probe (Eurorgenetec) straddling X1 and Cβ exon 1. Expression levels were normalized with ACTB and expressed as X1-Cβ/ACTB ratios obtained from ΔCq at identical thresholds, with verified amplification yields of 95% for both qPCR. We compared the levels of expression of the housekeeping genes ACTB, GAPDH, EEF1A1, and B2M in our samples [6] with the probe and primers indicated in Table 1, and observed the best correlations between the numbers of cells and ACTB expression levels.

**X1 promoter cloning**

Gene segments of 1125, 943, 242, 95, and 48 bp located upstream of the major Transcription Start Site (TSS) of exon X1 were obtained by PCR and cloned in Firefly luciferase vector pGL4.15 (Promega, Fitchburg, WI, USA), using Q5 (New England Biolabs) or the high fidelity DNA polymerase (Takara) in a final volume of 25 μl, with 35 cycles (annealing at 60°C). These products were analyzed after 24–40 h using the Dual-Glo Luciferase Assay System (Promega) and a Glomax Discover plate reader (Promega). The Firefly luciferase activity was normalized to that of the Renilla luciferase and the results compared to those obtained with a promoterless vector, providing the ratios shown in Figure 3A.

**Isolation of human thymocytes**

Postnatal thymuses were obtained from 0- to 12-year-old children that underwent cardiac surgery. Cord blood was obtained from the Navelstrengbloedbank UZ Gent. All human material was used following guidelines of the Medical Ethical Committee of the Ghent University Hospital (Belgium). Informed consent was obtained in accordance with the Declaration of Helsinki. A thymocyte suspension was made within 24 h after surgery. Cord blood mononuclear cells were obtained after density centrifugation. CD34+ cells were enriched by anti-CD34 magnetic activated cell sorting (MACS Miltenyi Biotech) to a purity of >90% and subsequently labeled and sorted in CD34+CD1−CD3−CD45+ and CD34+CD1+CD3−CD45+. CD4ISP (CD1+CD45−CD4+CD3−CD8−) and DP (CD3+CD4+CD8+) cells were sorted without pre-enrichment procedures. Anti-CD45, −CD34, −CD1, −CD3, −CD4, and −CD8a antibodies used for sorting were obtained from Milteny Biotech. The cells were sorted on a FACSaria II to a purity of >99%. RNA and cDNA were obtained as described above.

**Results**

**A new germline TCRB transcript contains a previously undescribed exon**

Starting from RNA extracted from eight melanomas that were infiltrated by T lymphocytes, we produced eight TCRB cDNA libraries using a TCR-Cβ reverse primer and the SMART oligonucleotide (Clontech). We sequenced about 1900 cDNA clones from these libraries. About 75% of the sequences corresponded to TCRB sequences; ±65% were in-frame rearranged TCRB sequences with a median length of 420 bp, ±15% were J-C sequences apparently initiated in front of Jβ2.3 (ENA LT626065) and other Jβ gene segments, ±10% were very short J-C products, and 5–10% were sequences of ±140 nucleotides, containing a Cβ1 (60%) or Cβ2 (40%) sequence preceded by 103 nucleotides not reported to be present in TCRB transcripts. In the human TCRB locus, this 103 bp sequence ends 174 nucleotides upstream of the Dβ1 gene segment. It is preceded by a potential TATA box and followed by a predicted [8] donor site of splicing (Fig. 1). These results suggested that this 103 nt sequence was the first exon, which we named TRBX1 (X1), of a new germline TCRB transcript that contained X1 and Cβ sequences.
To identify the 3' end of these X1-containing transcripts, RNA was extracted from 3 T cell clones and reverse-transcribed with an anchored oligo-dT primer. The resulting cDNA was used as a template for a PCR amplification with the anchor and primer 1 in exon X1 (Fig. 1) and the amplified products were sequenced. The sequences were about 860 bp long and corresponded to X1 spliced either with the first exon of Cβ1 followed by Cβ1 exons 2–4, or with the first exon of Cβ2 followed by Cβ2 exons 2–4. All sequences had a 3' polyA tail. The structure of the X1-Cβ transcripts is shown in Figure 1.

