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Differential usage of T-cell receptor Vβ gene families by $CD4^+$ and $CD8^+$ T cells in patients with $CD8^{hi}$ common variable immunodeficiency: evidence of a post-thymic effect

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SUMMARY

In this study, we report that differences between T-cell receptor (TCR) Vβ gene family usage in $CD4^+$ and $CD8^+$ T cells are significantly greater in a subgroup of patients with common variable immunodeficiency (CVI) and high levels of activated $CD8^+$ T cells ($CD8^{hi}$ CVI) than in controls ($P < 0.001$). In $CD8^{hi}$ CVI patients, such differences were also significantly greater for Vβ12 than for other Vβ families. As the causes of the differential usage of Vβ gene families by $CD4^+$ and $CD8^+$ T cells are under investigation, it was interesting that the combined differences between Vβ gene family usage in the $CD4^+$ and $CD8^+$ T-cell subpopulations as a whole were significantly lower than the combined differences between individual Vβ gene family usage in either $CD4^+$ or $CD8^+$ T-cell subpopulations ($P < 0.001$ in both control and $CD8^{hi}$ CVI patients). Further, the pattern of Vβ gene family usage in $CD4^+$ T cells was remarkably similar to that in $CD8^+$ T cells in both groups. These data strongly suggest that differences in Vβ gene family usage arising from co-selection by major histocompatibility complex (MHC) class I versus MHC class II restriction elements do not fundamentally distort 'basic' Vβ gene family usage patterns. They also support the concept that differences in $CD4^+$ and $CD8^+$ T-cell Vβ gene family usage, which were increased in $CD8^{hi}$ CVI, can arise from high-affinity interactions between disease-associated antigens or superantigens and T cells in the post-thymic T-cell compartment.

INTRODUCTION

Differences between T-cell receptor (TCR) Vα or Vβ gene family expression in mature $CD4^+$ and $CD8^+$ T cells probably arise because thymic selection mechanisms responsible for such expression rely on interactions between the TCR and self-peptide/major histocompatibility complex (MCH) restriction elements that differ for the two cellular subtypes. $CD4^+$ T cells are selected by peptides seen in the context of MHC class II elements, and $CD8^+$ T cells are selected by peptides seen in the context of MHC class I elements.[1–8] Direct evidence in support of this mechanism is inherent in the finding that the pattern of differences between $CD4^+$ and $CD8^+$ T-cell Vβ gene family expression were more constant among monozygotic twins than among unselected individuals.[9]

Another possible explanation of the differences between mature $CD4^+$ and $CD8^+$ T-cell Vβ gene family expression — one not exclusive of the possibility described above — is that environmental factors, i.e. antigens or superantigens, act on $CD4^+$ and $CD8^+$ T cells previously selected in the thymus to bring about expansion of certain Vβ gene families in either the $CD4^+$ or $CD8^+$ T-cell subsets. Support for this hypothesis comes from another study on monozygotic twins[10] in which it was found that although $CD4^+$ Vβ expression was highly concordant between twins, differences were noted for Vβ expression within the CD8 subset. Such differences were noticed between healthy twins, but there were increased differences between twins where one individual had an underlying disease. Because T cells from monozygotic twins are most probably influenced by the same thymic selection and genomic effects, this suggests that environmental factors influence the TCR repertoire of mature $CD8^+$ T cells.

Another way to test whether differences between $CD4^+$ and $CD8^+$ T-cell Vβ gene expression are influenced by environmental factors is to define such expression in disease states where it can be assumed that there is preferential activation of $CD4^+$ or $CD8^+$ T cells due to exposure to one or more antigens or superantigen. In such cases, one would assume that, if environmental influences were shaping the V gene repertoire, differences between $CD4^+$ and $CD8^+$ T cell Vβ gene family

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Table 1. Patient characteristics

	CD4		CD8	
	%	/μl	%	/μl
Patient 1	28·8	574	66·2	1319
Patient 2	10·7	161	71·0	1069
Patient 3	27·7	247	46·0	409
Patient 4	25·7	406	52·0	835
Patient 5	23·8	521	58·8	1288
Controls	32·6–58·9	480–1339	17·8–46·7	351–911

expression would be more pronounced in patients than in normal individuals.

In the present study, we took this approach by studying Vβ gene family usage in $CD4^+$ and $CD8^+$ T cells in a previously defined group of patients with '$CD8^{hi}$' common variable immunodeficiency (CVI), who express greatly elevated numbers and percentages of activated $CD8^+$ T cells.[11] We found that these $CD8^{hi}$ CVI patients do display increased differences in Vβ gene family expression in their $CD4^+$ and $CD8^+$ T-cell subsets. Furthermore, while the location of these increased differences generally varied from patient to patient (with respect to Vβ gene family), the $CD4^+/CD8^+$ differential expression of Vβ12 was particularly prominent in this group of patients.

