original paper
Haematologica 1997; 82:161-165

DETECTION OF CLONALITY BY HETERODUPLEX ANALYSIS OF AMPLIFIED JUNCTIONAL REGION OF T-CELL RECEPTOR γ IN PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS

GIOVANNI MARTINELLI, PATRIZIA FARABEGOLI, NICOLETTA TESTONI, CAROLINA TERRAGNA, ALESSANDRA VITTONE, DONATELLA RASPADORI, MARILINA AMABILE, SANTE TURA
Institute of Hematology "L. e A. Seràgnoli", University of Bologna, Italy

ABSTRACT

Background and Objective. Traditional gel electrophoresis of PCR amplification products of regions from T-cell receptor genes often does not differentiate monoclonal from polyclonal rearrangements. We used polymerase chain reaction (PCR) and heteroduplex analysis on polyacrylamide gels to improve the detection of monoclonal rearrangements.

Methods. We investigated heteroduplex analysis of the amplified Vγ-Jγ junctions of the rearranged T-cell receptor γ (TcR-γ) gene by electrophoretic separation on non-denaturing polyacrylamide gel (PAGE) in 8 T-cell acute lymphoblastic leukemia (T-ALL) patients analyzed at diagnosis.

Results. Clonal homoduplex and heteroduplex bands were present only in the T-ALL samples and not in controls. We confirmed clonality by direct sequencing of the Vγ-Jγ junction. In 2 instances the analysis was performed on samples obtained from the same patient at diagnosis and at relapse, respectively; the presence of the same clonal TcR-γ rearranged cell remained detectable during clinical progression of the disease.

Interpretation and Conclusions. Our heteroduplex analysis showed that separation of the PCR product by electrophoresis on non-denaturing PAGE is a rapid and convenient method for the detection of clonal TcR-γ rearrangements in T-ALL.

Key words: acute lymphoblastic leukemia, T-cell receptor γ gene, polymerase chain reaction

There are two types of antigen-specific T-cell receptors (TcR): the classical TcR-αβ, which is expressed in the majority (≥ 85%) of mature peripheral blood (PB) lymphocytes, and the alternative TcR-γ, which is expressed on a small fraction (≤ 15%) of PB lymphocytes.[1-4] The TcR molecule consists of two different glycoproteins (TcR-α and TcR-β, or TcR-γ and TcR-δ).[1-4] Each chain of the TcR heterodimer contains a variable antigen-recognizing region and constant (C) region. The variable region is encoded by a variable (V) gene segment, a joining (J) gene segment and a junctional region linking the V and J gene segments together.[1,3,4] This junctional region includes diversity (D) gene segments in the case of the TcR-β and TcR-δ genes.[1,3-6]

The TcR molecules from various T-lymphocytes differ from one another as a result of different combinations of V, D and J gene segments and different junctional regions that occur via gene rearrangement processes during early T-cell differentiation.[1,3-5] TcR-γ contains only 6 V and 5 J functional gene segments.[6,7] The available V and J gene segments determine the potentially different combinations. The junctional region determines the junctional diversity. It is formed by the presence of randomly inserted, so-called N-region nucleotides,[8-11] and the deletion of nucleotides through trimming of the ends of the involved gene segments (P-region nucleotides).[12] N-regions consist of nucleotides that are randomly inserted by enzyme terminal deoxynucleotidyl transferase (TdT) at the junctions of gene segments during the rearrangement process.

T-cell acute lymphoblastic leukemia (T-ALL) results from a clonal expansion of T-cells carrying identical copies of rearranged T-cell receptor genes. Therefore rearrangements of TcR genes are used as markers for clonality in human lymphoid tumors.[12] Since the junctional regions of rearranged TcR gene segments are unique for each T-cell clone, DNA sequences at the junction of V and J segments can be used as clone-specific markers in individual patients.[5,12-15]

One method, which has been commonly used for assessing the clonality of lymphocytes, involves the