X1-Cβ gene products can only be transcribed from TCRB loci that have not undergone a V-DJ rearrangement, as the latter deletes X1 (Fig. 1). We surmised that X1-Cβ1 transcripts originated from TCRB loci either in germline configuration or with Dβ1-Jβ1 rearrangements. Indeed, the first good acceptor site of splicing downstream of X1 is that of Cβ1 exon 1. In case of Dβ1-Jβ2 rearrangements, Cβ1 is deleted and the next good acceptor site is that of Cβ2 exon 1. We confirmed this hypothesis by establishing the TCRB genomic structure and Cβ usage of the X1-Cβ transcripts on a set of 12 T cell clones with a single V-DJ rearrangement. As expected, no X1-Cβ transcripts were detected in the three clones with two V-DJ rearrangements (data not shown).

Exon X1 contains no ATG initiation codon and is therefore not expected to be translated. Translation of a protein from the Cβ segment of X1-CB appears unlikely. The largest ORF is 141 nt long and ends 53 nt upstream of the exon 3/exon 4 junction. It is preceded by very short ORFs, a feature that disfavors its translation. In addition, translation ending in the second last exon at a distance greater than 50–55 nt from the last exon-exon junction precludes the ribosome from removing the complex which then recruits RNAse and ubiquitin [9]. We provisionally conclude that the X1-Cβ transcripts are sterile.

We conclude to the presence in human T lymphocytes of previously undescribed TCRB mRNAs that contain ±860 nt and consist of a new exon, X1, followed by Cβ1 or Cβ2 exons.

**Expression of X1-Cβ transcripts**

We screened various cell types using a RT-qPCR amplification with primers 1 and 2 located in X1 and Cβ1, respectively, and a probe straddling the X1-Cβ junction (Fig. 2A and Table 1). From a set of 44 T cell clones previously established in our laboratory, only 24 (55%) expressed X1-Cβ transcripts (Fig. 2B). This proportion was expected: X1 is lost during the V-DJ recombination process and X1-Cβ transcripts are therefore absent from T cells that have undergone bi-allelic TCRB rearrangements. In murine lymph node-derived T cells, the proportion of cells with a single rearranged TCRB locus was estimated at 57% [10]. X1-Cβ transcripts could also be detected in about 50% of T cell clones using a conventional RT-PCR yielding a larger amplicon whose sequence could be verified (Fig. 2C). In cultured T cell clones, we estimated the levels of expression of X1-Cβ and complete TCRB transcripts at 2–10 and 100–300 mRNA molecules per cell, respectively [11] and data not shown). Thus in mature T cells the number of X1-Cβ transcripts is considerably lower than that of the TCRB-encoding mRNAs.

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**Figure 1.** Exon X1 within the human TCRB locus. Representation of the 3' end of the unarranged TCRB locus with the location of exon X1. The structure of the two most abundant germline X1-Cβ transcripts is indicated. The complete sequence of X1 (103 bp) is boxed. A TATA box and a donor site of splicing (DS) are indicated. Primer 1 in X1 was used in PCR amplifications. Sequences are accessible at European Nucleotide Archive: Exon X1 (LT601549) and X1-Cβ1 transcript (LT601550).
We detected $X1-C\beta$ expression in freshly isolated blood CD4 or CD8 T cells, in Phytohemagglutinin-A activated blood T cells and in leukemic T cells including Jurkat E6.1. No expression was detected in non-T cells such as fibroblasts, keratinocytes, monocytes, CD34$^+$ cord blood cells, Epstein-Barr virus-transformed B cells and 35 tumor lines from various non-T histological types (Fig. 2B and data not shown).

In sorted sub-populations of thymocytes, $X1-C\beta$ expression increased concurrent with $\beta$-selection: expression was low in pre-$\beta$ selection CD34$^+$CD1a$^-$ and CD34$^+$CD1a$^+$ double negative (DN) cells (Fig. 2B) and 10-times higher in post-$\beta$ selection CD3$^+$CD4$^+$ (immature single positive cells: iSP4) and CD3$^+$CD4$^+$CD8$^+$ DP populations (Fig. 2B).

We conclude that $X1-C\beta$ transcripts are T cell-specific and appear when $TCRB$ gene rearrangements are initiated. They are absent from T cells with biallelic $V-DJ$ rearrangements.