MATERIALS AND METHODS

Patients

$CD8^+$ and $CD4^+$ T-cell subpopulations from five previously characterized patients belonging to a recently defined subgroup of patients with CVI having high $CD8^+$ T-cell levels were studied.[12] This $CD8^{hi}$ CVI subgroup is characterized by a low CD4/CD8 ratio due to an increased absolute number of $CD8^+$ T cells. In addition, $CD8^+$ T cells from these patients manifest an increased expression of HLA-DR and CD57, a decreased expression of CD45RA, and an abnormal pattern of $CD8^+$ T-cell proliferation and cytokine production.[11] All patients were on regular intravenous immunoglobulin therapy (1 month). There was no clinical or laboratory evidence for acute viral infection. Five normal volunteers served as control individuals. The percentage of CD4 and CD8 lymphocytes on total lymhocytes and absolute CD4 and CD8 numbers/μl blood of patients (individual values) and controls (95% confidence intervals) were as outlined in Table 1.

Cell separation

Peripheral blood mononuclear cells (PBMC) were separated from Blood Bank leukapheresis packs obtained from normal volunteer donors or patients by lymphocyte separation medium (LSM; Organon-Teknika, West Chester, PA) density gradient centrifugation. A population of T cells enriched in $CD2^+$ cells was obtained by rosetting with neuraminidase-treated sheep erythrocytes.[13] $CD4^+$ and $CD8^+$ T cells were purified by negative selection using immunomagnetic beads, as described elsewhere.[14] Briefly, rosette-positive T cells at 2×10^7 cells/ml were treated for 30 min at 4° with an antibody cocktail of 10F7 (anti-human erythrocyte glycophorin) 10 μg/ml, 63d3 (anti-CD14) 2 μg/ml, 3C10 (a non-CD monocyte marker) ascites diluted 1 : 1000, THB5 (anti-CD21) 1 μg/ml, 3G8 (anti-CD16) 5 μg/ml and OKT4 (anti-CD4) 4 μg/ml or OKT8 (anti-CD8) 2 μg/ml. The cells were washed twice in coating media consisting of RPMI-1640, 10% fetal calf serum (FCS), 10 mM HEPES and 0·005 M EDTA, and then suspended for 15 min at 4° in coating media containing 70 Advanced Magnetic goat anti-mouse immunoglobulin beads (Advanced Magnetics Inc., Cambridge, MA) per anticipated target cell to be removed. The suspension was then exposed to a magnet to remove the beads and their targets. The remaining bead-negative cells were resuspended at $1-2 \times 10^7$ cells/ml in coating media and subjected to a second round of negative selection using Dynal goat anti-mouse immunoglobulin beads (Dynal Inc., Great Neck, NY) at a ratio of two beads/cell. The resulting $CD4^+$ T-cell populations were > 95% Leu-3^+ ($CD4^+$) and > 97% Leu-4^+ ($CD3^+$); the resulting $CD8^+$ T-cell populations were > 90% Leu-2^+ ($CD8^+$) and > 95% Leu-4^+ ($CD3^+$). Fewer than 1% of cells stained with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse immunoglobulin, Leu-M3 (anti-CD14), Leu-11c (anti-CD16) or B1 (anti-CD20).

RNA extraction

Total cellular RNA was extracted using a modification of the technique of Chomczynski & Sacchi.[15] Briefly, cells were lysed in a denaturing solution (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7, 0·5% sarcosyl, 0·1 M 2-mercaptoethanol) at 10^6 cells/100 μl volume. Then 10 μl 2 M sodium acetate, pH 4, 100 μl phenol (H_2O saturated) and 20 μl chloroform were added to each 100-μl volume of lysis buffer; the mixture obtained was shaken vigorously, and the latter was then cooled on ice for 15 min and centrifuged at 15 000 *g* for 10 min at 4°. The aqueous layer was then isopropanol precipitated, resuspended in lysis buffer, reprecipitated with isopropanol, and washed in 75% ethanol. Finally, the total cellular RNA extracted was pelleted by centrifugation and resuspended. The quality and quantity of total RNA were determined by spectrophotometry. Some samples were isolated using 20 μg of tRNA as carrier, which had been added after the first isopropanol precipitation step.

