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Population study of T cell receptor Vβ gene usage in peripheral blood lymphocytes: differences in ethnic groups

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SUMMARY

The T cell receptor (TCR) Vβ repertoire in peripheral blood lymphocytes (PBL) of a large number of healthy individuals was analysed by quantifying Vβ-specific mRNA using the method of anchored multiprimer DNA amplification and a reverse dot blot assay. Among 16 Vβ gene families examined, particular Vβ genes were noted to be unequally expressed in the PBL of 70 healthy donors. The frequently used genes belong to the Vβ 4, 5, 6, 8 and 13 (12) families, while Vβ 1, 9 and 15 were the least frequently used gene families. This bias in gene usage was observed in all individuals. Marked deviation from the mean percentage usage was noted for some Vβ genes in individuals when their PBL were examined serially, but the common pattern of biased usage was not grossly distorted. When the TCR repertoire of different ethnic groups was examined, a lower mean frequency of Vβ 3.2 was seen in the repertoire of 19 Caucasians compared with 25 age-matched Samoans ($P < 0.003$). Conversely, the expression of Vβ 5.1 and of Vβ 5.3 was higher in Caucasians than in 51 age-matched Polynesians (Maoris and Samoans, $P < 0.003$). Considering the 20% co-efficient of variation in the estimate of Vβ gene usage, our data from 70 unrelated individuals suggest that in PBL, individual variations in the TCR repertoire were superimposed upon a common biased usage of Vβ genes in the general population.

Keywords T cell receptor TCR Vβ gene usage Polynesian

INTRODUCTION

The repertoire of T cell responses is derived from the rearrangement of the α, β, γ and δ T cell receptor (TCR) genes. The number of TCR genes, their V(D)J gene families and subfamilies, as well as the variability of recombination sites provide for a large rearranged repertoire [1]. However, not all possible recombinations are expressed in mature T cells. Precursor T cells with rearranged TCR genes are positively selected during their development in the thymus if their TCR were capable of recognizing antigen presented by self-MHC. There is evidence that those T cells which are potentially autoreactive are deleted and fail to leave the thymus [2]. As this selection is influenced by self-MHC, individuals of different MHC haplotypes may be expected to select for discordant sets of TCR gene rearrangements. The expressed T cell repertoire is also affected by environmental factors such as antigens presented by MHC molecules to T cells [3]. In addition, superantigens in combination with MHC are known to activate and expand T cell clones which bear particular TCR Vβ chains [4]. It is thus not surprising that identical twins with the same TCR and MHC genes express non-identical TCR repertoires [5,6]. Diverse individual patterns of gene usage are therefore generated and presumably contribute to individual differences in immune responses or susceptibility to disease [7,8]. As populations exposed to the same environmental factors but with differing MHC haplotypes may express dissimilar T cell repertoires, the selection of specific Vβ genes by distinct ethnic groups could explain in part ethnic predisposition to immune-mediated disease [9,10].

In this study we describe the TCR Vβ gene usage in the peripheral blood of healthy individuals and in the Polynesian and Caucasian populations in New Zealand. As a direct method of measuring Vβ-specific TCR-bearing T cells is limited by the lack of MoAbs for most Vβ chains, Vβ-specific mRNA levels were quantified [11]. The rapid analysis of the Vβ repertoire in a large number of individuals is now possible with the Fraser modification of the anchored polymerase chain reaction assay. The Vβ repertoires of 70 individuals from three ethnic groups were not identical, but individual variations were superimposed upon a common biased usage of Vβ genes by peripheral blood T cells in all healthy individuals.

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SUBJECTS AND METHODS

Subjects

Twelve unrelated healthy volunteers were recruited from staff and students of the Auckland Medical School (Auckland, New Zealand). To compare Vβ gene usage between ethnic groups, 70 unrelated, healthy, senior High School student volunteers were recruited. They were thus of the same age group and lived in the same vicinity of Auckland. Their blood samples were drawn within a period of 2 months in the summer. This sampling involved 19 Caucasian, 26 Maori and 25 Samoan students. Approval for this study was granted by the Auckland Area Health Board Ethics Committee.

