ANALYSIS OF T CELL CLONALITY BY CDR3 SIZE OF T-CELL ANTIGEN RECEPTOR Vβ REPERTOIRE IN HCL AND c-ALL*

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ABSTRACT

Objective: To analyze the distribution and clonality of TCR Vβ subfamily T cells in hairy cell leukemia (HCL) and common-acute lymphoblastic leukemia (c-ALL). Methods: Peripheral blood mononuclear cell samples from 3 cases of HCL and 1 case of c-ALL were investigated for analysis of complementarity determining region 3 (CDR3) size of T cell receptor Vβ repertoire using reverse transcriptase-polymerase chain reaction (RT-PCR). The products were further analyzed by genescan to identify T cell clonality. Results: Some Vβ subfamily PCR products from 4 patients contained monopeak (monoclone) or a dominant peak (oligoclone). In contrast, multipeak (polyclone) distributions were found in all Vβ subfamily PCR products from normal control cases. Conclusion: T cell clonal expansion may be found in HCL and c-ALL cases that may indicate a host response directed against leukemia related antigen. In addition, it may be useful to detect the minimal residual disease.

Key words: T cell receptor Vβ, CDR3 leukemia, T cell clonality, Genescan.

During T cell development, the V, D and J region of TCR completely rearranged to compose a functional TCR gene. In addition, in this process the nucleotides (N region) were randomly added between V–D and D–J and compose a high variable VNDNJ region, which is called complementarity determining region 3 (CDR3). The CDR3 length and sequence would be different, if the TCR rearrangement occurs at different clonal T cells. Thus, the detection of CDR3 length and sequence could be a specific method for identification of T cell clonality. It has been identified that TCR β gene contains 24 Vβ subfamily genes (Vβ1–24), 2 Dβ segments (Dβ1.1–Dβ2.1) and 13 Jβ segments (Jβ1.1–1.6 and Jβ2.1–2.7). According to the TCR Vβ repertoire and the diversity of CDR3 length, identification of T cell clonality using the analysis CDR3 length of TCR Vβ repertoire is a new established method[1, 2]. In the present study, the method was used to analyze the T cell clonality from peripheral blood in 3 patients with hairy cell leukemia (HCL) and a case with common-acute lymphoblastic leukemia (c-ALL).

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MATERIALS AND METHODS

Samples

Three cases with hairy cell leukemia (HCL) and a case with common-acute lymphoblastic leukemia (c-ALL), diagnosed based on to cytomorphology, cyto-chemistry, immunohistochemistry and analysis of electron-microscopy, were selected in this study. Peripheral blood mononuclear cells (PBMCs) from 8 normal individuals served as controls.

RNA Extraction and cDNA Synthesis

RNA was extracted from peripheral blood mononuclear cells of the patients and normal individuals and cDNA was synthesed first with single-strand cDNA using the standard methods.

Oligonucleotides

The 24 Vβ primers as sense primers were designed according to the sequences of 24 TCR Vβ subfamilies. The antisense primer Cβ was selected for complementary to both Cβ1 and Cβ2 genes. In addition, the labeled primer was labeled at 5' end with fam fluorophore (Cβ-fam, 5'-fam-CACAGCGACCTCGGGTGGG) for gene-

can analysis. All primers were purchased from TIB MOLBIOL GmbH, Berlin, Germany[1].

Polymerase Chain Reaction (PCR)

PCR was performed as previous described[1,3]. The total volume of PCR reaction mixture was 25 μl containing 1 μl cDNA, 0.1 mM dNTP (including dATP, dCTP, dGTP and dTTP), 0.5 μm sense primer (one of the 24 Vβ primer) and 0.5 μM antisense Cβ primer, 1.25 U Taq polymerase (Perkin Elmer). The cDNA from T cell lines served as positive controls in each Vβ PCR reaction, and negative controls in each reaction were performed without cDNA. The PCR products were analyzed in 2.5% agarose gel stained with ethidium bromide.