Reverse transcription (RT)

Total cellular RNA (1 μg/14 μl) from separated $CD4^+$ or $CD8^+$ T cells of individual patients was mixed with 10-fold dilutions of the internal TCR Cβ standard, as described previously.[16] For each experiment, double-distilled (dd) water instead of RNA was used as a negative control. Samples were then heated at 65° for 5–10 min. RT mix (16 μl/μg sample RNA), consisting of 3 μl oligo(dT)16 (0·5 mg/ml; Sigma, St Louis, MO), 150 U M-MLV RT (BRL, Gaithersburg, MD) and 40 U RNasin (Promega, Madison, WI) in 50 mM Tris–HCl, pH 8·3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT and 125 μM each dATP, dTTP, dGTP, dCTP (Boehringer Mannheim, Indianapolis, IN), was then added to achieve a final volume of 30 μl. RT was carried out at 39° for 1 hr, after which samples were heated at 65° for 5–10 min to terminate RT.

Polymerase chain reaction (PCR)

PCR amplification of TCR Vβ cDNA from the various $CD4^+$ and $CD8^+$ T-cell preparations was performed in a manner that

ensured that, in each case, amplification occurred in a linear range. To this end, the total amount of TCR Cβ mRNA was measured, first using a highly sensitive, quantitative RT PCR technique, as previously described.[16] The TCR Cβ mRNA content of the total RNA extracted from $CD4^+$ and $CD8^+$ T-cell samples was then calculated and adjusted to the same basis. Briefly, for each RNA derived from $CD8^+$ or $CD4^+$ T cells, cDNA (5 μl) containing a constant amount of sample RNA and 10-fold dilutions of TCR Cβ internal standard RNA was added to a PCR mix (45 μl) consisting of 1 × PCR buffer [10 mM Tris–HCl, pH 8·3, 50 mM KCl, 1·5 mM $MgCl_2$, 0·01% (w/v) gelatin], 50 pmoles each of the 5′ (AGGTCGCTGTGTTTGAGC-CATCA) and 3′ (ATGGTGGCAGACAGGACCCCTTG) primers, 100 μmoles each of dATP, dTTP, dGTP, dCTP (Boehringer Mannheim), 5 μCi of [^{32}P]dCTP (Amersham, Arlington Heights, IL) and 1·25 U Taq polymerase (Boehringer Mannheim). Tubes were then overlaid with a drop of mineral oil (Aldrich, Milwaukee, WI), and PCR amplification was performed in a DNA Thermal Cycler (Perkin Elmer, Applied Biosystems, Foster City, CA) for 25 sequential cycles at 94° for 45 seconds, 60° for 60 seconds and 72° for 90 seconds, with a 72° extension step for 7 min after the last cycle. Samples were then electrophoresed on a Seakem GTG 3% agarose gel (FMC Bioproducts, Rockland, ME), and the gels obtained were washed in water for 30 min to reduce background. Specific DNA bands were visualized with 1 μg/ml ethidium bromide and cut from the gel together with adjacent appropriate-size gel pieces for background determinations. [^{32}P]dCTP incorporation was determined in a liquid scintillation counter (Beckman, Fullerton, CA). TCR Cβ mRNA content was determined at the equivalence concentration as previously described.[15] Total RNA samples from a single $CD4^+$ or $CD8^+$ T-cell preparation were pooled and diluted according to the calculated differences in TCR Cβ mRNA content. Patient-matched $CD4^+$ and $CD8^+$ T cell cDNA was then amplified within the same PCR run to determine TCR Vβ gene expression. The 22 Vβ primers used were taken from a panel of published sequences[17] that have been modified in order to achieve similar melting temperatures and to include the TCR Vβ 8.3 subfamily. The Cβ primer was chosen to recognize the Cβ1 and Cβ2 sequences 5′(CCTTTTGGGTGTGGGAGATCTCT)3′. Southern hybridization to show specificity of the primers was performed following standard techniques using an internal Cβ probe, 5′(TTCTGATGGCTCAAACACAGCGAC)3′. Finally, quantitative RT-PCR for TCR Vβ families 2, 6, 7, and 14 was performed as described before, using a compound internal standard for TCR Vβ sequences.[15]

Statistical methods

The data were subjected to a three-way analysis of variance using Vβ gene family, T-cell subset ($CD4^+$ or $CD8^+$ cell) and type of subject (CVI patient or control) as factors.[18] The analysis was carried out assuming that the data followed a lognormal rather than a normal distribution. In this way (1) the variances of the mean values representing averages over Vβ gene families were stabilized, (2) additivity in the effects due to subject and T-cell subset and to subject and Vβ gene family was assured (in fact, none of the two-way or three-way interactions was significant at the 20% level), and (3) outlying points in the plot of the residuals were much less numerous and none was significant.