Preparation of RNA

Peripheral blood lymphocytes (PBL) were separated from 7 ml of venous blood on Ficoll–Hypaque gradients. PBL were lysed with guanidinium thiocyanate/SDS and total RNA was obtained by acid phenol and chloroform extraction [12]. To remove residual DNA, the RNA preparation was treated with 0·5 U of RNase free DNase (Promega). Following phenol-chloroform extraction and ethanol precipitation, the RNA was resuspended in 30 μl of diethyl pyrocarbonate (DEPC)-treated water and stored at −70°C. Vβ mRNA levels in these samples were quantified using a modification of the anchored multi-primer amplification method of Hudson *et al.* [11].

Preparation of TCR Vβ cDNA

cDNA was prepared from 10 μl of RNA using 0·5 μg of RNAse-free oligo dT15 and 100 U of murine-Maloney leukaemia virus (M-MLV) reverse transcriptase (BRL). Template for DNA amplification was prepared by heating the reaction for 5 min at 100°C in the presence of 5 μg DNase-free RNase and 1 μg of redundant oligonucleotide primers which hybridize to a conserved region in all TCR Vβ cDNA transcripts. These primers 5′-GACTAGTITI(CT)TGGTA(CT)(AC)(AG)(AGT)(AC)AG, 5′-GACTAGTITI(CT)TGGTA(CT)(AC)(AG)(AG)CAA, and 5′-GACTAGTITI(CT)TGGTATCGACGT (for Vβ 16) were used at a mixture of 48:16:1 respectively. The mixture was then cooled in wet ice, 100 U of M-MLV reverse transcriptase were added and incubated for 30 min at 37°C. Excess primers were removed from the reaction using the Magic DNA clean-up system (Promega).

DNA amplification

The two primers used to amplify DNA were 5′-GTGACTAGTCTG(CT)TGGTA, which is homologous to the 5′ end of the redundant oligonucleotides, and the primer 5′-TCAGGCAGTATCTGGAGTCA, which is complementary to a sequence 200 bp into the TCR Cβ gene. Samples of 50 μl containing 50 mM KCl, 10 mM Tris (pH 8·4), 1·5 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol (0·2 μM final) of each primer, 5 μl of template and 1 U of Taq polymerase (Boehringer) were amplified for 30 cycles in a Perkin Elmer-Cetus Thermocycler under the following conditions: 94°C denaturation for 30 s, annealing of primers at 55°C for 120 s, and extension of primers at 72°C for 60 s. The amplified Vβ-Dβ-Jβ-Cβ product of approximately 500 bp was purified by 1% agarose gel electrophoresis and labelled with ^{32}P-dCTP (Multiprime DNA; Amersham) by random priming.

Reverse dot blot assay

Vβ, Cβ 1 and Cβ 2 gene sequences from human TCR cDNA clones were subcloned into pBluescript (BS)(Stratagene) and sequenced to confirm their identity. Vβ nomenclature is as defined in previously published reports by Concannon *et al.* [13] and Kimura *et al.* [14]. Vβ 13.2 and Vβ 14.1 have also been called Vβ 12.3 and Vβ 3.3 respectively by Toyonaga & Mak [1]. These plasmids, in 1-μg aliquots, were immobilized on nylon filters (Hybond N^+; Amersham) using a dot blot apparatus (Bio-Rad) according to the manufacturer's instructions. BS was immobilized on each strip as a negative control. A mixture of plasmids containing Cβ 1 and Cβ 2 sequences was also included so that Vβ gene usage could be calculated as a percentage of Cβ. The test strips were prehybridized at 65°C for 1 h with 5 × SSPE, 5 × Denhardt's solution, 0·5% (w/v) SDS and 20 μg/ml salmon sperm DNA (1 × SSPE = 0·18 mol/*l* NaCl, 10 mmol/*l* sodium phosphate, pH 7·7, and 1 mmol/*l* EDTA). Before being added to the prehybridization solution, the labelled product was denatured by heating to 100°C for 5 min. Following hybridization overnight, the test strips were washed twice in 2 × SSPE/0·1% SDS at room temperature for 10 min, and once at high stringency in 0·2 × SSPE/0·1% SDS at 65°C for 20 min. Test strips were exposed for 2 h to Kodak X-Omat AR film before the dots were counted in a liquid scintillation counter. The data for each Vβ family were expressed as a percentage of Cβ counts ((Vβ ct/min − BS ct/min)/(Cβ ct/min − BS ct/min) × 100) or as a percentage of total Vβ counts recovered ((Vβ ct/min − BS ct/min)/Σ(Vβ ct/min − BS ct/min) × 100).