CDR3 Size Analysis (the Analysis of T Cell Clonality)

Labeling of PCR Products (Runoff Reaction)

The asymmetric and semi-nest PCR amplification was performed with a fluorescence labeled Cβ-fam antisense primer for labeling PCR products. Aliquots of the unlabeled PCR products (2 μl) were mixed in final 10 μl reaction volume containing 0.1 μM Cβ-fam primer, 3 mM $MgCl_2$, 0.2 mM dNTP and 0.25 U Taq polymerase[1,3]

Genescan Analysis

The labeled PCR products (2 μl) were loaded on 6% polyacrylamide gels and electrophorezed on a 373 A DNA sequencer with Genescan 672 analysis software (ABI, Perkin Elmer). The results from genescan should show the different size, intensity and configuration of peaks corresponding to the different size, intensity and the homology of the PCR products[1,3].

RESULTS

RT-PCR Analysis Result

For eight normal blood samples amplified, except Vβ 20, all Vβ family-specific PCR reactions gave a signal. For four leukemia cases investigated, PCR reactions could not be found in all Vβ subfamily products, only 10 to 15 of 24 Vβ families gave products (Table 1).

Genescan Analysis Result

All Vβ subfamily PCR products from eight normal samples displayed multi-peaks picture (polyclonal) (Table 1, Figure 1). But some PCR products from four leukemia samples displayed unique or dominant peaks corresponding to a mono- or oligoclonal expansion T cells (Table 1, Figure 2).

Table 1. Identification of T cell clonality in peripheral blood samples by analysis of CDR3 length of TCR Vβ using genescan analysis

Primer	Control	Nr. 1 (HCL)	Nr. 2 (HCL)	Nr. 3 (HCL)	Nr. 4 (c-ALL)
Vβ1/Cβ	Poly*	Poly	Poly	Poly	
Vβ2/Cβ	Poly	Poly	Poly	Poly	Oligo**
Vβ3/Cβ	Poly		Poly	Oligo	Poly
Vβ4/Cβ	Poly	Poly	Poly		
Vβ5/Cβ	Poly	Oligo	Poly		Poly
Vβ6/Cβ	Poly	Poly		Poly	
Vβ7/Cβ	Poly	Poly			Poly
Vβ8/Cβ	Poly				
Vβ9/Cβ	Poly		Oligo		
Vβ10/Cβ	Poly				Poly
Vβ11/Cβ	Poly	Poly	Poly		
Vβ12/Cβ	Poly				
Vβ13/Cβ	Poly	Poly	Poly	Poly	Poly
Vβ14/Cβ	Poly	Poly	Poly	Poly	Poly
Vβ15/Cβ	Poly	Poly	Poly		
Vβ16/Cβ	Poly		Poly		Poly
Vβ17/Cβ	Poly			Poly	
Vβ18/Cβ	Poly	Poly	Poly	Poly	Poly
Vβ19/Cβ	Poly	Poly	Poly	Oligo	Oligo
Vβ21/Cβ	Poly	Poly	Mono***	Poly	
Vβ22/Cβ	Poly			Poly	
Vβ23/Cβ	Poly		Poly		
Vβ24/Cβ	Poly			Oligo	Poly