For each patient (or control) and each Vβ, there were two c.p.m. values corresponding to the $CD4^+$ and $CD8^+$ T cells, respectively. The ratio formed by dividing the smaller of these two values into the larger was called the T-cell $CD4^+/CD8^+$ subset distance (TSD) and is a measure of the inequality of the two values. Note that the logarithm of a given subset distance is equal to the corresponding absolute CD4–CD8 difference.

Dixon's test for an outlier was applied to the data shown in Fig. 5.[19]

RESULTS

Overall pattern of Vβ gene family expression in $CD4^+$ and $CD8^+$ T cells

Expression of TCR Vβ gene families 1–20 was measured in purified $CD4^+$ and $CD8^+$ T cells of five normal control individuals and five patients with $CD8^{hi}$ CVI using a semi-quantitative RT-PCR technique (see the Materials and Methods). Figure 1 shows the relative V gene frequencies of the αβ TCR repertoire of the $CD4^+$ and $CD8^+$ T-cell subsets from each of the control individuals and patients, as determined by this method.

As shown in Fig. 2, initial analysis of these data with regard to variability of $CD4^+$ and $CD8^+$ T-cell Vβ gene family usage between individual TCR Vβ families showed that V gene usage frequencies for individual TCR Vβ gene families averaged over controls and patients varied considerably. However, as shown in Table 2, determination of standard deviations demonstrated that this variability was similar in controls and $CD8^{hi}$ CVI patients (0·733 versus 0·707). It is also evident from Fig. 2 that TCR Vβ gene usage frequencies of individual Vβ families followed a pattern that was not only preserved between $CD4^+$ and $CD8^+$ T cells but also between the control and the $CD8^{hi}$ CVI patient groups.

Further analysis of these data with regard to variability of $CD4^+$ and $CD8^+$ T-cell Vβ expression within individual TCR Vβ families showed that these CD4/CD8 T-cell subset differences were considerably smaller than differences in TCR Vβ gene usage frequencies between Vβ families. Thus, comparison of standard deviations generated by these differences (Table 2) showed that the variation between control $CD4^+$ and $CD8^+$ T-cell Vβ values was fourfold smaller than among individual mean Vβ values (0·733 versus 0·162; $P < 0·001$). A similar picture was obtained for $CD8^{hi}$ CVI patients; in this case, while variation in $CD8^{hi}$ CVI Vβ values between $CD4^+$ and $CD8^+$ subsets was greater than corresponding control Vβ values (Table 2; see further discussion below), the former variation was still threefold less than that among individual $CD8^{hi}$ CVI Vβ values (0·707 versus 0·232; $P < 0·001$).

Thus, despite the fact that $CD4^+$ and $CD8^+$ TCR recognize antigen within different MHC contexts, $CD4^+$ and $CD8^+$ T cells displayed a remarkably consistent array of Vβ values compared with the much larger differences in Vβ values observed between individual Vβ gene families.

CD4/CD8 T cell Vβ expression differences in controls and $CD8^{hi}$ CVI patients

Despite the consistency of Vβ expression in $CD4^+$ versus $CD8^+$ T cells relative to variation in expression among

