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T cell receptor Vβ variable gene family expression in human peripheral blood lymphocytes at the mRNA and membrane protein level

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SUMMARY

A polymerase chain reaction (PCR) was developed, using combinations of an oligonucleotide primer for a T cell receptor Vβ gene family and one for the constant Cβ gene segments, to assess the expression of each of 20 Vβ gene families in RNA after reverse transcription into cDNA. The detection was done after agarose gel electrophoresis of PCR products and ethidium bromide staining. The positive identification of the PCR products was done by hybridization with a Jβ oligonucleotide probe. For T cell lines, a signal was observed in the Vβ8 combination for Jurkat cells, Vβ5a in HSB cells, Vβ2 and Vβ12a in Molt-3 cells and Vβ2, Vβ5a and Vβ12a in Molt-4 cells. Using mixtures of RNA from different cell lines, the sensitivity of the method was in the range of 0·1–0·5%. In peripheral blood mononuclear cells from four donors, taken at three different occasions, all Vβ families were detectable. The intensity of the PCR product varied between various Vβ gene families. Flow cytometric analysis of blood mononuclear cells from the same donors with a restricted series of Vβ gene family-specific antibodies also revealed the presence of all families. The approach to assess Vβ gene family expression in heterogeneous populations opens the possibility to study T cell receptor variable gene expression in relation to physiology and pathologic processes.

Keywords T cell receptors Vβ families flow cytometry polymerase chain reaction

INTRODUCTION

The classical receptor moiety used by the majority of human T cells is the so-called αβ T cell receptor (TCR) heterodimer [1,2]. Both α and β chains contain a variable (V), junction (J), and constant (C) region, and in addition there is a diversity (D) region in the β chain [3]. The genome encoding the variable region of the receptor chains is organized in 'families', which show 75% or higher homology in nucleotide sequence. For the β chain 20 families have been established, each comprising one to seven members [4]. The two human β chain constant region (Cβ) genes are highly homologous with respect to both nucleotide sequence and intron/exon organization [3].

During the development of lymphoid precursor cells into immunocompetent T cells distinct V–D–J combinations are generated at the genomic (DNA) level followed by transcription, splicing in V–D–J–C combination and translation into protein. These combinations can be measured at the subcellular level, e.g. by Southern blotting techniques using appropriate restriction endonucleases and probes. This clonal analysis has a sensitivity of about 1–5% in a heterogeneous population. Therefore its application in the clinical setting is restricted to the evaluation of advanced T cell malignancies [5].

The analysis of variable gene family expression was originally developed in the mouse, using antibodies to family-specific epitopes; subsequently, molecular biologic methods have been applied. In specified mouse strains restrictions in expression of variable gene families do occur, among others related to the haplotype of major and minor histocompatibility antigens or superantigens like Mls^a [1,6]. The reactivity of the TCR is pivotal in causing this restriction of expression. Positive and negative selections during intrathymic processing of precursor T cells determine the outcome of variable gene family expression, starting from the rearrangement at the germline configuration [7]. In humans, an association between variable gene family and reactivity of the TCR has also been documented, e.g. the recognition of superantigens of *Staphylococcus aureus* toxin [8,9] is related to some specified Vβ families. In certain disease conditions like sarcoidosis [10], rheumatoid arthritis [11,12], multiple sclerosis [13] and autoimmune thyroid disease [14], a restricted or preferential expression of distinct Vβ or Vα families has been reported. Despite the fact that the association of this preferential expression of some variable gene families and antigen recognition remains to be elucidated, these data have the prospect of a potential value of the detection of

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Table 1. Oligonucleotide sequences of T cell receptor β chain