Monoclonal antibodies and flow cytometry

Staphylococcus aureus-derived toxic shock syndrome toxin (TSST) was obtained from Toxin Technology (Madison, WI).

Five murine MoAbs specific for the human variable region of the TCR β chain were used. MPB2/C11 (reactive with Vβ2) and 42/1C1 (reactive with Vβ 5.3) were a gift from A.W. Boylston [15]. LC4 (reactive with Vβ 5.1) was a gift from R. Levy [16]. C305 (reactive with Vβ 8.1) was a gift from A. Weiss [17]. S511 (reactive with Vβ 12) was a gift from D.N. Posnett [18]. The anti-CD3 directly conjugated to PE (anti-CD3-PE) and fluorescein-conjugated $F(ab')_2$ fragments of sheep anti-mouse immunoglobulin were obtained from Silenius Laboratories (Hawthorne, Australia).

PBL were cultured at 2×10^6 cells/ml in RPMI medium supplemented with 10% fetal calf serum (FCS) in 50-ml tissue culture flasks and stimulated with either phytohaemagglutinin (PHA) at 1 μg/ml or bacterial enterotoxins at 1 ng/ml. After 5 days, viable cells were harvested on a Ficoll–Hypaque density gradient. Cells were kept at 4°C throughout. They were incubated for 30 min with a TCR-specific antibody in PBS supplemented with 3% AB serum. After three washes they were incubated with fluorescein-conjugated $F(ab')_2$ fragments of sheep anti-mouse immunoglobulin for 20 min. Following a further three washes, anti-CD3-PE was added and the cells were incubated for 20 min. They were washed three times and analysed in a FACS 440 (Becton Dickinson, Mountain View, CA) and a MicrovaxII computer using Consort 40 software (Becton Dickinson). A total of 2×10^6 cells per sample were analysed and the dead cells were gated out by staining with propidium iodide. The fluorescein and PE background was less than 0·5%.

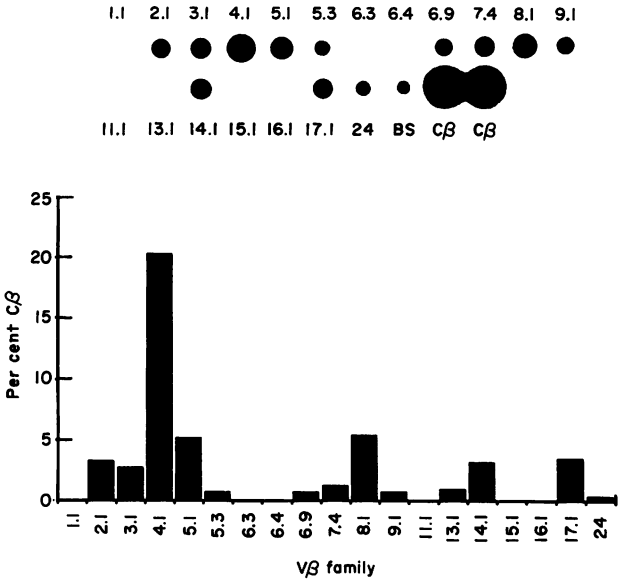

Fig. 1. T cell receptor (TCR) Vβ gene usage in peripheral blood lymphocytes (PBL) of a healthy individual. The autoradiograph shows Vβ-specific dots for Vβ 1.1 through to Vβ 24 (in the order as shown in the graph) beginning from the left of the top row. The dot labelled BS is the Bluescript control. The last two dots of the lower row are duplicates of Cβ. Vβ gene usage was calculated as the percentage ratio of amplified Vβ:Cβ mRNA.