Note: *Polyclone; **Oligoclone; ***Monoclone

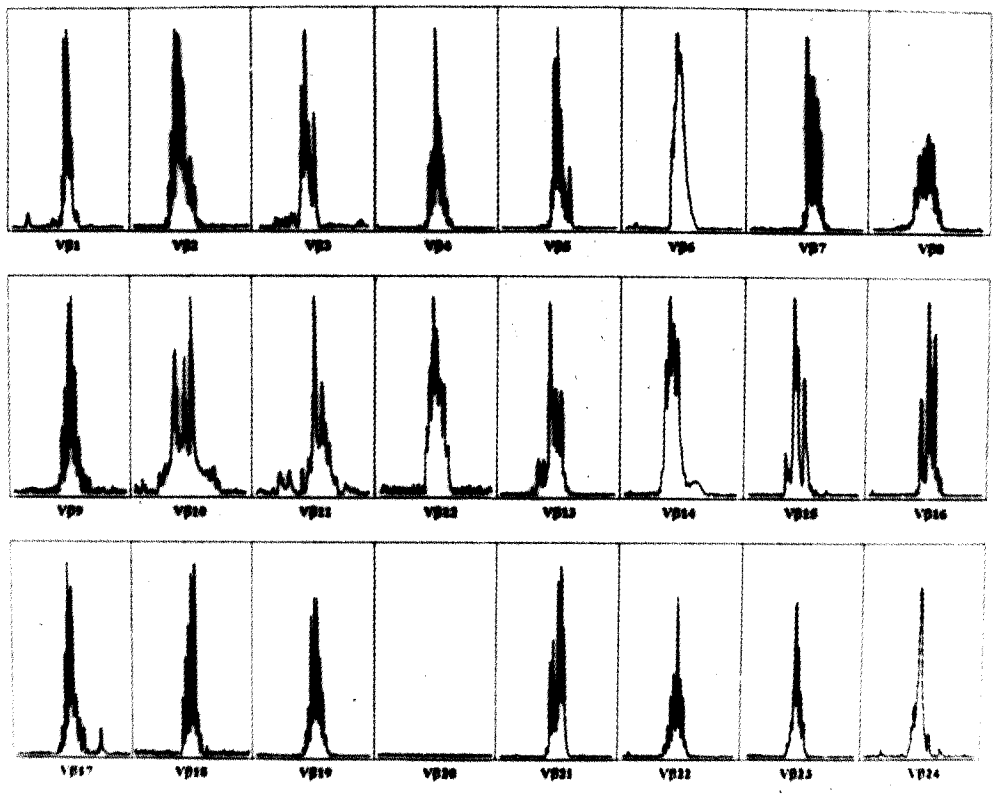

Fig. 1. The genescan analysis results from PCR products of TCR Vβ subfamilies in normal individual. Multi-peaks could be found in all Vβ subfamily PCR products corresponding to polyclonal T cells.

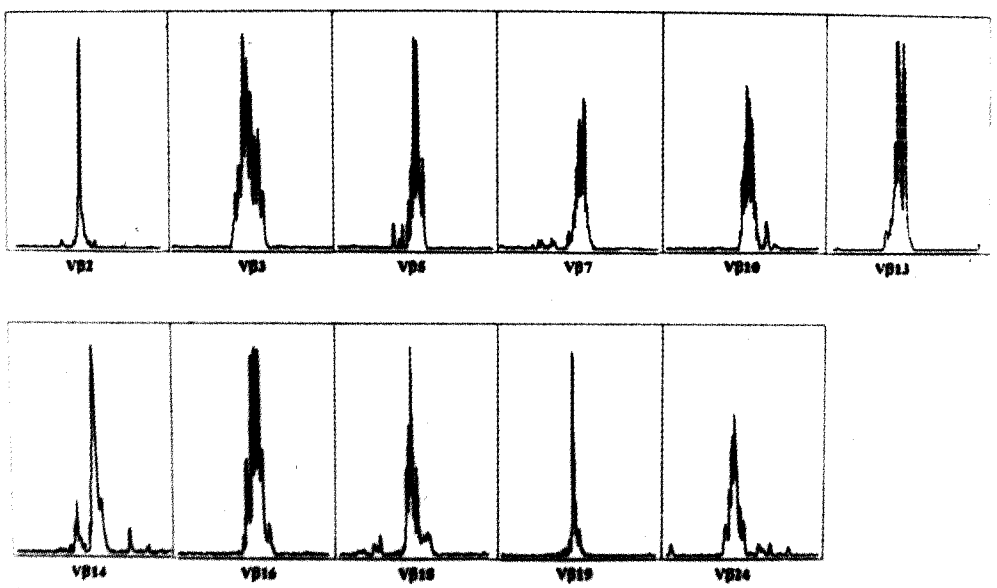

Fig. 2. The genescan analysis results from PCR products of TCR Vβ subfamilies in a patient with c-ALL (case 4). Two dominant peaks could be found in Vβ2 and Vβ19 subfamilies corresponding to the oligoclonal T cells. The other Vβ subfamily PCR products displayed multi-peaks (polyclonal).