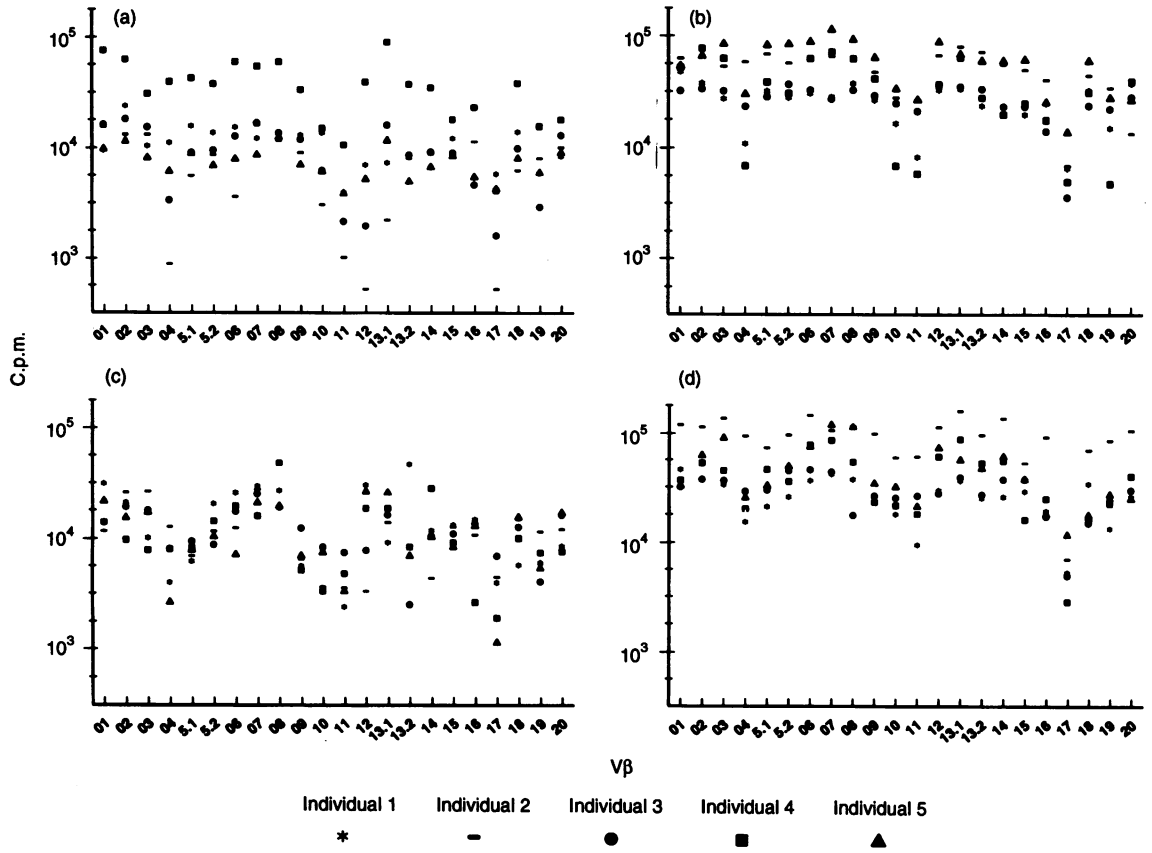

Figure 1. Vβ gene expression in patients with $CD8^{hi}$ CVI (a and c) and in control individuals (b and d). Data points represent individual Vβ family values obtained by semi-quantitative RT-PCR (see the Materials and Methods). Vβ gene family expression is shown for patients with CVI, $CD4^+$ T-cell subset (a) and $CD8^+$ subset (c), and for control individuals, $CD4^+$ subset (b) and $CD8^+$ subset (d).

individual Vβ, there did exist substantial differences in $CD4^+$ versus $CD8^+$ T-cell Vβ expression in some Vβ subsets (Figs 1 and 2). This finding is consistent with the fact that differences in TCR Vβ gene usage frequencies between $CD4^+$ and $CD8^+$ T-cell subsets have been reported previously.[2,4] In an analysis of such variation, we first calculated CD4–CD8 differences (with signs preserved) across individual Vβ. When this was done for individual control or $CD8^{hi}$ CVI patients, as seen in Table 3, the values obtained were not significantly different from zero, indicating that CD4 > CD8 and CD8 > CD4 differences tend to balance out. This finding was expected, as greater usage of a Vβ subset by both $CD4^+$ and $CD8^+$ T cells

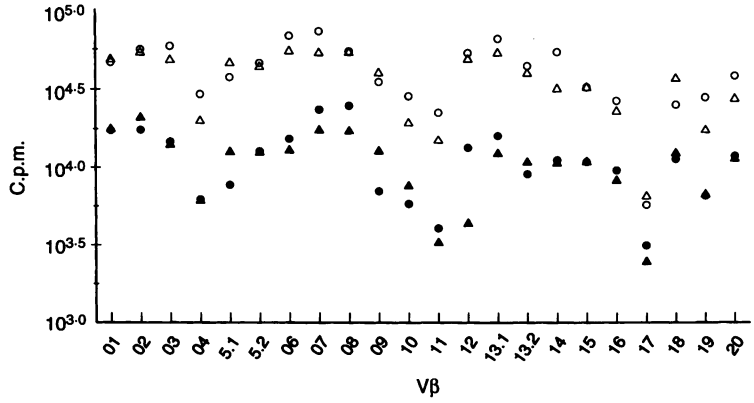

Figure 2. Mean values for Vβ gene family expression in patients with $CD8^{hi}$ CVI, $CD4^+$ (▲), $CD8^+$ (●), and in control individuals, $CD4^+$ (△) and $CD8^+$ (○). Each data point represents the geometric mean across the corresponding five individual subject values shown in Fig. 1. Note that patient values are consistently lower than control values due to differences in specific activity of [^{32}P]dCTP used in $CD8^{hi}$ CVI patient and control RT-PCR determinations; this difference does not affect subsequent statistical analyses.