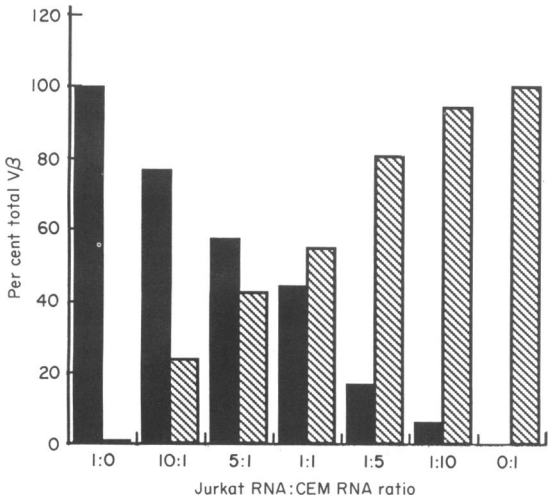

Fig. 2. Relative Vβ mRNA levels from Vβ 8 Jurkat (■) and Vβ 9 CEM (▨) RNA mixed at varying ratios. Vβ mRNA levels were calculated as a percentage of total Vβ counts recovered.

Statistical analysis

All amplified cDNA was analysed for TCR Vβ expression in triplicate. TCR Vβ expression in the three ethnic groups was analysed by multiple analysis of variance (MANOVA) using the SAS statistics program (SAS Institute Inc., Cary, NC).

RESULTS

The TCR reverse dot blot assay

The expression of TCR Vβ genes in PBL was measured following the single tube anchored multiprimer amplification of cDNA in a reverse dot blot assay [11]. The variable expression of individual Vβ gene families is shown by the different intensities of the dots on the autoradiograph in Fig. 1. The Vβ gene usage was expressed as a percentage of Cβ, and a profile of the repertoire is shown in the histogram. The total ct/min for the 16 Vβ gene families that were measured amounted to approximately 50% of total Cβ ct/min. When corrected for the known discrepancy in the G/C content of the amplified Vβ and Cβ products, the total Vβ gene usage in this analysis corresponded

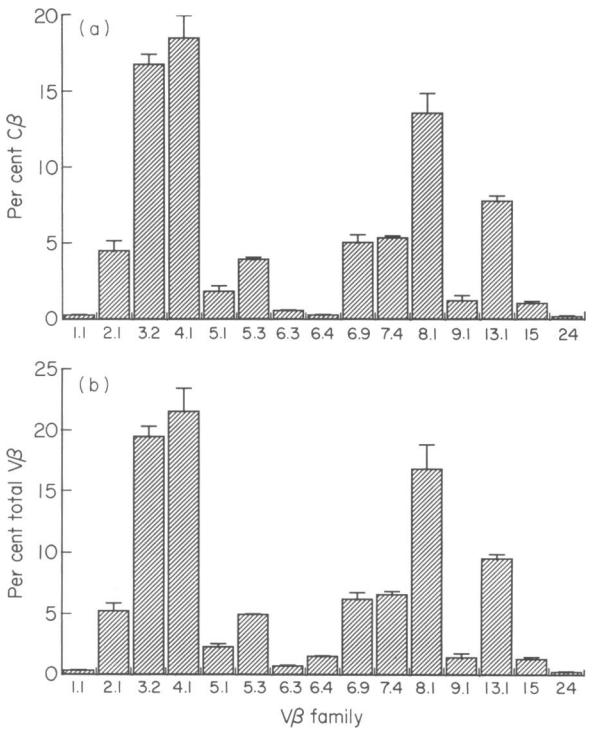

Fig. 3. Reproducibility of T cell receptor (TCR) Vβ gene usage analysis. Vβ usage was assayed six times in blood drawn from one donor. Vβ usage was expressed as (a) percentage of total Cβ, and (b) percentage of total Vβ counts recovered. Error bars represent s.e.m.

