Analysis of the Clonal Expansion of TCR Vβ T Cells in Patients with CML after DLI

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Objective To investigate the clonal expansion of T cell receptor (TCR) Vβ subfamily T cells which were considered as GVL effective cells after donor lymphocytes infusion (DLI) in patients with relapse chronic myelogenous leukemia (CML) after allogeneic bone marrow transplantation (allo-BMT).

Methods The CDR3 of TCR Vβ24 subfamily genes were amplified in samples of peripheral blood mononuclear cells at different time points before and after DLI, which were drawn from 2 cases of relapse CML treated by allo-BMT, to observe the usage of TCR Vβ repertoire. The PCR products were further labeled with fluorescent and analyzed by gencscan technique for identification of the CDR3 size, to evaluate the clonality of the detectable TCR Vβ T cells.

Results Only 4 – 11 Vβ subfamily T cells could be identified in CML cases before DLI, and 12 – 21 Vβ subfamily T cells could be detected in samples of CML which display remission after DLI. Genescan analysis showed that new clonal expansion TCR Vβ subfamily T cells could be found in samples after DLI.

Conclusion The skew distribution of TCR Vβ subfamily T cells could be found on patients with relapse CML after allo-BMT, and this skewing pattern may stage to stage to normal pattern during the complete remission. The GVL effect may exert through some clonal expansion TCR Vβ subfamily T cells during the treatment of DLI in relapse CML.

Key words donor lymphocytes infusion; chronic myelogenous leukemia; T cell receptor Vβ gene; T cell clonality; allogeneic bone marrow transplantation

D onor lymphocyte infusions (DLI) has demonstrated to induce clinical responses in patients with relapsed chronic myelogenous leukemia (CML) after allogeneic bone marrow transplantation (allo-BMT). About 60% – 80% of patients achieved a complete hematologic response. But the immunologic mechanisms involved have not been well characterized, suggesting that some clonal expansion T cells have the ability of a graft versus leukemia cells (GVL) activity. To identify changes in the T cell populations and clonality associated with DLI, we performed a molecular analysis of the distribution and clonality of T cell receptor (TCR) Vβ 24 subfamilies in 2 patients with relapsed CML who received DLI and achieved a complete molecular remission.

Materials and methods

Samples

Two patients with relapsed CML after allo-BMT 3 years or 2 years respectively received DLI from HLA-identical sibling donors followed by clinical remissions. Samples were collected from the patients before and after DLI at different time points. For case 1 (C1) 4 blood samples were obtained and named as C1-A, C1-B, C1-C and C1-D corresponding to the time of 3 months before CML relapse, at the time of CML relapse, 4 months and 7 months after DLI respectively. For case 2 (C2) 4 blood samples were obtained and named as C2-A, C2-B, C2-C and C2-D corresponding to the time of 3 months before CML relapse, 1.5 months, 3 months or 5 months after DLI respectively. The samples from donor and normal individuals served as controls. T-cell lines Jurkat served as monoclonal control. The evidence of relapse or remission was detected by RT-PCR for detection of bcr-abl fusion. Bcr-abl fusion in cDNA could be detected in the samples of C1-A, C1-B, C2-A, C2-B and C2-C, whereas negative for the remained sample was demonstrated.

RNA extraction and cDNA synthesis

RNA was extracted according to the instruction of the RNeasy kit and reversely transcribed into the first single-strand cDNA with the use of random hexamer primer and reverse transcriptase Superscript II Kit (Gibco, BRL).

Polymerase chain reaction

24 Vβ and a Cβ primers used in unlabeled PCR and a fluorescent primer labeled at its 5’ end with fam fluorophore (Cβ-fam) for runoff reaction were purchased from TIB MOLBIOL GmbH, Berlin, Germany. PCR was performed as previous studies, using a panel of 24 Vβ primer detecting Vβ families 1 – 24 and a Cβ primer. Aliquots of the cDNA (1 µl) were amplified in a 25 µl reaction system and the final reaction mixture contained 0.5 µmol/L sense primer (Vβ) , 0.5 µmol/L Cβ primer, 0.1 mmol/L dNTP, 1.25 U Taq polymerase (Perkin Elmer) and 1 × PCR buffer containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂ and 0.001% (w/v) gelatin. The amplification was performed on a DNA thermal cycler (Perkin Elmer). After 3 min of denaturation at 94 °C, 40 PCR cycles were performed.
performed, each cycle consisting of reactions at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, and the last extension at 72 °C lasted for 10 min. PCR products were electrophoresed through 2.5% agarose gel stained with ethidium bromide.