Correspondence: Giovanni Martinelli MD, Institute of Hematology and Medical Oncology "Seràgnoli", Policlinico S. Orsola, via Massarenti 9, 40138 Bologna, Italy. Tel. international +39.51.6363680. Fax. international +39.51.398973.
Acknowledgements: this work was supported by the Italian Cancer Research Association (A.I.R.C.), by a C.N.R. "A.C.R.O." no. 93.02257.PF39, 94.02111.PF39, "Detection of bcr-abl transcript by capillary electrophoresis" no.952206 C.N.R. and by "30 ore per la vita" target projects.
We wish to thank Dr. Marco Bottaro, Dr. Andrea Biondi and Dr. Nicola Mingone for their helpful discussion on TcR-γ heteroduplex analysis and results, and for providing primer sequences. We are also grateful to Mr. Robin Cooke for his editorial assistance.
Received October 8, 1996; accepted February 19, 1997.

detection of clonal antigen receptor gene rearrangements by Southern blot hybridization,[5] and TcR-γ rearrangements have in fact been reported in all cases of T-ALL.[5,16,17] Furthermore, polymerase chain reaction (PCR)-mediated amplification and subsequent direct sequencing[15,16,18] or temperature-gradient gel electrophoresis (TGGE)[18] have been used effectively to detect the clonal origin of junctional region sequences of TcR-γ in T-ALL. Nevertheless, the Southern blot method and the hybridization techniques all suffer from several technical disadvantages, including the long time needed to obtain results, the use of radiolabeled probes, and the need for a large amount of DNA. Moreover, both TGGE and the method employing PCR plus sequencing are susceptible to various artefacts, such as the use of long and CG-rich primers needed for cloning procedures. An alternative approach using heteroduplex analysis of amplified TcR-γ gene rearrangements has been developed and applied to the assessment of the clonality of cutaneous T-cell lymphomas.[16] Furthermore, we tried to determine whether this approach could also offer significant advantages for diagnosis or monitoring of T-ALLs.

Materials and Methods

Patient and cell samples

Eight adult patients with a diagnosis of T-ALL were included in this study after giving informed consent. Cells were obtained from bone marrow samples from all patients at initial diagnosis before any treatment and, in two cases, at relapse as well (#3 and 9, 4 and 10 in Table 1). Mononuclear cells (MNC) were isolated from bone marrow by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Immunophenotypic data on the T-ALL were studied as previously described.[15,17,20] Some of the immunophenotypic and molecular data from these patients are summarized in Table 1.

Immunologic marker analysis

MNC from the T-ALL patients were analyzed for nuclear expression of terminal deoxynucleotidyl transferase (TdT), for cell membrane expression of the T-cell markers CD1, CD2, CD3, CD4 CD5, CD7, CD8 and CD34, for intracytoplasmic expression of CyCD3, and for reactivity with a panel of monoclonal antibodies (MoAbs) (Becton Dickinson, Mountain View, CA) reported in the literature.[15,17,20] Immunofluorescence staining was performed as described[20] and evaluated with Zeiss fluorescence microscopes (Carl Zeiss, Oberkochen, Germany) and/or a FACScan flow cytometer (Becton Dickinson).[20]

PCR amplification analysis

High molecular weight DNA was isolated from MNC as described previously.[21,22] Amplification of the TcR-γ locus V-J junctional region was essentially performed as described previously with few modifications.[16] The oligonucleotides were synthesized on a 391 DNA Synthesizer (Applied Biosystems, Foster City, CA, USA) using the solid-phase phosphotriester method in our laboratory. The oligonucleotide primers used are listed in Table 2. The primer pairs used in our amplification strategy were: P126 and P129, P126 and P130, P127 and P129, respectively; their positions are illustrated in Figure 1. We used the same primers as in ref. #16.

The PCR mixture was subjected to 35 serial cycles of denaturation (96°C for 1 min), annealing (60°C for 1 min) and extension to control the reactions (72°C for 1 min). A 15 μL aliquot of each reaction was analyzed on 2% agarose gel (Bio-Rad Laboratories, Hercules, CA, USA)[22,23] and/or on 12% polyacrylamide gel visualized by ethidium bromide staining and further stained with silver.[24]

Vγ-Jγ heteroduplex analysis

We analyzed the TcR-γ PCR products by heteroduplex analysis performed as previously described[16] with a few modifications; 20 μL of each amplified product was heated at 96° for 10 min, left to cool at room temperature for 15 min and then loaded. We called this procedure *heteroduplex analysis*. Samples were run for 4 hours on 12% non denaturing polyacrylamide gel

Table 1. Results of immunophenotypic and molecular analysis by PCR of the T-ALL patients studied. Phase of disease, immunological phenotype assessment and PCR results of eight T-ALL patients at time of diagnosis (#1 to 8) and at relapse time (#9 and 10) are given. All the results are from bone marrow samples (BM); N.D. = not done, Immunological marker analysis: (+) means that ≥75% of all the cells are positive; (–) means that ≤25% of the cells are positive. Molecular analysis: (+) = clonal PCR product; (–) = polyclonal PCR product. The type of rearrangement and the primers used for each PCR are given in the boxes at the top of each column.