Table 1. Biased Vβ gene usage in stimulated T cells*

	PHA		TSST	
Vβ family	FACS Per cent $CD3^+$	PCR Per cent Cβ	FACS Per cent $CD3^+$	PCR Per cent Cβ
2	11·5	5·7	43·9	27·6
5.1	5·4	5·6	2·6	2·8
5.3	7·6	7·8	0	0
8	5·7	11·4	0·1	0·5
12	4·2	3·3	0	0

* PBL were cultured with phytohaemagglutinin (PHA) or toxic shock syndrome toxin (TSST). Vβ gene usage was expressed as the percentage of $CD3^+$ cells by flow cytometry and as the percentage ratio of Vβ-specific mRNA to Cβ mRNA.

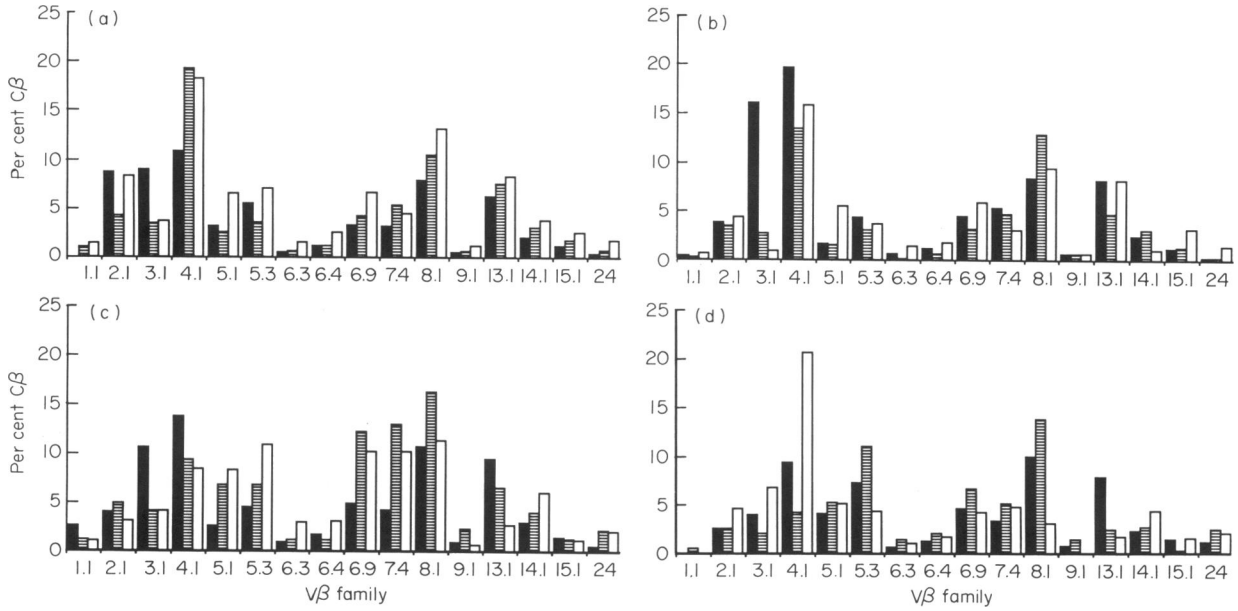

Fig. 4. Serial assays of Vβ usage in peripheral blood lymphocytes (PBL) from four healthy individuals. ■, Initial sample; ▤, after 5 weeks; □, after 8 weeks.