**Analysis of T cell clonality**

*Runoff reactions (labeled PCR products)* Aliquots of the unlabeled PCR products (2 μl) were separately added to a final 10 μl reaction system containing 0.1 μmol/L Cβ-fam primer, 3 mmol/L MgCl₂, 0.2 mmol/L dNTP, 0.25 U Taq polymerase and PCR buffer (Perkin Elmer). After a 3 min denaturation at 94 °C, 25 cycles of amplification were carried out (1 min at 94 °C, 1 min at 66 °C and 1 min at 72 °C and a final 10 min extension at 72 °C).

*Genescan analysis (CDR 3 length analysis)* The fluorescent labeled PCR products (2 μl) were heat-denatured at 94 °C for 4 min after addition of 2.5 μl formamide, 0.5 μl of Genescan-500 Tamra Size Standards (ABI, Perkin Elmer) and 0.5 μl of loading buffer (Dextran 50 mg/ml, EDTA 25 mmol/L, Genescan-500 Tamra Kit) and were then loaded on 6% polyacrylamide gel for size and fluorescence intensity determination by Genescan 672 analysis software on 373A DNA sequencer. Since the positions of the Vβ and Cβ primers are fixed, the length distribution observed in the PCR Vβ-Cβ products depends only on the size of the rearrangement of V-D, D-J gene segment and the randomly inserted nucleotides (V₃ D₃ J). After electrophoresis on an automated sequencer and subsequent computer analysis, the products of different size could be separated and expressed as different peaks.

**Results**

**TCR Vβ RT-PCR analysis**

For the donor, as well as the normal blood samples amplified, all Vβ subfamily transcripts could be detected, but only 4 – 11 Vβ subfamilies could be detected in samples before DLI, whereas 19 – 21 Vβ subfamilies were found in the samples after DLI at different time points (Table 1), primary RT-PCR for the Jurkat T cell lines gave a positive result in only one of the Vβ 8 subfamily.

**Genescan analysis**

Fluorescent labeled-PCR products were separated on a polyacrylamide gel and analyzed by automatic fluorescence quantification and size-determination by using the computer program Genescan-672 software. Because of the hypervariable character of rearranged TCRβ V-N(D)-N-J junctions, the size distribution pattern of a given PCR product should represent the characteristics of the corresponding T cell population. Products derived from homogeneous clonal cell populations, such as the T cell lines, should display one sharp and dominant peaks of fluorescence corresponding to the PCR-amplified clonally rearranged alleles. Consequently, mRNA extracted from polyclonal T cells should yield a fluorescence spectrum of DNA bands composed of polyclonal PCR fragments of different sizes. In the present study, all Vβ family PCR products in donor and normal control samples displayed multi-peak pattern (polyclonal). Mono-peak pattern (monoclonal), however, was found in the Jurkat T cell line PCR product. Most of the PCR products from the both patients also displayed polyclonality, whereas part of the PCR products from some samples displayed a dominant peak or bi-peak corresponding to a oligoclonal or biclonal CDR3 size, strongly suggesting clonal expansion of their T cells. Some clonal T cells expansion could be identified after DLI. And some TCR Vβ subfamily T cells sustainedly displayed clonal expansion before and after DLI (Vβ15 and Vβ18) (Table 1, Fig. 1 -2).

**Discussion**

The ability of DLI to antileukemia cells in patients with relapse CML after allo-BMT in vivo confirms the important role played by donor lymphocytes in the eradication of residual leukemia cells after allogeneic BMT. Several clinical observations have previously provided indirect evidence in support of this graft-versus-leukemia (GVL) effect. However, infusion of large numbers of allogeneic T cells frequently results in significant GVHD, which is the most common serious toxicity associated with DLI occurring in 40% – 60% of the patients. Therefore, it is very important to distinguish the T cell subfamilies that mediate GVL or GVHD response after DLI, which can provide the basic data for target subfamily T cells depletion to reducing GVHD.

In the present study, both patients showed to have significant antileukemia activity after DLI, and were able to achieve a complete remission documented by the gradual disappearance of cells expressing the bcr-abl gene rearrangement in blood. The considerable evidence further supports the hypothesis that elimination of residual leukemia is mediated primarily by donor T cells. The series analysis for distribution and clonality TCR Vβ subfamily T cells was performed in samples of peripheral blood mononuclear cells at different time points before and after DLI, which were drawn from 2 cases with relapse CML treated by allo-BMT. The first observation of our analysis of TCR Vβ repertoire was a markedly skewed expression in peripheral blood from two patients before and after relapse, 3 – 4 Vβ subfamilies were detected in case 1, whereas 11 Vβ subfamilies were found in case 2. The feature of skewed distribution of TCR Vβ subfamily T cells was simi