	Clinical data			*Immunophenotype*									*Heteroduplex analysis*			
No	*Patient*	*Phase of disease*	*Sample*	*TdT*	*CD1*	*CD2*	*CD3*	*CD4*	*CD5*	*CD7*	*CD8*	*CyCD3*	*TcR-γ (VI/J1/2) [P126/P129]*	*TcR-γ (VI/JP1-P2) [p126/p130]*	*TcR-γ (VII/J1/2) [P127/P129]*	*Mono/ Biallelic*
1	C.D.	diagnosis	BM	+	+	+	+	+	+	+	–	+	+	–	–	Monoallelic
2	M.G.	diagnosis	BM	+	–	+	+	+	N.D.	+	+	+	+	–	–	Monoallelic
3	C.G.	diagnosis	BM	+	+	+	–	+	+	+	+	N.D.	+ +	–	–	Biallelic
4	B.R.	diagnosis	BM	–	–	–	+	–	N.D.	+	–	N.D.	+	+	–	Biallelic
5	B.E.	diagnosis	BM	+	+	+	+	–	N.D.	+	–	N.D.	+	–	–	Monoallelic
6	BE.FE.	diagnosis	BM	+	–	+	+	+	N.D.	+	+	N.D.	+	+	–	Biallelic
7	A.V.	diagnosis	BM	+	+	+	+	+	+	+	+	N.D.	–	+	–	Monoallelic
8	BA.FU.	diagnosis	BM	+	–	+	+	–	+	+	–	N.D.	+ +	–	–	Biallelic
9	C.G.	relapse	BM	+	+	+	–	+	+	+	+	N.D.	+ +	–	–	Biallelic
10	B.R.	relapse	BM	–	–	–	+	–	+	+	–	N.D.	+	+	–	Biallelic

Name	Code	PCR/SEQ	Sequence	Reference
P126	Vγl (V2-5, V7, V8,)	PCR	5' - TACATCCACTGGTACCTACACCA - 3'	16
P127	VγII (V9)	PCR	5' - TCATACAGTTCCTGGTGTCC - 3'	16
P128	VγIII (V10/11)	PCR	5' - TCATTCACTGGTACCGGCAGAAACCAAA - 3'	16
P129	Jγ1/2	PCR	5' - CCCGTCGACTACCTTGGAAATGTTGTATTCTTC - 3'	16
P130	JP1/JP2	PCR	5' - CCAGGTGAAGTTACTATGAG - 3'	16
P131	JγP	PCR	5' - AAGCTTTGTTCCGGGACCAA - 3'	16

Vγl: a consensus oligo recognizing all rearrangeable members of the first Vγ family, i.e. five functional (V2-5 and V8) and four non functional (V1, V5, V6 and V7).

Table 2. Oligonucleotide primers used in PCR and direct sequencing analysis of the TcR-γ gene. Sequence information used to design the oligonucleotide primers was derived from the indicated literature references.

(37.5:1 acrylamide: bisacrylamide) in Tris-Borate EDTA (TBE) 1 x buffer at 250 volts and 4°C (Protean II Cell apparatus, Bio-Rad Laboratories, Hercules, CA, USA), visualized by ethidium bromide staining and further stained with silver. Electrophoretic analysis on agarose gel gave rise to sharp bands in both leukemic and normal samples; by contrast, on polyacrylamide gel, when no clonal rearrangement was present in the sample subjected to heteroduplex analysis, the migration pattern appeared as a homogeneous smear. The absence of homoduplex and heteroduplex bands represents the pattern of a polyclonal T-cell population.[16] In the presence of a major clone, when both TCR-γ alleles are rearranged in a given T-cell clone (biallelic) and often amplifiable in a single reaction (e.g. by VI-J1/2 primers), four double-strand chains predominate at the end of the PCR; of these, two are the original rearranged chains (homoduplexes; no mispairing at the VJ junction), and two originate from the exchange of the respective positive and negative strands, which result in mismatched V-J regions (heteroduplex). When only one clonal rearrangement is amplified by a given primer pair (monoallelic), only a single homoduplex fragment appears, without any heteroduplex.