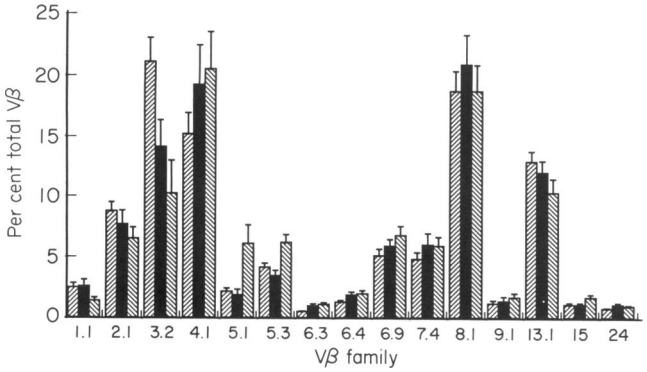

Fig. 5. Vβ gene usage in peripheral blood lymphocytes (PBL) of healthy age-matched individuals from three ethnic groups. Error bars indicate s.d. ▩, Samoan; ■, Maori; ▧, European.

to approximately 75% of Cβ, consistent with the fact that not all specific Vβ gene plasmids were present on the dot blots.

Using this method, two human T cell lines, Jurkat and CEM, were separately analysed for Vβ expression. Jurkat was 100% Vβ 8, while CEM was 100% Vβ 9 (data not shown). To confirm that this assay accurately reflected the levels of Vβ mRNA transcripts in the original RNA, RNA extracted from these two cell lines were mixed at varying ratios and the corresponding ratios of amplified Vβ 8 to Vβ 9 were calculated. For this comparison, Vβ usage was expressed as a percentage of total Vβ counts recovered. The results in Fig. 2 show a linear relationship between the percentage of TCR Vβ detected and the proportion of each Vβ RNA specie in the mixture.

The reproducibility of the technique was determined by analysing RNA from six blood samples drawn from the same person at the same time. Highly reproducible profiles for Vβ gene usage were obtained, whether expressed as per cent of Cβ (Fig. 3a) or as per cent of total Vβ counts recovered (Fig. 3b). The mean co-efficient of variation was 20% for values of Vβ expression greater than 1%. Values below 1% were outside the sensitivity of the method. These results indicate that there was no significant bias in the extraction of RNA, synthesis of cDNA, amplification and quantification. In addition, the Vβ profiles from either RNA or cDNA stored at −70°C for at least 9 months (not shown) were identical with those obtained from fresh samples.

Vβ usage and flow cytometry analyses of a less frequently used Vβ gene family

To demonstrate that a low frequency gene family could be amplified, and also compare the dot blot analysis with flow cytometry measurement of Vβ-bearing T cells, the repertoire of cultured PBL stimulated with TSST was analysed. This toxin is known to stimulate Vβ 2-bearing T cells [4]. As shown in Table 1, TSST stimulation led to the high usage of Vβ 2 as detected by the anchored multiprimer amplification. Using a limited number of MoAbs, a corresponding high proportion of Vβ 2-bearing T cells were detected in TSST-stimulated PBL. In contrast, low levels were noted as expected for Vβ 5.1, 5.3, 8 and 13 (also known as 12.3) in the dot blot assay and with the use of Vβ-specific MoAbs. When PHA was used to stimulate PBL from the same individual, the relative Vβ family frequencies were more comparable to an unstimulated Vβ repertoire. Both assays gave similar but not identical results, presumably because the specificities of the MoAbs (MPB/C11 and C305) for individual Vβ 2 or Vβ 8 family members respectively, differed from those detected by the Vβ probes.

TCR Vβ gene usage in healthy individuals

To examine the variation in TCR gene usage over time within the same individual, PBL from four healthy donors were assayed on three occasions over a period of 2 months. As shown in Fig. 4, the variable usage of Vβ gene families is not random. In each case, Vβ 4, 5, 6, 8 and 13 (12) were the most commonly used families (approximately 10% of Cβ), whereas Vβ 1, 9 and 15

were infrequently expressed (<2·5% of Cβ). Within the Vβ 6 gene family, 6.9 was always more frequently used than 6.4 or 6.3 by all four individuals on all three occasions.

From these studies, a clear pattern of Vβ usage emerged that appeared to be independent of the individual. Deviations from the profile were noted for individual gene families over the 2-month period. For instance, individual D had a markedly elevated Vβ 4 level at 8 weeks and a correspondingly low level of Vβ 8.1 (Fig. 4). However, the infrequently expressed families (Vβ 1, 9 and 15) were never used to the same extent as the high frequency families. On occasions, usage of a moderately frequent family such as Vβ 3.2 did show an increase from the usual 5% to exceed 10% or 15%. Conversely, the usual frequency of Vβ 13.2 (12) at 10% was noted to fall below 2·5% on three occasions in two individuals.