Table 2. Comparison of variation in T-cell Vβ gene family usage among individual Vβ with that between T-cell subsets within Vβ

Source of variation in T-cell usage	$CD8^{hi}$ CVI patients	P	Control individuals	P
Among individual Vβ	0·707*	< 0·001	0·733*	< 0.001
Between subsets ($CD4^+$ versus $CD8^+$) within individual Vβ	0·232**		0·162**	

* Standard deviation based on the 22 individual Vβ mean log c.p.m. (21 df).

** Standard deviation based on subset differences within the 22 individual Vβ (21 df).

Table 3. Differences in $CD4^+/CD8^+$ T-cell Vβ gene family usage among $CD8^{hi}$ CVI patients and control individuals

Type of $CD4^+/CD8^+$ differences in T-cell usage	Mean difference ± SE: $CD8^{hi}$ CVI patients	Mean difference ± SE: Control individuals
Ordinary [sign (+ or −) considered]	0·025 ± 0·048	0·062 ± 0·043
	$P > 0·30$	
Absolute	0·312* ± 0·024	0·164* ± 0·015
	$P < 0·001$	

* Significantly greater than zero ($P < 0·001$).

must be reflected by lesser usage of another Vβ subset if total Vβ usage by $CD4^+$ and $CD8^+$ T cells is to remain equivalent. In contrast, when absolute CD4–CD8 differences (differences without regard to sign) were averaged across individual Vβ for controls or for $CD8^{hi}$ CVI patients, the mean value obtained for each group was greater than would be predicted by the null hypothesis ($P < 0·001$), indicating that differences in $CD4^+/CD8^+$ T-cell Vβ gene usage do occur (see Materials and Methods). In a further analysis we compared $CD4^+/CD8^+$ T-cell Vβ gene usage differences obtained in $CD8^{hi}$ CVI patients with that in control individuals. In this case we determined such differences by calculating the $CD4^+/CD8^+$ subset distance, i.e. the ratio formed by dividing the smaller of the Vβ subset values (CD4 or CD8) by the larger values. As shown in Fig. 3, in which the CD4—CD8 distances (averaged over the study groups) for the individual Vβ are plotted for $CD8^{hi}$ CVI patients and control individuals, the patient distances were significantly greater than the control distances ($P < 0·001$). Similarly, as shown in Fig. 4, averages of CD4–CD8 distances (averaged across individual Vβ) were greater in $CD8^{hi}$ CVI patients than in control individuals ($P < 0·03$).

An abnormality in Vβ12 expression in $CD8^{hi}$ CVI patients

As shown in Fig. 1, the data obtained on individual Vβ subset expression were sufficiently variable among patients and control individuals to preclude the direct identification of an abnormality in a particular Vβ subset. Indeed, we felt it was premature to assign a particular Vβ subset abnormality on the basis of the relative Vβ CD4–CD8 differences, as individual $CD4^+$ or $CD8^+$ T-cell Vβ abnormalities may be obscured by compensatory effects in the opposite T-cell subset. In addition, there was no clear cut-off point to allow the assignment of abnormality to a particular group of Vβ.

To approach this problem in another way, we reasoned that the CD4–CD8 distances for given Vβ gene families in control individuals should be concordant with the distances in $CD8^{hi}$ CVI patients, although the latter, as indicated above, were larger. In other words, the concordance, defined as the ratio of the T-cell subset counts ($CD8^+/CD4^+$) of each Vβ for the $CD8^{hi}$ CVI patients divided by the corresponding ratio for the control individuals and then log transformed, should yield a normally distributed set of values with a common mean. This analysis was performed and is shown in Fig. 5. It can be seen that the concordances were normally distributed except for a

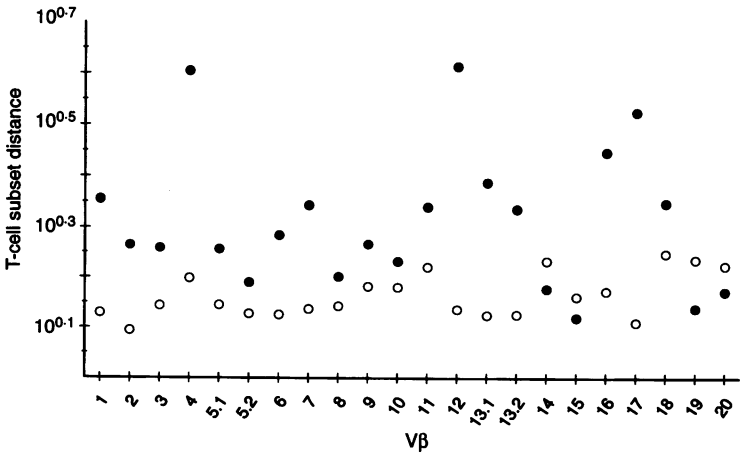

Figure 3. Mean T-cell subset distances in Vβ gene family expression among $CD8^{hi}$ CVI patients (●) and control individuals (○). Each data point represents an average over five subjects.