DNA sequencing analysis

The homoduplex bands were cut out, minced, left overnight at 37°C in 400 mL of elution buffer [500 mM $(NH_4)CH_3COO$, 10 mM $Mg(CH_3COO)_2$, 1 mM Na_2EDTA, 0.1% SDS], and centrifuged for 5 min at 12,000 rpm in a microfuge. The DNA was then precipitated in ethanol and resuspended in Tris-EDTA (TE), buffer pH 7.6.

Twenty ng of DNA were employed for the sequencing reaction with an Amplicycle Sequencing Kit (Perkin Elmer, Cetus, Norwalk, CT, USA). The sequencing primers used were Jγ1/2 for the positive strand and VγI and VγII for the negative strand.

Results

T-ALL phenotypes

All cases were regarded as unequivocally malignant by morphologic standards and immunophenotypic studies (Table 1).

Seven samples (#1-3, 5-8) were TdT+ and four (#1, 3, 5, 7) $CD1^+$. The patients differed in their CD4/CD8 phenotype. Four T-ALL demonstrated a $CD4^+/CD8^+$ phenotype (#2, 3, 6, 7), 3 a $CD4^-/CD8^-$ phenotype (#4, 5, 8), and one was $CD4^+/CD8^-$ (#1). Interestingly, the phenotypes of patients #3 and 4 were the same at relapse as at diagnosis.

Electrophoretic analysis of the Vγ-Jγ PCR product

The PCR products were approximately 300 bp long, depending on the type of primer pair used. After electrophoresis on 2% ethidium bromide-stained agarose gel, the PCR products obtained with different primer combinations appeared as intense sharp bands not only with the products derived from T-ALL DNA samples, but also with those from healthy DNA controls (Figure 2). Moreover, the samples were positive with all the primer pairs used.

When we analyzed the same TcR-γ PCR products on non-denaturing polyacrylamide gel electrophoresis (without previous denaturation = heteroduplex analysis) following silver staining, the polyclonal Vγ-Jγ junctions of non-neoplastic cells were again indistinguishable from the monoclonal ones, with amplification producing sharp bands for both (data not shown), even though the polyclonal PCR products in the polyacrylamide gels were generally broader and less sharp than the monoclonal counterparts.

When we followed the PCR-based approach, which takes advantages of the heterogeneity of the Vγ-Jγ junctional sequences and of the different migration properties on polyacrylamide gel of the Vγ-Jγ segments containing a few mismatches (heteroduplex) as compared to fully matched Vγ-Jγ junctions (homoduplex), polyacrylamide gel analysis of PCR products from specimens lacking clonal TcR-γ gene rearrangements persistently revealed a homogeneous smear without the presence of any band. By contrast, each PCR sample containing a clonal rearrangement showed one (monoallelic Vγ-Jγ rearrangement) or 2 (biallelic Vγ-Jγ rearrangements) prominent bands in either ethidium bromide or silver staining (homoduplex pattern) (Figure 3). When a bi-allelic rearrangement was present and amplified with the same primer pairs, two additional bands became visible in the upper part of the gel (heteroduplex bands).

Using this method and these combinations of primers, we were able to identify TcR-γ rearrangements in all samples; the TcR-γ gene rearrangements occurred in 16 out of 20 alleles. All 16 rearranged alleles contained at least one rearranged gene of the VI family; twelve had the Jγ1/2 gene and 4 the JγP1/P2 gene. Two patients (#3 and 8), who had both alleles rearranged to the Jγ1/2 gene, showed two fast migrating bands (homoduplex) accompanied by two fainter, slower migrating fragments (heteroduplex).