**A promoter sequence in front of $X1$**

To examine the regulatory elements governing $X1$ transcription, we cloned the genomic DNA immediately upstream of $X1$ in a luciferase-encoding vector and transfected the construct into HEK293T cells. We compared the promoter activities of five sequences of decreasing sizes (Fig. 3A). The highest promoter activities were observed for the two largest fragments, of 1125 and 943 bp, with similar inductions of 23- and 21-fold versus that of the promoterless construct. These inductions were approximately sixfold lower than that observed with a $V\beta$ 7.2 promoter (Fig. 3A). An antisense construct of 943 bp had no activity (Fig. 3A). The shortest $X1$ promoter fragment with a detectable activity was 95 bp long (7.5-fold induction).

We also transfected several of these constructs in the leukemic T cell clone Jurkat E6.1, with or without the 393 bp core sequence of the $TCRB$ gene enhancer ($E\beta$) which was shown to enhance the transcription of several $TRBV$ genes [7]. Transfection of the 943 bp long $X1$ promoter with $E\beta$ stimulated transcription (Fig. 3A). However, in the absence of $E\beta$ the stimulation of transcription was minimal, which was expected considering the role of $E\beta$ for $TCRB$ transcription in T cells.

We conclude that a promoter sequence is present immediately upstream to the $X1$ sequence. In line with...
this conclusion, a DNase I hypersensitivity region straddling the TSS of X1 is observed almost exclusively in T cells, according to the ENCODE project (Fig. 3B and Materials and Methods). Moreover, in T cells the X1 promoter is controlled by Eβ.

The sequence of the X1 promoter followed by X1 appears to be the human ortholog of the murine Dß1 Promoter (PDß1), a 377 bp sequence located immediately upstream of the TRBD1 gene segment [12] (Fig. 3B). Actually, this 377 bp sequence appears to consist in a promoter followed by the first 200 nt of germline transcripts controlled by this promoter. Indeed, reported transcripts contain the PDß1 last 200 nt sequence followed by Dß1 usually rearranged with a Jß spliced to a Cß segment [13] (and murine EST database).

Figure 3. Characterization of the X1 promoter region. (A) Genomic DNA fragments of indicated sizes, immediately preceding the TSS of exon X1, were cloned in front of the Firefly luciferase gene in vector pGL4.15, with or without Eß as indicated. The constructs were co-transfected in HEK293T cells or Jurkat cells with vector pGL4.75 containing the Renilla luciferase sequence. Control constructs included pGL4.15 without promoter, with a X1 promoter sequence cloned antisense (as) and with a Vß7.2 promoter sequence. One day after transfection, both luciferase activities were measured. The results, means of 2–9 independent assays, are expressed relatively to those obtained with the pGL4.15 promoterless construct. Sequence of the 1125 bp promoter fragment: ENA LT601551. (B) Sequence homologies between the human and murine pre-Dß1 regions. Promoter and transcribed sequences are shown as closed and open boxes, respectively. The PDß1 sequence proposed here is shorter at its 3'-end than in the original description by Sikes [12], taking into account the longest germline transcripts reported by Doty [13] or present in Genbank (EST CB598216, and BB587363). The indicated DNase I hypersensitivity region straddling the TSS of X1 is described for human T cells by the ENCODE project.
The homology between the human and murine DNA sequences upstream of Dß1 is above 70% immediately 5' to Dß1, remains at 60% for X1, and the last 135 bp of its promoter, then drops sharply to 40% for upstream sequences (Fig. 3B).

Sp1 and GATA3 have been shown to contribute to PDB1 activity [12] and binding sites for these transcription factors are present also in the X1 promoter. Seven GATA3 sites are present upstream of the human and murine Dß1 sequences (Fig. 4). While a single and important Sp1 site is present in the mouse, three sites are present in the X1 promoter (Fig. 4). Interestingly, 16 nucleotides positioned 91–77 nt upstream to the TSS of X1 are perfectly conserved between human and murine sequences. They are contained in the 377 bp core PDB1 promoter reported by Sikes [12]. They contain no GATA3 or Sp1 site, but a consensus AP-1 site indicated with arrows on Figure 4. This AP-1 site might participate together with a Sp1 site in the promoter activity present between nucleotides −48 and −95 of the human X1 promoter (Fig. 3A).

**Discussion**

In the mouse, several germline transcripts have been described that originate from the IG and TCR loci [14–16]. They are usually expressed, when the gene segments from which they are derived are poised to rearrange [17]. IG and TCR germline transcriptions have been considered as facilitating gene segment rearrangements by contributing to the accessibility of the loci to the recombinase complex [18], as deeply reviewed by Oltz and colleagues [19, 20].