Population analysis of TCR Vβ gene usage

To compare Vβ gene usage in individuals of different ethnic backgrounds, PBL from unrelated, healthy high school students from three ethnic groups were analysed. The same common variable usage of frequent *versus* infrequent gene families was conserved in the analysis of 70 individuals from the three ethnic groups (Fig. 5). However, one Vβ family, Vβ 3, appeared to be significantly different between the three races. This difference was well outside the coefficient of variation. Vβ 3.2 expression was higher in Samoans (21·1±9·5%) than in Caucasians (10·2±11·9%; $P<0.003$) and conversely the expression of Vβ 5.1 and Vβ 5.3 genes was higher in Caucasians (6·1±6·8% and 6·2±2·8% respectively) than the Samoans (2·2±1·6% and 4·1±2·1%) or Maoris (1·8±2·6 and 3·5±2·4%; $P<0.003$). These data indicate that while most of the Vβ families were used at very much the same frequencies irrespective of ethnic origin, both Vβ 3 and Vβ 5 expression were exceptional.

DISCUSSION

Several strategies using the polymerase chain reaction (PCR) to amplify cDNA from reverse transcribed RNA have been used to study TCR Vβ gene expression [4,5,19,20]. The need to rely on multiple primers, multiple reactions or extensive sequencing has made precise measurement with these techniques difficult for studying large numbers of samples. In this study we have adapted and validated the single tube approach of Fraser [11] for use in population studies.

A feature of this assay was the use of redundant primers to obtain the repertoire of Vβ-specific cDNA templates, but only one pair of primers was used in a single PCR reaction to amplify DNA [11]. Possible bias from variable primer efficiency or multiple PCR reactions was thus eliminated [19]. The efficacy of the technique was confirmed by proportional mixing of two RNA species from the Jurkat and CEM T cell lines. When used to analyse human PBL, every Vβ family RNA specie on the dot blot has been detected in screening more than 100 individuals, indicating that where there is low Vβ gene usage, this is not due to failure of the redundant oligonucleotides to prime the mRNA. Although Vβ9 was infrequently used in PBL, it was readily detected as the only Vβ gene used by the CEM cell line. We have also shown that Vβ 2, a low frequency gene family, was easily detected at high frequency if PBL were cultured with the TSST superantigen. This high usage of Vβ 2 following TSST stimulation was comparable to the high proportion of Vβ 2-bearing T cells. However, as emphasized in the first description of the assay, Vβ gene expression was quantified essentially as the relative ratio of Vβ mRNA to Cβ mRNA, and did not indicate the percentage of Vβ-bearing cells. Finally, the reproducibility of the technique was demonstrated by independently analysing Vβ gene usage in six blood samples drawn from the same individual at the same time. In this last experiment we determined the co-efficient of variation of this assay to be 20%.

The usage of particular Vβ genes in an individual repertoire was not random. When Vβ usage was analysed in a large number of individuals, a remarkable similarity in the variable usage of Vβ gene families was observed. All individuals expressed a common pattern of usage in which some gene families predominate. Although we have not measured all known Vβ families, this pattern of usage appears to result from the frequent use of the Vβ 4, 5, 6, 8 and 13 (12) and infrequent use of Vβ 1, 9 and 15 gene families. Rosenberg and colleagues described the variable usage of Vβ genes in five outbred northern European individuals and a pair of identical twins, and showed a pattern of variable usage very similar to our healthy subjects [5]. Malhotra *et al.* analysed PHA-stimulated PBL of 19 individuals from three pedigrees and also noted a basic trend in which Vβ 1, 2, 6, 7, and 13 (12) were the most frequently used and Vβ 10 through to Vβ 19 were the least frequently used genes [21]. This bias is partly explained by the relative size of some Vβ gene families such as the Vβ 6 family [1]. Variation in usage of particular Vβ genes may also be due to polymorphisms. In their three human pedigrees, Malhotra *et al.* identified two alleles of Vβ 20 [21]. Low, intermediate or high expression of Vβ 20 depended on the homozygosity or heterozygosity for the alleles. The selection of some Vβ genes may provide another explanation for their biased usage. The Vβ framework structure is known to affect the positioning and thus the antigen-binding characteristics of the third complementarity determining region (D-J) of the TCR [22]. Some Vβ genes may therefore be more frequently selected by common factors such as ubiquitous antigens, self-antigens or superantigens.