In order to confirm the finding that it is possible

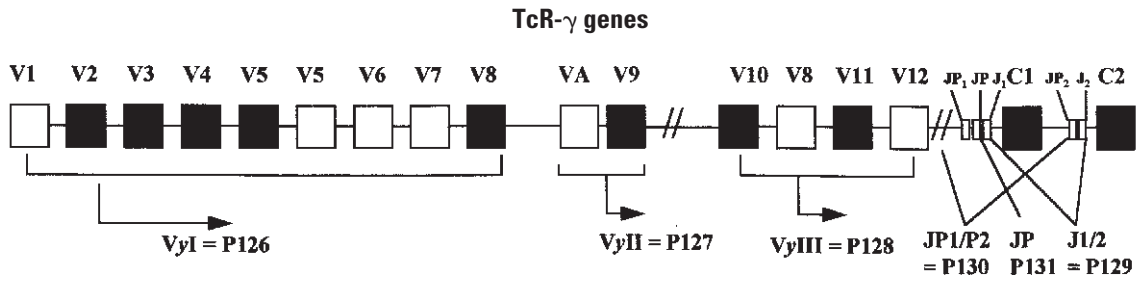

Figure 1. Schematic representation of TcR-γ region. The picture shows the position of Vγ TcR-γ genes (filled big boxes), and TcR-γ pseudogenes (open big boxes), the Jγ TcR-γ gene (small open rectangular), and the position of the primers used during PCR procedures. The names of the individual TcR-γ genes are listed at the top of the boxes. At the bottom of the boxes the TcR-γ families are reported (VγI, VγII, VγIII) with the name of the primers used for PCR procedures (P126, P127, P128 for VγI, VγII and VγIII, respectively, and P129, P130, P131 for J1/2, Jp1/Jp2, JP, respectively). V = variable gene; J = junctional gene; C = constant gene; // = double bars represent intronic region of undetermined size.

to distinguish accurately between monoclonal and polyclonal TcR rearrangements with non denaturing PAGE, we sequenced some samples showing sharp bands. From two samples (#4 and 5) we directly sequenced the homoduplex fragments once they were dissolved in the polyacrylamide gel. This sequencing approach confirmed the specificity of PCR, showing Vγ8-Vγ1/2 (case #2) and Vγ2-Jγ1/2 (case #5) junction sequences in the samples analyzed.

Discussion

Amplification by PCR of the rearranged TcR-γ gene has been described for the detection of clonal T-cell populations.[5,14-16,18,19] We and other investigators[5,14,16] have selected universal consensus Vγ primers corresponding to homologous regions in the Vγ1-8 genes, in the Vγ9 and in the Vγ10 genes. While our sequencing results clearly showed that these consensus primers successfully amplify the rearranged genes, we also found that the presence of distinct bands after electrophoresis of TcR-γ PCR products on agarose gel alone cannot be interpreted as a reliable marker of clonality.

In our experience, heteroduplex analysis on PAGE was capable of separating identical (i.e. clonal) PCR fragments in a mixture of partially homologous (i.e. polyclonal) PCR products. It therefore appears to be an ideal method for identifying clonal PCR products. Because of the diversity of TcR-γ junctions, with heteroduplex analysis on PAGE[16] a mixture of multiple heteroduplexes bearing extensive mismatch regions should be formed by denaturation (96°C) and reannealing of the TcR-γ PCR product from a polyclonal sample. This mixture of multiple heteroduplexes will migrate and appear as a diffuse smear. On the other hand, TcR-γ PCR

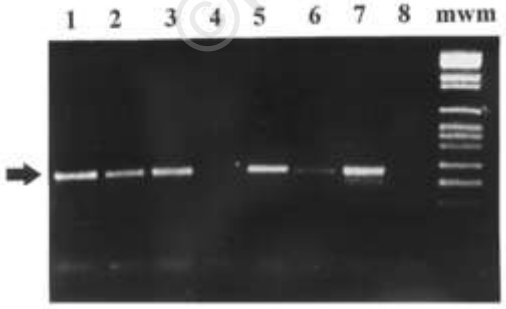

Figure 2. Ethidium bromide staining on 2% agarose gel of PCR products obtained with the TcR-γ primers VγI and VγP1/P2 and DNA from polyclonal control (lane 7) and from 5 cases with T-ALL (lanes 1, 2, 3, 5, 6; respectively, patients MG, BR, BE, CG, CD). PCR reagent controls are in lanes 4 and 11. MWM: Molecular Weight Marker VI, Boheringer Mannheim, Italy. Note that intense and sharp bands are observed with polyclonal as well as clonal PCR products.