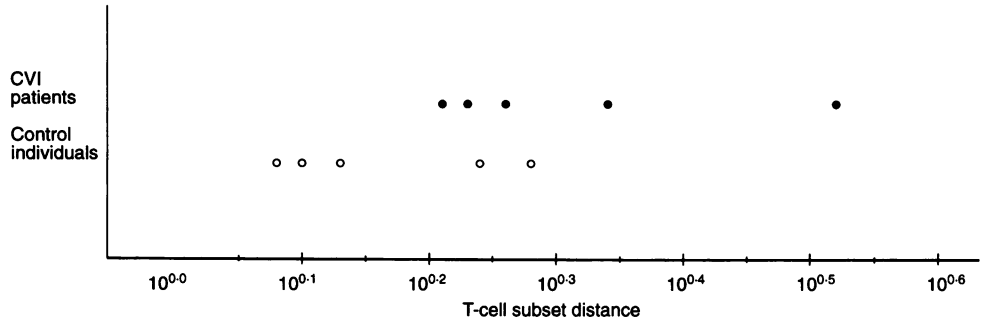

Figure 4. Mean T-cell subset distances among $CD8^{hi}$ CVI patients and control individuals. Each data point represents an average over the corresponding 22 Vβ values.

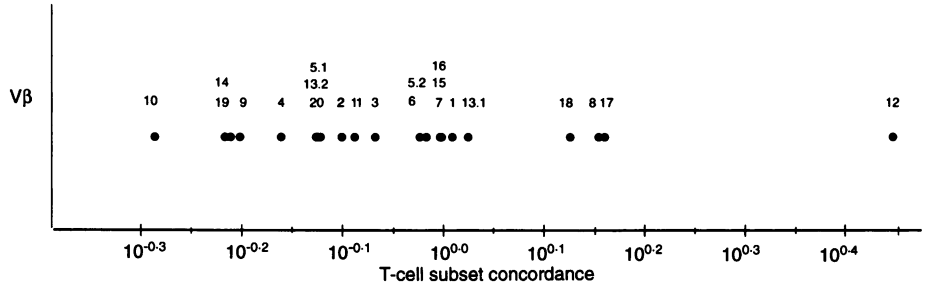

Figure 5. T-cell subset concordance between $CD8^{hi}$ CVI patients and control individuals. For each Vβ, the ratio of the T-cell subset counts ($CD8^+/CD4^+$) for the patients is divided by the corresponding ratio for the controls. Log transformation of the resulting quantities yields a set of normally distributed values (concordances) with a common mean except for Vβ12, which is a significant outlier ($P < 0{\cdot}001$).

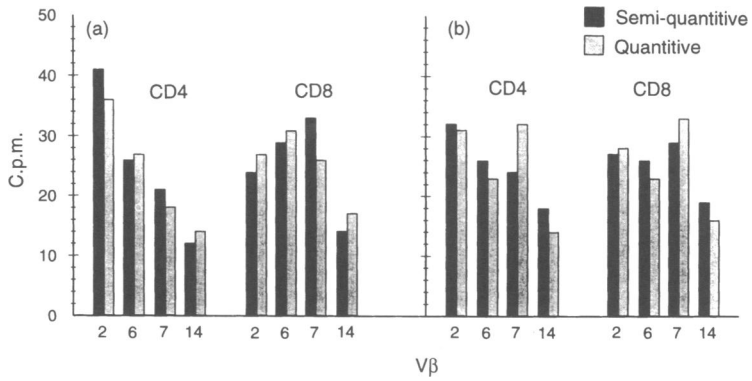

Figure 6. Comparison of $CD4^+$ and $CD8^+$ Vβ2, 6, 7, and 14 expression measured by a semi-quantitative and a quantitative RT-PCR method (see text). (a) Measurements in a patient with $CD8^{hi}$ CVI. (b) Measurement in a control individual. $CD4^+$ and $CD8^+$ T-cell Vβ values in both methods are strikingly similar.

single outlying point (Vβ12) that was significantly greater than the other concordances ($P < 0{\cdot}001$) (Dixon's r_{10} statistic). This finding is also reflected by Fig. 2, which indicates that the discrepancy in Vβ12 CD4–CD8 differences is conspicuously greater than the corresponding pattern of differences for the remaining Vβ. In a further analysis we determined the difference between the Vβ12 value and the mean Vβ values for CD4 and CD8 in both controls and patients.Whereas control differences for CD4 and CD8 were very similar, the patient CD4 difference was greater and opposite in direction from the patient CD8 difference as well as the control differences. This indicates that a decreased CD4 Vβ12 is likely to be present in CVI patients rather than an increased CD8 Vβ12.