For the TCRß locus, the best-studied germline transcription occurs upstream of the murine Dß1 gene segment and is controlled by a promoter named PDB1 [12]. The accessibility of Jß1, Dß2-Jß2, and the proximal Vß gene segments is controlled by the enhancer Eß [14], but both Eß and PDB1 are required for Dß1-Jß rearrangements to occur [21]. Deletion of PDB1 or its displacement downstream toward Jß1 was shown to prevent Dß1-Jß recombinations [21, 22]. However, Dß1-Jß recombinations persisted after PDB1 inversion, indicating that it is not the germline transcription through the Jß segments that is important for Dß1-Jß recombination [22]. Finally, mutations of GATA3 or Sp1 binding sites within PDB1 strongly impaire d Dß1 recombinations [12, 13]. Thus, like Eß, PDB1 is an accessibility control element (ACE), even though it controls a much shorter genomic interval than Eß does. The accepted model is that the interaction of PDB1 with Eß leads to the recruitment of additional factors that ultimately favor recombination by locally reorganizing the chromatin structure. One of these factors is a component of the SWI/SNF complex, which is required in murine thymocytes to open the Dß1 region prior to recombination [23]. Very little is known about IG and TCR germine transcription in human cells [24, 25]. We describe a new germline transcript from the human TCRß locus, with a new exon, X1, located upstream of Dß1 and spliced with the first exon of Cß1 or Cß2. X1-Cß transcription is controlled by a promoter that appears to be the ortholog of PDB1, for two reasons. First, both are localized in front of germline transcripts initiated in the pre-Dß1 region. Indeed in the mouse, the PDB1 sequences described by Sikes [12] and Whitehurst [21] contain more than a promoter, as their 3' halves can be transcribed [13] (and Genbank EST database). Accordingly the core promoter in PDB1 is less than 200 bp long and starts 377 bp upstream of Dß1 (Fig. 3B). In the human TCRß locus, the shortest tested sequence with X1-promoter activity is...
95 bp long and starts 371 bp upstream of Dβ1. Second, the 410 nucleotides upstream of murine and human Dβ1 are homologous. The homology is maximal (77%) next to Dβ1, is maintained at about 60% until the 5′ end of the core promoters, then drops sharply to 40% (Fig. 3B).

In line with their role of ACEs, the murine PDb1 and the human X1 promoter are activated at the earliest stages of thymocyte maturation, that is prior to Dβ rearrangements. Indeed, PDb1-controlled transcripts have been detected in thymocytes of Rag−/− animals [13, 14], and we have detected X1-Cβ transcripts in human CD34+ CD1a− CD4− CD8− CD45+ (DN) thymocytes, which contain no proteins with a Β1 domain [26] and very few if any Dβ-Jβ recombination products [2].

Our results suggest that X1-Cβ expression is 10-times higher in late stages of thymocyte differentiation (ISP4 and DP) than in earlier stages (DN). The timeframe for V-DJ rearrangements at the human TCRB locus extends until the DP stage [2, 3, 26, 27]. Accordingly, a high level of X1-Cβ transcript at the time of V-DJ recombination may contribute to this stochastic process by increasing the accessibility of the 5′RSS of the recombining Dβ1 gene segment. Mutations in X1 promoter might cause biases in the TCR repertoire, with a higher proportion of Vβ-Dβ2 rearrangements.

We observed that X1-Cβ expression persisted in mature T cells without two complete TCRB rearrangements. This persistence contrasts with what has been observed for other IG or TCR germline transcripts such as J-Ck, I-Cμ, T early alpha (TEA), and Vβ, which are expressed mainly in immature lymphocytes [18, 28, 29].

X1-Cβ was also expressed in some acute B cell leukemias (data not shown). This expression is linked to an accessible TCRB locus leading to an incomplete β-rearrangement process [30–32]. X1-Cβ is expressed at low levels in the erythroleukemia line K562, which is one of the rare non-T cell lines with some DNase I hypersensitivity around the TSS of X1 (ENCODE project).

Allelic inclusion at the TCRB locus, that is leading to the presence of two functional TCRB chains on the cell surface, has been reported to occur in about 1% of human T cell clones, suggesting that allelic exclusion at this locus is not an absolute rule [33, 34]. The difficulty of these genetic analyses is to ensure that the analyzed cell populations are clonal. In this context, an RT-PCR assay for X1-Cβ can help: in a given clone the detection of X1-Cβ transcripts excludes the presence of two complete TCRB rearrangements (except for the rare Vβ30-Dβ2-Jβ2 rearrangements).