Primer	Sequence	Family member	Size of PCR product*
Vβ	5′	3′	
1	GCAAAAGGAAACATTCTTGAACG		278±8
2	AGAGTCTCATGCTGATGGC		318±20
3	AGGACGGGAGAGAAAGTTTTTC		403±18
4	GGCCACATATGAGAGTGGATTTGTC		281±11
5a	CAGTGAGACACAGAGAAAC	5.1; 5.4	303±13
5b	CCCTAACTATAGCTCTGAGCTG	5.2; 5.3	225±17
6	GGGGCAGGGCCCAGAGTTTCTAAT		321±26
7	CTTAAACCTTCACCTACACGC		230±10
8	CTTTAACAACAACGTTCCG		319±23
9	CAGTTCCAAATCGCTTCTCAC		276±18
10	GGATTGTGTTCCTATAAAAGC		366±16
11	CCACTATTCCTATGGAGTTAATTCC		303±11
12a	GTCACCAGACTGAGAACCACCGCTA	12.1; 12.2	371±13
12b	GATTCATTACTCAGTTGGTG	12.3; 12.4	313±21
13	GCATGACACTGCAGTGTGCCC		346±16
14	ACCCAAGATACCTCATCACAG		401±7
15	TCTCAGACTAAGGGTCATGATAGA		346±16
16	CTGTTACATTTTGTGAAAGAGTC		296±12
17	CTCACAGATAGTAAATGACTTTC		290±12
18	AGCCCAATGAAAGGACACAGTCAT		365±15
19	ACCCCCGAAAAAGGACATACTTTT		365±15
20	GAGGGAACATCAAACCCCAACCTA		365±15
Cβ	GGCCTTTTGGGTGTGGGAGATCTCTGCTTCTGATGGC		
Jβ	CCCTGGCCCGAAGAACTGCTCATTGTAGGA		

* Size is measured from results (±s.d.).

specified families in the diagnostic evaluation of patients with immunologic abnormalities. Further, most of these studies lack the analysis of the whole spectrum of V-gene families, because antibodies to V-gene family products were used, which are not available for every V-gene family.

We initiated studies on the assessment of TCR variable gene families in heterogeneous (blood) cell populations. The assessment of the whole spectrum of families has thus far been done at the DNA or mRNA level. To this end, a polymerase chain reaction (PCR) was developed, using Vβ family-specific oligonucleotide primers in combination with an oligonucleotide primer for constant Cβ1 and Cβ2 gene segments. For RNA analysis this PCR was done after preparation of cDNA by reverse transcription. The technique was developed and validated using mRNA from T cell lines as substrate, and subsequently applied on material from normal healthy blood donors. The data were compared with those obtained in flow cytometry using variable gene family-specific antibodies.

MATERIALS AND METHODS

Cells

Cells of T-leukaemic cell lines Jurkat, HSB, Molt-3 and Molt-4 were cultured in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS). Cells were counted, pelleted and the supernatant was removed; then the cells were immediately frozen at −70°C. Peripheral blood mononuclear cells from four healthy volunteers were isolated by Ficoll–Hypaque density centrifugation. Part of the cells was used for suspension immunofluorescence, and another part was stored in liquid nitrogen till RNA isolation. The RNA analysis was performed two or three times for each donor with an interval of 4 months. The assessment by flow cytometry was done twice.

RNA isolation

RNA was prepared using a single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction [15]. The RNA concentration was measured using a spectrophotometer (Hitachi U-200, Tokyo, Japan).

Primers

One Cβ and 22 Vβ primers which are specific for 20 different Vβ families have been selected using the program DNASIS (created by LKB-Pharmacia; Table 1). For the Vβ families 5 and 12, two primers were selected, respectively, because no primer sequence common to all family members was found. The primer oligonucleotides were synthesized using a DNA autosynthesizer (ABI 381A, Foster City, CA) using β-cyanoethylphosphoramide chemistry, based on 1 OD = 30 μg/ml. The primers were purified following the method of the ABI user's bulletin and diluted to 0·5 μg/μl.

cDNA Synthesis

The RNA sample was incubated at 42°C for 1 h with 30 μ cDNA-reactive buffer (100 mM Tris–HCl, pH 8·3, measured a 42°C; 140 mM KCl, and 10 mM $MgCl_2$) containing 2·5 μg C primer, dNTPs (1 mM in each as final concentration), DTT (1 mM as final concentration) and 25 U avian myeloblastosis viru reverse transcriptase (Promega Corporation). After incubation 3 μl 0·2 M EDTA were added to stop the reaction. Then 70 μ

MilliQ water and 100 μl phenol/chloroform mixture (1:1) were added and mixed thoroughly. After 5 min of centrifugation at 14000 *ca/g*, the water phase was transferred to another fresh Eppendorf tube. This step was repeated twice, and for the second time 100 μl of chloroform/isoamylalchohol mixture (49:1) was used instead of phenol/chloroform. Then 10 μl 3 M sodium acetate and 200 μl 100% ethanol were added to the tube and kept at −70°C for 30 min. The tube was centrifuged for 15 min at 14000 *ca/g*, and the supernatant was discarded carefully. The pellet was washed with 70% ethanol and dried in a Speedvac concentrator (Savant, NY). Finally, the pellet was dissolved in MilliQ water to reach a concentration of 1·0 μg/μl cDNA.

