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TRAV and *TRBV* repertoire, clonality and the proliferative history of umbilical cord blood T-cells

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Abstract

Umbilical cord blood (CB) has been used successfully as a source of hematopoietic stem cells for transplantation. But the distribution and clonality of T-cell receptor alpha variable region (*TRAV*) and T-cell receptor beta variable region (*TRBV*) subfamily T-cells, the naïve T-cells level and the diversity of thymic recent output function in CB has not been yet clearly defined. In order to characterize the repertoire of CB T-cells, the CDR3 of 29 *TRAV* and 24 *TRBV* subfamily genes were analyzed in T-cells from 12 cord blood samples, using RT-PCR and genescan technique. To determine the proliferative history of CB T-cells, quantitative analysis of δRec-ψJα signal joint T-cell receptor excision circles (sjTRECs) was performed in mononuclear cells, CD3+, CD4+ and CD8+ T-cells from 20 CB samples by real-time PCR. In addition the analysis of 23 *TRBV–TRBD1* sjTRECs in 10 cases of CB CD4+ T-cells and CB CD8+ T-cells was performed by semi-nested PCR. We found a marked restriction of *TRBV* expression pattern in CBMCs compared to peripheral blood mononuclear cells (PBMC), which expressed all 24 *TRBV* genes. All PCR products of *TRAV* and *TRBV* subfamilies from CB, except for 3 cases, displayed polyclonal rearrangement pattern. The δRec-ψJα sjTRECs counts were significantly higher in CB, than in PB samples. Also the number of detectable *TRBV* sjTRECs was higher in CB than in peripheral blood. In conclusion, our results indicate polyclonal but restricted repertoire and a very short proliferative history of CB T-cells. The incomplete repertoire and naivety of CB T-cells might be the reason that CB hematopoietic stem cells transplant recipients are less likely to develop graft *vs* host disease.

Keywords: T-cells; Cord blood; *TRAV*; *TRBV*; TREC; *TRBV–TRBD1*sjTREC

1. Introduction

Umbilical cord blood is a valuable alternative source of hematopoietic stem cells for transplantation of patients who lack a suitable sibling donor [1]. And CB T-cells are resourceful for production the specific CTL to use in leukemia immunotherapy.

Since CB T-cells are not exposed to foreignantigens, they are almost exclusively naïve and express the $CD45RA^{+}/CD45R0^{-}$ immunophenotype. The naive phenotype of CB T lymphocytes is most likely responsible for the reduced alloreactivity observed after CB transplantation. T-cell receptors (*TCR*) are heterodimers comprising either α/β or γ/δ chains. Genes encoding for the variable domains of the *TCR* α and β heterodimer chains are assembled by somatic recombination from variable (V), diversity (D, only for β chain), and joining (J) segments and compose three hypervariable or complementarity-determining regions (CDR1, CDR2, CDR3) of the *TCR*. The CDR3 is involved in response for the specific interaction with the antigenic peptide. The majority of V, D and J regions in human *TCR* have been identified. The *TCRA* is known to contain at least 50 functional *TRAV* gene segments and *TCRB* at least 75 functional *TRBV* subdivided into 29 *TRAV* and 24 *TRBV* subfamilies. Analysis of the *TRAV* and *TRBV* repertoire provides a global picture of the distribution and clonal expansion of *TCR* α/β subfamilies in T-cells from different samples. Several methods for analysis of *TCR* repertoire involve

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the direct amplification of cDNA between V-region and C-region specific primers, or genomic DNA between V region and J region specific primers, followed by the analysis of labeled PCR products by sequencing or by single strand conformation polymorphism (SSCP) analysis. A more flexible and quantitative approach involves an initial PCR reaction followed by "run-off" reaction with fluorescent *TRAC/TRBC* primer [2].

The generation of *TCR* diversity occurs in the thymus through recombination of gene segments encoding the variable parts of the *TCR* α and β chains. During these processes, by-products of the rearrangements are generated in the form of signal joint T-cell receptor excision circles (sjTRECs), which is considered as a very valuable tool to estimate thymic function [3–5].

Quantitative analysis of δRec-ψJα sjTRECs provides the information about total thymic output, but it doesn't allow to determine the proliferative history of a particular *TRBV* subfamily. This can be assessed by quantitative analysis of *TRBV–BD* sjTRECs specific for each *TRBV* subfamily [6,7].

The main objective of our study was to investigate the distribution and clonal expansion of *TRAV* and *TRBV* subfamilies, and the naïve T-cells status in cord blood T-cells.

2. Materials and methods

2.1. Samples

Cord blood was obtained at the delivery from 20 full-term healthy pregnancies after the mother's consent. 17 normal peripheral blood samples (6 males and 11 females, median age: 28 years, range 25–51) served as control.

2.2. Mononuclear cells isolation

Mononuclear cells were isolated from 20 cord blood samples (CBMC) or 17 peripheral blood samples (PBMC) by Ficoll–Hypaque gradient centrifugation.

2.3. Immunocytochemical determination of $CD3^+$ cells content

The PLP-fixed cytospin preparations were incubated with 200 μg/ml of murine anti-CD3 mAb (Boster Biological Technology Ltd, Wuhan , China), washed and incubated with 1:50 dilutionof fluorescein labelled goat anti-mouse Ig (Boster Biological Technology Ltd, Wuhan , China). The slides were counterstained with Mayer's hematoxylinfor 30 min. All slides were blindly evaluated on the fluorescent microscope (Nikon WFX-II, Nikon Ltd, Japan). 200 hundred cells were counted.