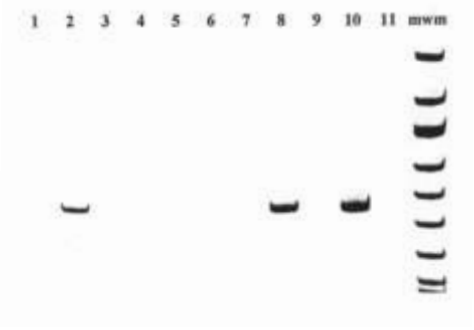

Figure 3. PAGE analysis of the PCR products obtained with the VγI and JγP1/P2 primers and DNA from polyclonal control (lane 7), and 8 cases with T-cell lymphoproliferations (lanes 1, 2, 3, 5, 6, 8, 9, 10; respectively, patients MG, BR, BE, CG, CD, BeFe, BaFu, AV). PCR reagent controls are in lanes 4 and 11. MWM: Molecular Weight Marker VI, Boheringer. Note that intense and sharp bands are observed only with clonal PCR products.

products containing a significant percentage of monoclonal (i.e. identical) sequences after denaturation and reannealing should result in a high proportion of matched homoduplexes running as sharp single or double bands, with monoallelic and biallelic rearrangements, respectively.

Our strategy for detecting monoclonal TcR-γ junctions presents several practical advantages. It requires less work than other approaches (Southern blot, subcloning of PCR products); it is also less time-consuming than Southern blot analysis, providing results in just a few days. PCR products can be obtained even from partially degraded DNA, whereas degradation can be a major problem in Southern blot hybridization. Moreover, heteroduplex analysis does not require radioactivity, thus reducing costs and eliminating the need for special disposal of radioactive and contaminated materials. Finally, the heteroduplex system appears to be less susceptible to the artefacts often associated with Southern blot hybridization.

With regards to sensitivity, PCR may again have advantages over Southern blot analysis of antigen receptor gene rearrangements. In particular, Southern blot seems to have an absolute threshold of detection of 1-5% neoplastic cells per sample, while PCR followed by heteroduplex analysis and silver stain achieved a sensitivity of 0.1-1%.[25-27] In addition, our procedures could be further shortened and simplified by a more rational use of primers. In fact, most one-rearranged-allele samples can be amplified using a single pair of primers (i.e. VγI and Jγ1/2) because of non-random V-J gene usage.

Heteroduplex analysis of the TcR-γ gene also has several advantages over other methods employing PCR, such as agarose gel electrophoresis analysis[28] or TGGE.[18] In the former, a nested PCR with internal sequence specific primers is usually necessary, while in the latter a long stretch of GC-rich base tail is needed inside the primer for better resolution of the amplified primer. By contrast, heteroduplex analysis is relatively simple and rapid.

In conclusion, we think that the heteroduplex analysis approach described herein could represent a step forward in the evaluation of T-cell clonality in T-ALL.