Polymerase chain reaction

The PCR cocktail was 50 μl PCR buffer (NaCl 50 mM, Tris--HCl 10 mM (pH 8·3), $MgCl_2$ 2 mM) containing dNTPs (0·2 mM for each as final concentration), 1·0 μg cDNA, 0·25 μg Cβ primer, 0·25 μg one of the Vβ primers and 0·5 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The cDNA reversely transcribed from the RNA of Jurkat cells and the Vβ8 primer were always used as positive control. The negative control was the same as the positive control except that 1 μl MilliQ water was used instead of cDNA. To prevent evaporation, the cocktails were covered with three droplets of mineral oil, and then amplified by an automated DNA thermal cycler (Perkin Elmer Cetus) for 35 cycles. The amplification cycle profile was as follows: denaturation for 1 min at 95°C; annealing for 1 min at 60°C; and extension for 2 min at 72°C. Twenty microlitres of PCR product from each tube were electrophoresed in 2% agarose gel. The gel was stained with 1% ethidium bromide and visualized under u.v. light. Markers used were Φ-X DNA digested with *Hae*III, and λDNA digested with *Hind*III. The intensity of individual bands was scored as very faint (±); faint (+); clear (++); and strong (+++).

Positive identification of the PCR product was done using a Jβ-specific oligonucleotide (Table 1). The oligonucleotide probe was labelled with ^{32}P-γATP using T4 polynucleotide kinase, and purified by Sephadex G25 chromatography. Gels after electrophoresis were dried in a gel drier (Hoefer Scientific Instruments, San Francisco, CA). The gel was soaked for 30 min in a solution containing 0·5 M NaOH and 0·15 M NaCl, and then for 30 min in a buffer containing 0·5 M Tris, 0·15 M NaCl, pH 8·0. Pre-hybridization was done at 55°C for 60 min, followed by probe hybridization overnight. Washing was done for 10 min in a solution of 6 × SSC supplemented with 0·1% SDS. The gel was exposed to Kodak X-OMAT S Film 100 (Eastman Kodak, Rochester, NY) overnight at −80°C, followed by development.

Sensitivity test of PCR

After establishment of the TCR Vβ family (or families) of the T cell lines, mixtures were prepared of RNA from HSB and Molt-3, Jurkat and HSB, and Molt-3 and HSB. The mixtures were prepared on the basis of cell numbers put into RNA isolation. Then cDNA was synthesized and PCR was performed as described above.

Flow cytometry

MoAbs to human TCR variable regions were obtained from T Cell Sciences (Cambridge, MA), including antibodies recognizing Vβ5.1 (LC4) [16]; Vβ5.2 and Vβ5.3 (1C1) [17]; Vβ5.3 (W112) [18]; a Vβ6.7 allotypic determinant (OT145) [19,20]; Vβ8 (16G8) [18]; and Vβ12 (S511) [21]. The Vβ5.1 determinant is encoded by the Vβ5a segment, and the Vβ5.2 and Vβ5.3 determinants by the Vβ5b segment (Table 1). In a two-step procedure, peripheral blood mononuclear cells ($0·5–1·0 \times 10^6$ cells) were incubated with 10 μl antibody, subsequently with FITC-conjugated rabbit $F(ab')_2$ immunoglobulin fragments to mouse immunoglobulins (Dakopatts, Glostrup, Denmark). Analysis was performed using the FACSCAN (Becton Dickinson, Mountain View, CA). In computerized analysis of data, only lymphocytes were scored by appropriate gating. The analysis was supplemented with the detection of CD3-expressing cells using flow cytometry with FITC-conjugated CD3 antibody (Becton Dickinson).