DISCUSSION

It is a new developmental method for identification of T cell clonality that analyze the distinction of CDR3 size using runoff reaction for fluorescence labeled PCR products and genescan analysis according to the features of variation CDR3 length in different T cell clones. This method was first used in a foreign country, and it has not

been introduced into our country[1,2]. It combines the feature of 24 TCR Vβ repertoire and the diversity of CDR3 length, and introduces the sensitive approach of genescan analysis which could provide the objective and intuitional results for determination of T cell clonality by computer manipulation[3]. The approach has been rapidly accepted and introduced into analysis of immune status of autoimmune disease and malignancy[1, 4, 5] during its development. As an objective and sensitive method, it may be useful in clinical study and should be introduced into our country.

The present study showed that almost all TCR Vβ subfamily T cells could be detected in peripheral blood samples from 8 normal individuals by RT-PCR, which display the picture of multi-peak in all PCR products by genescan analysis. The result indicated that the size of the DNA segments in PCR products is different, and since that the CDR3 length is different, and it means that the PCR products were obtained from different clonal T cells (polyclonal T cells). The rearrangement of TCR in T cells is a random formation in normal individuals, and the T cells display multi-subfamily and polyclonality, which have not been activated by any antigen and should not show as specific monoclonal T cells responding for some specific antigens. However, the result from 4 leukemia cases showed that only a part of TCR Vβ subfamily T cells could be detected, and the genescan analysis showed that mono-peak or a dominant peak pictures could be identified in some TCR Vβ subfamily T cells, which indicates that the size of the DNA segments in PCR products is complete or almost homogenous, and considered that the CDR3 length is complete or almost identical. This means that the PCR products were obtained from monoclonal or a dominant clonal T cells (oligoclonal T cells), both are called clonal expansion T cells.[2,5] Only a few studies have mentioned about the functional feature of cytoimmune in HCL, and the research on the clonality of T cells has not been reported in HCL and c-ALL. Our study, for the first time provides the evidence that the clonal expansion T cells could be found in peripheral blood from the patients with HCL or c-ALL, suggesting that it may be the immune response of host T cells to the leukemia associated antigen. The clonal expansion Vβ subfamily T cells are predominantly in Vβ 2, 3, 5, 9, 19, 21 and 24. Only Vβ 19 clonal expansion T cells could be found in 2 patients, the other clonal expansion T cells could be distributed in different Vβ subfamilies. It is difficult to find the common feature, which Vβ clonal expansion is related to with the anti-leukemia cells T cells by an analysis of the present one study in 4 patients. The studies of anti-leukemia Vβ clonal T cells have been preformed in a so few little patients and they are not enough to find the relationship between the Vβ subfamily and anti-leukemia cells clonal expansion T cells. But the clonal expansion T cells with different TCR Vβ rearrangement in different patients may be unique due to the individual is immune feature.

It could not be made clear whether the clonal expansion T cells act as the function of cytotoxicity against leukemia cells. One may look for further research on culture of clonal T cells and the detection of cytotoxicity *in vitro* in the future. On the other hand, the feature of clonal expansion T cells may be used as a mark for detection of the minimal residual disease.

REFERENCES

[1]. Puiseux I, Even J, Pannetier C, et al. Oligo-clonality of tumor-infiltrating lymphocytes from human melanomas. J Immunol 1994; 153: 2807.

[2]. Pannetier C, Evwen J, Kourilsky P, et al. T-cell repertoire diversity and clonal expansions in normal and clinical samples. Immununol Today 1995; 16:176.

[3]. Li YQ, Wang MC, Siegert W, et al. Genescan analysis of T cell clonality by CDR3 size of T cell antigen receptor V β repertoire in acute myeloid leukemia. Tumor (Shanghai) 1997; 17: 435.

[4]. Lim A, Toubert A, Pannetier C, et al. Spread of clonal T-cell expansions in rheumatoid arthritis patients. Human Immunol 1996; 48:77.

[5]. Prevost-Blonded A, Ostankovitch M, Melle J, et al. CDR3 size analysis of T cell receptor V beta transcripts: follow-up study in a patient with T cell acute lymphoblastic leukemia. Leukemia 1995; 9:1711.