References

1. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988; 334: 395-9.
2. Borst J, Van Dongen JJM, Bolhuis RLH, et al. Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. J Exp Med 1988; 167:1625-9.
3. Raulet DH. The structure, function, and molecular genetics of the γ/δ T cell receptor. Ann Rev Immunol 1989; 7:175-9.
4. Van Dongen JJM, Comans-Bitter WM, Wolvers-Tettero ILM, Borst J. Development of human T lymphocytes and their thymus-dependency. Thymus 1990; 16:207-11.
5. Van Dongen JJM, Wolvers-Tettero ILM. Analysis of immunoglobulin and T cell receptor genes. Part I: basic and technical aspects. Clin Chim Acta 1991; 198:1-4.
6. Yoshikai Y, Clark SP, Taylor S, et al. Organization and sequences of the variable, joining and constant region genes of the human T-cell receptor α-chain. Nature 1985; 316:837-41.
7. LeFranc M-P, Forster A, Baer R, Stinson MA, Rabbitts TH. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. Cell 1986; 45:237-41.
8. Quertermous T, Strauss WM, Van Dongen JJM, Seidman JG. Human T cell γ chain joining regions and T cell development. J Immunol 1987; 138:2687-91.
9. Forster A, Huck S, Ghanem N, Lefranc M-P, Rabbitts TH. New subgroups in the human T-cell rearranging Vγ gene locus. EMBO J 1987; 6:1945-9.
10. Lefranc M-P. The human T-cell rearranging γ (TRγ) genes and the γ T-cell receptor. Biochimie 1988; 70:901-4.
11. Van Dongen JJM, Wolvers-Tettero ILM, Seidman JG, et al. Two types of gamma T cell receptors expressed by T cell acute lymphoblastic leukemias. Eur J Immunol 1987; 17:1719-23.
12. Lafaille JJ, DeCloux A, Bonneville M, Takagaki Y, Tonegawa S. Junctional Sequences of T cell receptor gd genes: implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining. Cell 1989; 59:859-63.
13. Hata S, Satyanarayana K, Devlin P, et al. Extensive junctional diversity of rearranged human T cell receptor δ genes. Science 1988; 240:1541-5.
14. Macintyre E, E'Auriol L, Amesland F, et al. Analysis of junctionl diversity in the preferential Vδ1-Jδ1 rearrangement of fresh T-acute lymphoblastic leukemia cells by in vitro gene amplification and direct sequencing. Blood 1989; 74:2053-7.
15. Breit TM, Wolvers-Tettero ILM, Hählen K, Van Wering ER, Van Dongen JJM. Limited combinatorial repertoire of γδ T-cell receptors expressed by T-cell acute lymphoblastic leukemias. Leukemia 1991; 5:116-20.
16. Bottaro M, Berti E, Biondi A, Migone N, Crosti L. Heteroduplex analysis of T-cell receptor γ gene rearrangements for diagnosis and monitoring of cutaneous T-cell lymphomas. Blood 1994; 83:3271-5.
17. Van Dongen JJM, Wolvers-Tettero ILM, Wassenaar F, Borst J, Van den Elsen P. Rearrangement and expression of T-cell receptor δ genes in T-cell acute lymphoblastic leukemias. Blood 1989; 74:334-8.
18. Borgouin A, Tung R, Galili N, Sklar J. Rapid, non radioactive detection of clonal T-cell receptor gene rearrangements in lymphoid neoplasms. Proc Natl Acad Sci USA 1990; 87:8536-40.
19. Macintyre EA, D'Auriol L, Duparc N, Leverger G, Galibert F, Sigaux F. Use of oligonucleotide probes directed against T cell antigen receptor γδ variable-(diversity)-joining junctional sequences as a general method for detectiong minimal residual disease in acute lymphoblastic leukemias. J Clin Invest 1990; 86:2125-9.
20. Lauria F, Raspadori D, Martinelli G, et al. Increased expression of myeloid antigen markers in adult acute lymphoblastic leukemia patients: diagnostic and prognostic implication. Br J Haematol 1994; 87:286-92.
21. Guerrasio A, Martinelli G, Ambrosetti A, et al. Different suppression of the Ph1 positive hemopoiesis induced by intensive chemotherapy in lymphoid and myeloid blast crisis of CML. Haematologica 1991; 76:126-30.
22. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning, a laboratory manual. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1989.
23. Martinelli G, Farabegoli P, Buzzi M, et al. Fingerprinting of HLA class I genes for improved selection of unrelated bone marrow donors. Eur J Immunol 1996; 23:55-65.
24. Benini C, Bandini G, Motta MR, et al. Donor origin of hematopoiesis after a case of allogenic transplantation with cryopreserved marrow. Haematologica 1993; 78:414-5.
25. Martinelli G, Testoni N, Farabegoli P, et al. Amplification of third-complementary-determining-region (CDR-III) of heavy chain immunoglobuline gene (IgH) and cytogenetical analysis in one hundred adult acute leukemias. Leuk Lymphoma 1997; in press.
26. Martinelli G, Remiddi C, Visani G, et al. Molecular analysis of PML-RARa fusion mRNA detected by reverse transcription-polymerase chain reaction assay in long term disease free acute promyelocytic leukemia patients. Br J Haematol 1995; 90:966-8.
27. Testoni N, Martinelli G, Farabegoli P, et al. A new method of "in cell RT-PCR" for the detection of bcr-abl transcript in chronic myeloid leukemia patients. Blood 1996; 87:3822-7.
28. Bahler DW, Berry G, Oksenberg J, Warnke RA, Levy R. Diversity of T-cell antigen receptor variable genes used by mycosis fungoides cells. Am J Pathol 1992; 140:1-4.