RESULTS

Cell lines: sensitivity assessment

RNA isolated from the T cell lines was transcribed to cDNA, which was subsequently used as substrate in the PCR with various family-specific oligonucleotide primers and a Cβ primer (specific for Cβ1 and Cβ2). A signal was observed for Jurkat cells in the Vβ8 combination, and for HSB in the Vβ5a (Fig. 1). For Molt-3 we observed signals in two combinations, Vβ2 and Vβ12a; for Molt-4 cells, three combinations gave a signal: Vβ2, Vβ5a and Vβ12a (data not shown). The size of the amplified fragments detected after PCR differed for various combinations of family-specific Vβ and Cβ oligonucleotide primers, according to the place in the V region where the specific oligonucleotide was selected.

The sensitivity of the PCR approach was assessed for HSB (Vβ5a) in various mixtures with Molt-3, for Jurkat (Vβ8) in mixtures with HSB (Fig. 1b), and Molt-3 (both Vβ2 and Vβ12a) in combinations with HSB (Table 2). A signal was detectable at percentages of ⩾0·5% cells for Jurkat, and the Vβ2 detection of Molt-3 was also at 0·1%. In combinations with lower percentages of the cellular RNA to be detected (⩽0·05%) no signal was observed.

TCR Vβ families in peripheral blood of healthy donors

For the four healthy blood donors investigated, all combinations of a family-specific oligonucleotide primer and Cβ primer gave a signal (Fig. 2), except for the Vβ10 combination in donor A at one of the three occasions tested. The size of amplified fragments was measured from the ethidium-bromide-stained gels with respect to marker Φ-X DNA digested with *Hae*III: mean data from four gels are presented in Table 1. Family-specific bands were identified in a positive way using hybridization with an internal Jβ primer (Fig. 3). In addition, extra bands were detected in the ethidium-bromide-stained electrophoresis pictures. The scoring of the intensity of Vβ family PCR products of different donors assessed at different times is presented in Table 3. The intensity of the signal observed varied for different family-specific combinations, e.g. the Vβ10 and Vβ19 signal being generally lowest and that of Vβ1, Vβ4, Vβ5 and Vβ9 being highest.

Flow cytometry for cells expressing TCR of different Vβ families

Cells from the four donors that were investigated for Vβ family RNA expression, were also used to test Vβ family expression at the protein level (Table 4). The proportions of Vβ family