**Conflict of Interest**

The authors declare no commercial or financial conflict of interest.

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**References**

1. Spicuglia, S., A. Pekowska, J. Zacarias-Cabera, and P. Ferrier. 2010. Epigenetic control of Tcrb gene rearrangement. Semin. Immunol. 22(6):330–336.
2. Dik, W. A., K. Pike-Overzet, F. Weerkamp, D. de Ridder, E. F. de Haas, M. R. Baert, P. van der Spek, E. E. Koster, M. J. Reinders, J. J. van Dongen, et al. 2005. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. J. Exp. Med. 201(11):1715–1723.
3. Taghon, T., J. Van de Walle, G. De Smet, M. De Smedt, G. Leclercq, B. Vandekerckhove, and J. Plum. 2009. Notch signaling is required for proliferation but not for differentiation at a well-defined beta-selection checkpoint during human T-cell development. Blood 113(14):3254–3263.
4. Lurquin, C., B. Lethé, E. De Plaen, V. Corbière, I. Théâtre, N. van Baren, P. G. Coulie, and T. Boon. 2005. Contrast frequencies of antitumor and anti-vaccine T cells in metastases of a melanoma patient vaccinated with a MAGE tumor antigen. J. Exp. Med. 201(2):249–257.
5. Matz, M., D. Shagin, E. Bogdanova, O. Britanova, S. Lukyanov, L. Diatchenko, and A. Chenchik. 1999. Amplification of cDNA ends based on template-switching effect and step-out PCR. Nucleic Acids Res. 27(6):1558–1560.
6. Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, et al. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55(4):611–622.
7. Gottschalk, L. R., and J. M. Leiden. 1990. Identification and functional characterization of the human T-cell receptor beta gene transcriptional enhancer: common nuclear proteins interact with the transcriptional regulatory elements of the T-cell receptor alpha and beta genes. Mol. Cell. Biol. 10(10):5486–5495.
8. Senapathy, P., M. B. Shapiro, and N. L. Harris. 1990. Splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to genome project. Methods Enzymol. 183:252–278.
9. Schweingruber, C., S. C. Rufener, D. Zund, A. Yamashita, and O. Muhlemann. 2013. Nonsense-mediated mRNA decay—mechanisms of substrate mRNA recognition and degradation.
in mammalian cells. Biochim. Biophys. Acta 1829 (6–7):612–623.
10. Khor, B., and B. P. Sleckman. 2005. Intra- and inter-allelic ordering of T cell receptor beta chain gene assembly. Eur. J. Immunol. 35(3):964–970.
11. Lennon, G. P., J. E. Sillibourne, E. Furrie, M. J. Doherty, and R. A. Kay. 2000. Antigen triggering selectively increases TCRBV gene transcription. J. Immunol. 165(4):2020–2027.
12. Sikes, M. L., R. J. Gomez, J. Song, and E. M. Oltz. 1998. A developmental stage-specific promoter directs germline transcription of D beta J beta gene segments in precursor T lymphocytes. J. Immunol. 161(3):1399–1405.
13. Doty, R. T., D. Xia, S. P. Nguyen, T. R. Hathaway, and D. M. Willerford. 1999. Promoter element for transcription of unrearranged T-cell receptor beta-chain gene in pro-T cells. Blood 93(9):3017–3025.
14. Mathieu, N., W. M. Hempel, S. Spicuglia, C. Verthuy, and P. Ferrier. 2000. Chromatin remodeling by the T cell receptor (TCR)-beta gene enhancer during early T cell development: implications for the control of TCR-beta locus recombination. J. Exp. Med. 192(5):625–636.
15. Engel, H., R. Hrubl, C. J. Benham, J. Bode, and S. Weiss. 2001. Germ-line transcripts of the immunoglobulin lambda J-C clusters in the mouse: characterization of the initiation sites and regulatory elements. Mol. Immunol. 38(4):289–302.
16. Abarrategui, I., and M. S. Krangel. 2009. Germline transcription: a key regulator of accessibility and recombination. Adv. Exp. Med. Biol. 650:93–102.