2.4. CD4+ or CD8+ cells sorting

The CD4+ and CD8+ T-cells from 19 CBMC and 17 PBMC samples were sorted using CD4 and CD8 MoAb (Miltenyi Biotec, Germany) and MACS®Magnetic Cell sorting technique.

2.5. RNA isolation and cDNA synthesis

RNA was extracted from the CB and PB samples according to the manufacturer's recommendations (Trizol, Gibco): The quality of RNA was analyzed in 0.8% agarose gel stained with ethidium bromide. Two μg RNA was reversely transcribed into the first single-strand cDNA with random hexamer primers, using reverse transcriptase, Superscript II Kit (Gibco). The quality of cDNA was confirmed by RT-PCR for β2 microglubin gene amplification.

2.6. DNA extraction

Total DNA from distinct cell populations was extracted using QIAamp® DNA Blood Mini Kit (QIAGEN, Germany). The quality of DNA was analyzed by in 0.8% agarose gel stained with ethidium bromide and the

Table 1
The sequence of primers used for detection of *TRAV* and *TRBV* repertoire

Primer	Sequence
Vα1	5′-GGCATTAACGGTTTTGAGGCTGGA-3′
Vα2	5′-CAGTGTTCCAGAGGGAGCCATTGT-3′
Vα3	5′-CCGGGCAGCAGACACTGCTTCTTA-3′
Vα4	5′-TTGGTATCGACAGCTTCACTCCCA-3′
Vα5	5′-CGGCCACCCTGACCTGCAACTATA-3′
Vα6	5′-TCCGCCAACCTTGTCATCTCCGCT-3′
Vα7	5′-GCAACATGCTGGCGGAGCACCCAC-3′
Vα8	5′-CATTCGTTCAAATGTGGGCAAAAG-3′
Vα9	5′-CCAGTACTCCAGACAACGCCTGCA-3′
Vα10	5′-CACTGCGGCCCAGCCTGGTGATAC-3′
Vα11	5′-CGCTGCTCATCCTCCAGGTGCGGG-3′
Vα12	5′-TCGTCGGAACTCTTTTGATGAGCA-3′
Vα13	5′-TTCATCAAAACCCTTGGGGACAGC-3′
Vα14	5′-CCCAGCAGGCAGATGATTCTCGTT-3′
Vα15	5′-TTGCAGACACCGAGACTGGGGACT-3′
Vα16	5′-TCAACGTTGCTGAAGGGAATCCTC-3′
Vα17	5′-TGGGAAAGGCCGTGCATTATTGAT-3′
Vα18	5′-CAGCACCAATTTCACCTGCAGCTT-3′
Vα19	5′-ACACTGGCTGCAACAGCATCCAGG-3′
Vα20	5′-TCCCTGTTTATCCCTGCCGACAGA-3′
Vα21	5′-AGCAAAATTCACCATCCCTGAGCG-3′
Vα22	5′-CCTGAAAGCCACGAAGGCTGATGA-3′
Vα23	5′-CCTGAAAGCCACGAAGGCTGATGA-3′
Vα24	5′-CTGGATGCAGACACAAAGCAGAGC-3′
Vα25	5′-TGGCTACGGTACAAGCCGGACCCT-3′
Vα26	5′-AGCGCAGCCATGCAGGCATGTACC-3′
Vα27	5′-AAGCCCGTCTCAGCACCCTCCACA-3′
Vα28	5′-TGGTTGTGCACGAGCGAGACACTG-3′
Vα29	5′-GAAGGGTGGAGAACAGATGCGTCG-3′
Cα	5′-GTTGCTCCAGGCCGCGGCACTGTT-3′
Cα-fam	5′-Fam-ATA CAC ATC AGA ATC CTT ACT TTG-3′
Vβ1	5′-CCGCACAACAGTTCCCTGACTTGC
Vβ2	5′-GGCCACATACGAGCAAGGCGTCGA
Vβ3	5′-CGCTTCTCCCGGATTCTGGAGTCC
Vβ4	5′-TTCCCATCAGCCGCCCAAACCTAA
Vβ5	5′-AGCTCTGAGCTGAATGTGAACGCC
Vβ6	5′-TCTCAGGTGTGATCCAAATTCGGG
Vβ7	5′-CCTGAATGCCCCAACAGCTCTCTC
Vβ8	5′-CCATGATGCGGGGACTGGAGTTGC
Vβ9	5′-TTCCCTGGAGCTTGGTGACTCTGC
Vβ10	5′-CCACGGAGTCAGGGGACACAGCAC
Vβ11	5′-TGCCAGGCCCTCACATACCTCTCA
Vβ12	5′-TGTCACCAGACTGGGAACCACCAC
Vβ13	5′-CACTGCGGTGTACCCAGGATATGA
Vβ14	5′-GGGCTCGGCTTAAGGCAGACCTAC
Vβ15	5′-CAGGCACAGGCTAAATTCTCCCTG
Vβ16	5′-GCCTGCAGAACTGGAGGATTCTGG
Vβ17	5′-CTGCTGAATTTCCCAAAGAGGGCC
Vβ18	5′-TGCCCCAGAATCTCTCAGCCTCCA
Vβ19	5′-TCCTCTCACTGTGACATCGGCCCA
Vβ20	5′-AGCTCTGAGGTGCCCCAGAATCTC
Vβ21	5′-TCCAACCTGCAAGGCTTGACGACT
Vβ22	5′-AAGTGATCTTGCGCTGTGTCCCCA
Vβ23	5′-GCAGGGTCCAGGTCAGGACCCCCA
Vβ24	5′-CCCAGTTTGGAAAGCCAGTGACCC
Cβ	5′-CGGGCTGCTCCTTGAGGGGCTGCG
Cβ-fam	5′-Fam-CACAGCGACCTCGGGTGGG