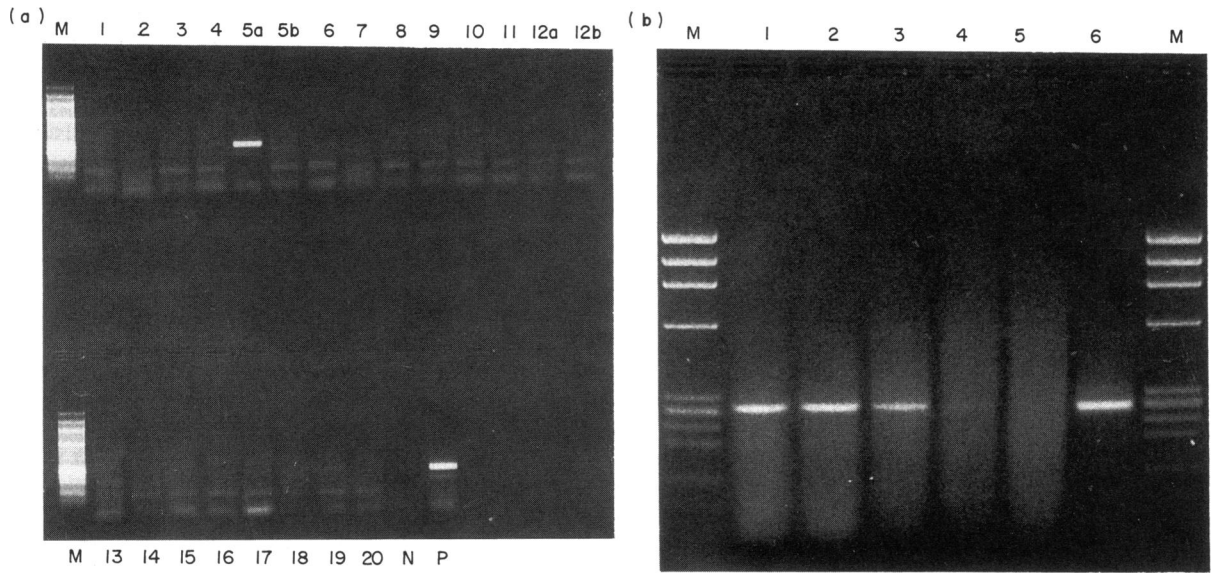

Fig. 1. (a) Agarose gel electrophoresis of PCR products for various combinations of Vβ gene family-specific and Cβ oligonucleotides primers, for cDNA derived from RNA of the HSB cell line. Different lanes represent marker DNA (M, Φ-X DNA) digested with *Hae*III, individual Vβ families (1-20), a negative (N) control of Vβ8 and Cβ primer without cDNA, and a positive (P) control comprising cDNA from Jurkat cells with a Vβ8–Cβ primer combination. A positive signal is observed for HSB cDNA and the Vβ5a-Cβ primer combination. In other combinations, only primer–dimer products are observed; (b) agarose gel electrophoresis of PCR products for mixtures of RNA isolated from Jurkat cells and from HSB cells, amplified in combinations of a Vβ8 and Cβ oligonucleotide primers. In lanes 1–5 different mixtures are shown with the following percentages of Jurkat RNA: 1, 5%; 2, 1%; 3, 0·5%; 4, 0·1%; 5, 0·05%. In lane 6, a control of pure HSB-derived cDNA with a Vβ5a-Cβ oligonucleotide combination is shown. Lanes M represent the Φ-X marker DNA.

Table 2. Sensitivity of RNA-PCR for TCR Vβ gene family analysis

Combination	5%	2·5%	1%	0·5%	0·1%	0·05%
HSB (Vβ5a)/Molt-3	ND	+	+	+	−	−
Jurkat (Vβ8)/HSB	+	ND	+	+	+	−
Molt-3 (Vβ12a)/HSB	+	ND	+	±	−	−
Molt-3 (Vβ2)/HSB	+	ND	+	+	+	−

For each combination the Vβ gene family assessed is indicated within brackets. The RNA preparations were mixed on the basis of original cell numbers put into RNA isolation: the percentage denotes the cell population which is analysed.

ND, not done.

expressing cells in percentage of lymphocytes varied between 2% and 17%. The Vβ12 family manifested the lowest percentage, and the highest percentages were scored for the combined expression of Vβ5.2 and Vβ5.3. The proportions of Vβ5.1-positive cells was lower than that of Vβ5.2+Vβ5.3-positive cells, but formed a significant part of all Vβ5-positive cells. Vβ6.7-positive cells were found in percentages of lymphocytes ranging between 1% and 4%. The proportions of Vβ family-expressing cells, both in percentage of lymphocytes (data not shown) and in percentage of CD3-positive T cells, was similar for each donor analysed at different occasions. Exceptions were Vβ5 data in donor A, and Vβ8 data in donor D. The intra-individual variation was lower than the inter-individual variation, but the largest variation was observed between various TCR Vβ families.

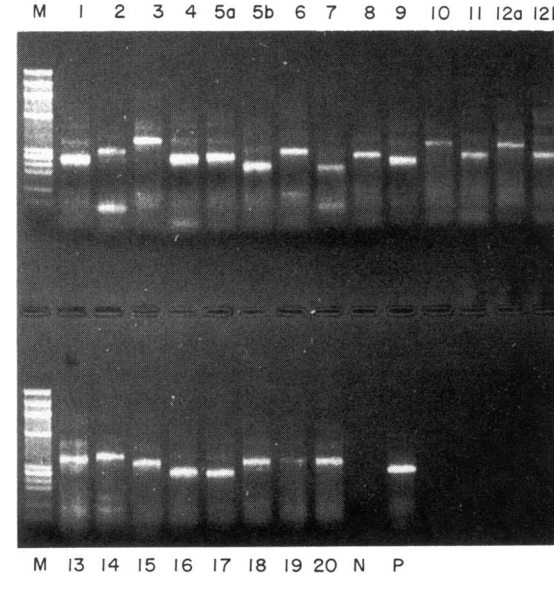

Fig. 2. Agarose gel electrophoresis of PCR products for various combinations of Vβ gene family-specific and Cβ oligonucleotide primers, for cDNA derived from blood mononuclear cells of donor C tested at first occasion (occasion a in Table 3). Different lanes represent marker DNA (M, Φ-X DNA digested with *Hae*III), individual Vβ families (1–20), a negative (N) control of Vβ8–Cβ primer without cDNA, and a positive (P) control comprising cDNA from Jurkat cells with a Vβ8–Cβ primer combination. A positive signal in varying intensity is observed for every Vβ family assessed. Apart from intense family-specific product, non-specific PCR products are visible.