17. Schlissel, M. S., and P. Stanhope-Baker. 1997. Accessibility and the developmental regulation of V(D)J recombination. Semin. Immunol. 9(3):161–170.
18. Yancopoulos, G. D., and F. W. Alt. 1985. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. Cell 40(2):271–281.
19. Cobb, R. M., K. J. Oestreich, O. A. Osipovich, and E. M. Oltz. 2006. Accessibility control of V(D)J recombination. Adv. Immunol. 91:45–109.
20. Thomas, L. R., R. M. Cobb, and E. M. Oltz. 2009. Dynamic regulation of antigen receptor gene assembly. Adv. Exp. Med. Biol. 650:103–115.
21. Whitehurst, C. E., M. S. Schlissel, and J. Chen. 2000. Deletion of germline promoter PD beta 1 from the TCR beta locus causes hypermethylation that impairs D beta 1 recombination by multiple mechanisms. Immunity 13(5):703–714.
22. Sikes, M. L., A. Meade, R. Tripathi, M. S. Krangel, and E. M. Oltz. 2002. Regulation of V(D)J recombination: a dominant role for promoter positioning in gene segment accessibility. Proc. Natl. Acad. Sci. U. S. A. 99(19):12309–12314.
23. Osipovich, O., R. M. Cobb, K. J. Oestreich, S. Pierce, P. Ferrier, and E. M. Oltz. 2007. Essential function for SWI-SNF chromatin-remodeling complexes in the promoter-directed assembly of Tcrb genes. Nat. Immunol. 8(8):809–816.
24. Calman, A. F., and B. M. Peterlin. 1986. Expression of T cell receptor genes in human B cells. J. Exp. Med. 164(6):1940–1957.
25. Berman, J. E., C. G. Humphries, J. Barth, F. W. Alt, and P. W. Tucker. 1991. Structure and expression of human germline VH transcripts. J. Exp. Med. 173(6):1529–1535.
26. Joachims, M. L., J. L. Chain, S. W. Hooker, C. J. Knott-Craig, and L. F. Thompson. 2006. Human alpha beta and gamma delta thymocyte development: tCR gene rearrangements, intracellular TCR beta expression, and gamma delta developmental potential-differences between men and mice. J. Immunol. 176(3):1543–1552.
27. Ramiro, A. R., C. Trigueros, C. Marquez, J. L. San Millan, and M. L. Toribio. 1996. Regulation of pre-T cell receptor (pT alpha-TCR beta) gene expression during human thymic development. J. Exp. Med. 184(2):519–530.
28. Duber, S., H. Engel, A. Rolink, K. Kretschmer, and S. Weiss. 2003. Germline transcripts of immunoglobulin light chain variable regions are structurally diverse and differentially expressed. Mol. Immunol. 40(8):509–516.
29. de Villartay, J. P., D. Lewis, R. Hockett, T. A. Waldmann, S. J. Korsmeyer, and D. I. Cohen. 1987. Deletional rearrangement in the human T-cell receptor alpha-chain locus. Proc. Natl. Acad. Sci. U. S. A. 84(23):8608–8612.
30. Pelicci, P. G., D. M. Knowles, 2nd, and R. Dalla Favaera. 1985. Lymphoid tumors displaying rearrangements of both immunoglobulin and T cell receptor genes. J. Exp. Med. 162(3):1015–1024.
31. Dombret, H., P. Loiseau, J. C. Bories, and F. Sigaux. 1992. Unexpected consistent involvement of V beta gene segments in inappropriate T-cell receptor beta gene gene rearrangements occurring in B-lineage acute lymphoblastic leukemias. Blood 80(10):2614–2621.
32. Szczepanski, T., M. J. Pongers-Willems, A. W. Langerak, and J. J. van Dongen. 1999. Unusual immunoglobulin and T cell receptor gene rearrangement patterns in acute lymphoblastic leukemias. J. Immunol. 162(8):809–816.
33. Davodeau, F., M. A. Peyrat, F. Romagne, A. Necker, M. M. Hallet, H. Vie, and M. Bonneville. 1995. Dual T cell receptor gene expression on human T lymphocytes. J. Exp. Med. 184(1):1391–1398.
34. Padovan, E., C. Giachino, M. Cella, S. Valitutti, O. Acuto, and A. Lanzavecchia. 1995. Normal T lymphocytes can express two different T cell receptor beta chains: implications for the mechanism of allelic exclusion. J. Exp. Med. 181(4):1587–1591.