In murine strains, Vβ genes are expressed unequally [23]. Their peripheral T cell Vβ repertoire is influenced by Mls/IE related decisions. However, for all Mls strains, some Vβ genes like Vβ 8.2 are used more frequently than others. It has also been noted that CD4/CD8 double negative thymocytes express Vβ genes unequally, indicating that high Vβ 8.2 usage occurs before thymic selection. Usage of other Vβ gene families in CD4/CD8 double negative thymocytes is more uniform, as expected of a repertoire before selection [23]. In contrast, when Vβ-specific RNA was examined in human fetal thymocytes, Vβ usage by CD4/CD8 double negative cells was reported to be similar to the more mature CD4 or CD8 positive thymocytes, indicating the absence of major Vβ-dependent deletions [24]. Hawes *et al.* have recently reported the differential usage of V gene segments in $CD4^+$ and $CD8^+$ subsets of T lymphocytes in monozygotic twins [25]. Usage of Vα 11, 17, 22 and Vβ 3, 9, 12, 18 were skewed to the $CD4^+$ subset, whereas Vβ 2, 6, 12, 15, 20 and Vβ 7, 14, 17 were skewed to the $CD8^+$ subset.

Deviations from the common pattern of usage did occur in individuals when they were analysed over 2 months in our studies. This usually affected a few gene families and did not radically perturb the rest of the repertoire, so that the underlying pattern of a common biased usage remained evident. Vβ mRNA levels can be expected to fluctuate in time, as certain clones of T

cells are activated in immune responses to antigens or superantigens. However, the normal immune response involves a relatively small number of T cell clones in PBL and the resting profile is not lost. Increased usage of some V gene families has been documented in disease states, but this relates to the T cell repertoire in tissues at the site of the lesion such as brain [26], synovium [27,28] or endocrine organs [29].

The pattern of biased usage of Vβ genes in three ethnic groups was remarkably similar. However, Samoans had a higher mean frequency of Vβ 3.2 usage compared with Caucasians, and the latter had a higher mean frequency of Vβ 5.1 and Vβ 5.3 usage than the two Polynesian races. We are presently investigating the role of MHC haplotypes in determining this ethnic variable usage, as preliminary data suggest that the predominance of Vβ 3.2 in Samoans is MHC-dependent. Using a restricted number of Vβ and Vβ-specific MoAbs, Gulwani-Akolkar and colleagues demonstrated the influence of human leucocyte antigens (MHC) on Vβ gene usage in family studies [6], and more recent results with monozygotic twins also suggest that the predominant influence shaping the repertoire is genetically predetermined [25]. Conversely, it was also shown that T cells expressing Vβ 8 did not predominantly recognize HLA-DR2 alloantigen [30]. It was perhaps surprising that more significant differences in the Vβ repertoire were not evident between the ethnic groups, as the gene frequency of DR 1, 2, 3, 7 and 10 are known to be significantly lower in Maoris than Caucasians [31].

Our data of Vβ gene usage analysis of a large number of individuals from different ethnic groups suggest that a 'normal repertoire' exists in PBL within the apparent 'chaos' of variable usage of Vβ genes in individuals. The repertoire oscillates around a constant mean percentage usage for most Vβ gene families and subfamilies in health. Significant deviations from the mean in a restricted number of specific Vβ genes are presumably part of the normal immune response. Significant deviations from the common profile of biased usage may well indicate a major disorder, as exemplified in the extreme by the emergence of a malignant clone of T cells.

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