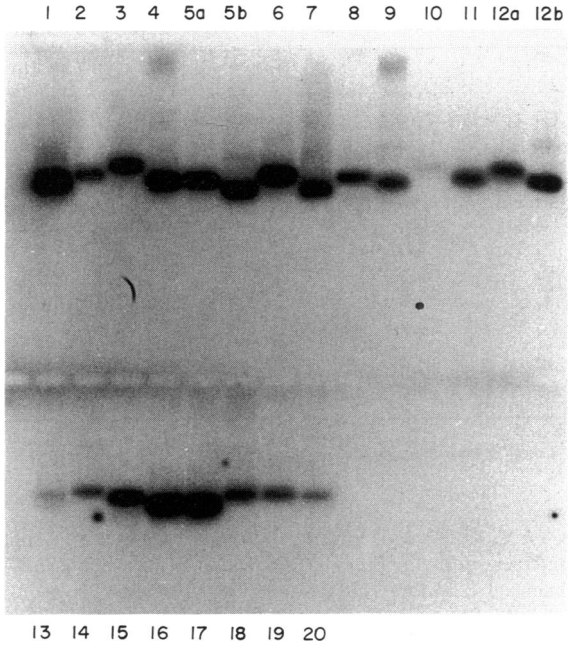

Fig. 3. Positive identification of PCR products by hybridization on dried gel with an internal oligonucleotide Jβ probe labelled with ^{32}P-γATP. The bands identified coincide with those of ethidium bromide staining (Fig. 2). All families but Vβ10 show a strong hybridization signal.

DISCUSSION

We have described a PCR for the assessment of TCR Vβ variable gene families at the mRNA level in heterogeneous cell populations. The reproducibility of the method is acceptable: when tested at different occasions, a similar banding pattern with similar intensities for various family-specific bands was found (data not illustrated). The approach followed is similar to that used by another group of investigators [8,9,12], but the composition of the oligonucleotide primers chosen is different, as well as the way in which the PCR product is detected. Due to the detection of the PCR product by chemical staining, primer–primer associations and other non-specific bands are observed (Figs 1 and 2), but this did not disturb the reading of the family-associated PCR product. Family-specific bands were identified in a positive way by hybridization with an internal Jβ oligonucleotide probe. The conditions of the method were chosen in such a way that all 20 families can be assessed, irrespective of the dependency of PCR conditions on factors like nucleotide composition of the primers and size of the fragment to be amplified. This use of the same conditions in PCR amplification of each Vβ family theoretically affected for instance the detection of Vβ10 and Vβ19, because the family-specific primers contained relatively low numbers of cytidine and guanidine. Generally the signal after PCR amplification for these families was rather low (Table 3). The size of PCR product of Vβ gene families after electrophoresis varied (Table 1), which is explained by the different location of Vβ primer in the V region of individual families. This variation adds to the specificity of the analysis shown by hybridization with an internal oligonucleotide probe.

This specificity was further indicated by the results with T cell lines. For instance, Jurkat and HSB cell-derived RNA (Fig. 1) manifested the presence of only one family out of the total series: for Molt-3 two families were detected and for Molt-4 cells three families. Both lines manifested Vβ2 and Vβ12a, which is in accord with the common origin from one patient [22]. There are several explanations for the presence of two families (Molt-3) and three families (Molt-4): it can be ascribed to (i) cross-hybridization; (ii) the rearrangement and transcription into RNA of both chromosomes in a single cell; and (iii) an oligoclonal and not monoclonal character of the cell lines. In this respect, it is worth mentioning that Southern blot analysis of these lines with a Cβ2 probe revealed two rearranged bands, and with a Jβ2 probe three bands [22].

The sensitivity of the PCR method was studied using mixtures with defined cell numbers of cell lines. The three combinations of lines tested yielded similar results. The lowest percentage at which a distinct family can be detected in a mixture of cells is between 0·1% and 0·5%. This is more sensitive than rearrangement studies with Southern blotting to detect clonal populations in heterogeneous populations (1–5%). The sensitivity is comparable to that of, e.g. flow cytometry in detection of marker-expressing cells. Since Molt-3 cells expressed two families, their mixtures with HSB cells enabled the direct comparison of sensitivity for Vβ2 and Vβ12a. The higher sensitivity observed for Vβ2 (Table 2) can be ascribed to PCR conditions being more favourable for Vβ2 than for Vβ12a, but it can also be ascribed to the difference in relative presence of single clones expressing Vβ2 and Vβ12a.

In peripheral blood samples from four donors tested on three occasions, all 20 Vβ families were present at the mRNA level (Table 3, Figs 2 and 3). These data differ from data coming from specified mouse strains, where deletions in the Vβ family spectrum occur related to the haplotype of major or minor histocompatibility antigens or presence of superantigens like Mls^a [6,7]. Apparently clonal deletion and expansion in shaping the T cell repertoire in humans has no overt effect resulting in the deletion of complete families. This may be related to the outbred genetic constitution of human individuals.

Using MoAbs, percentages of lymphocytes expressing Vβ family-associated epitopes were assessed. All families detected were present for each donor (Table 4). The donors differed in the percentages found and the variation between repeated analyses of single donors was low. The largest variation was observed between individual families (Table 4).

The PCR method described presents a specific, sensitive and reproducible but not a quantitative approach for the assessment of Vβ gene families in heterogeneous cell populations like peripheral blood mononuclear cells. Using this method, all Vβ families proved to be present in healthy adults. For some families antibodies to family-associated epitopes were available and applied in flow cytometry. Also in this analysis all detectable families were expressed in blood of healthy adults. The present approach opens the possibility to study TCR variable gene family expression in relation to physiology and pathologic processes.

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Table 3. Expression of T cell receptor Vβ family genes at the mRNA level in peripheral blood lymphocytes of healthy blood donors

Donor	1	2	3	4	5a	5b	6	7	8	9	10	11	12a	12b	13	14	15	16	17	18	19	20
A a	+++	+++	+++	+++	+++	+++	++	+	+	+++	+	++	+++	+++	+++	+++	++	++	++	++	+	+
b	++	++	++	+++	+	+	+	±	+	+	+	+	+	++	+	+	+	+	++	+	+	+
c	++	+	+	++	++	++	++	+	+	++	−	+	+	+	+	+	+	+	+	+	+	+
B a	++	+	++	++	++	++	+	+	+	++	+	++	++	++	+++	++	++	++	++	++	+	++
b	++	+++	++	+++	++	+++	+	+	±	+++	±	++	++	++	++	++	++	++	++	++	+	++
c	++	+	+	++	+	++	+	+	+	++	±	++	+	++	+	+	+	+	++	+	+	++
C a	+++	+	++	+++	++	++	+	+	++	++	+	++	++	++	+	++	++	++	++	++	+	++
b	+	++	+	+++	++	++	++	+	++	++	+	++	++	++	+	+	+	++	++	+	+	+
c	++	++	+	++	++	+++	++	+	+	++	+	++	++	++	+	+	+	++	++	+	+	++
D a	++	+	++	+++	++	+	+	+	+	++	+	+	+	+	+++	++	++	++	++	++	+	++
b	++	++	+	+++	++	++	++	++	±	++	+	++	++	++	+	+	+	++	++	+	+	+

* Donors A, B, C, D were assessed at three occasions: a; b, 4 months after a; and c, five months after a.

Table 4. Percentages of lymphocytes in peripheral blood mononuclear cells expressing Vβ gene family determinants in healthy blood donors

Donor	5.1	5.2+5.3	5.3	6.7	8	12
A b	5·0	11·7	11·1	4·9	8·6	2·6
c	4·6	12·0	3·9	5·1	11·4	2·0
B b	3·8	7·1	1·4	3·9	10·7	0·8
c	4·4	9·0	2·6	5·8	11·5	2·4
C b	3·7	7·7	3·5	1·9	9·1	1·1
c	5·3	6·8	3·1	4·4	6·3	2·3
D b	6·0	10·1	3·9	2·3	9·2	2·1
c	3·7	10·9	4·3	4·6	37·4	0·2

Data presented is percentage of $CD3^+$ cells. Donors A, B, C, and D were assessed at the same occasions (b and c) as the analysis of Vβ gene family by PCR (Table 3). The Vβ5.1 determinant is encoded by the Vβ5a segment, and the Vβ5.2 and Vβ5.3 determinants by the Vβ5b segment (Table 1).

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