Control studies verifying the Vβ quantitative method

As pointed out in the Materials and Methods, the RT-PCR method used here to quantify Vβ subsets was only semi-quantitative, although care was taken to perform RT-PCR in the linear portion of the amplification curve (see the Materials and Methods). To obtain additional verification of this technique, we performed quantitative RT-PCR on four Vβ subsets (Vβ2, 6, 7 and 14) in two patients using artificial mRNA constructs as internal controls (see the Materials and Methods). We then compared the results obtained with the quantitative method with those obtained with the semi-quantitative method. As shown in Fig. 6, good agreement was obtained, suggesting that the semi-quantitative

method was, in fact, a suitable method for this particular application.

DISCUSSION

In this study we used patients from a recently defined subgroup of CVI, who express high levels of activated $CD8^+$ T cells ($CD8^{hi}$ CVI), as a model to test the hypothesis that differences between $CD4^+$ and $CD8^+$ T-cell Vβ gene family expression are strongly influenced by environmental factors such as disease-related exposure to antigen and superantigen. Patients with $CD8^{hi}$ CVI were particularly suitable for this study because they have an acquired disease characterized by increased susceptibility to infection with encapsulated bacterial organisms and, possibly, with viruses.[20] In addition, they manifest a numerical increase in $CD8^+$ T cells containing a large subset of activated $CD8^+/CD57^+$ T cells, which are also increased both in immunologically normal individuals with viral infection and in other immunodeficient patients.[21–26] Thus these patients have an immunological profile suggestive of a prolonged and repeated T-cell response to one or more infectious agents.

In our initial analysis of Vβ gene family expression among $CD4^+$ and $CD8^+$ T cells (of both control individuals and $CD8^{hi}$ CVI patients), we found a remarkable similarity in the pattern of Vβ gene family usage frequencies between $CD4^+$ and $CD8^+$ T-cell subsets. Thus, while differences in mean Vβ gene family usage frequencies in $CD4^+$ versus $CD8^+$ T cells did exist, statistical analysis revealed that these differences were three- to four-fold smaller than those among individual Vβ gene families, i.e. Vβ gene families were similar to one another regardless of $CD4^+/CD8^+$ T-cell subset. This finding suggests that Vβ gene family usage, despite clear-cut evidence that it is influenced by MHC restriction elements,[4] is driven by a factor or factors that override such an influence.

One mechanism that may account for this fact could be that during positive T-cell selection using intermediate-affinity interactions in the thymus, antigen acts as the main TCR-selecting element, while MHC merely coselects from within a Vβ gene family. T cells positively selected in that fashion would be expected to express a Vβ gene family spectrum that is relatively unaffected by MHC. In this way, $CD4^+$ and $CD8^+$ T cells with differing MHC-selecting elements are nevertheless quite similar in their Vβ gene family patterns.

In previous studies, it was reported that Vβ gene frequencies could be related to HLA type, and the V gene usage patterns of HLA-identical individuals were more alike than those of partially or non-identical individuals.[4,9] It was concluded that HLA is the major genetic component influencing V gene frequency. It should be noted that this conclusion is compatible with the above proposal, as the latter still preserves the idea that HLA type is very important to the generation of differences in Vβ gene expression.

The surprising similarity in Vβ family gene usage in the $CD4^+$ and $CD8^+$ T-cell subsets should not be allowed to obscure the fact that differences in subset usage were definitely present in control individuals and were, in fact, increased in patients with $CD8^{hi}$ CVI.

In contrast to mechanisms of intrathymic T-cell selection discussed above, these differences might be a consequence of high-affinity interactions with exogenous antigens in the extrathymic environment. Here, in contrast to the situation in the thymus, high-affinity interactions do not result in cell death and, after contact with the appropriate antigen, T cells (both $CD4^+$ and $CD8^+$ T cells) emerge that manifest different Vβ gene family usage. Thus it is exposure to exogenous antigens and the high-affinity interactions that inevitably occur from such exposure that result in $CD4^+/CD8^+$ T-cell Vβ gene usage discrepancies.

Relating this to the findings in $CD8^{hi}$ CVI patients, one would say that, here again, there is selection of T cells via high-affinity interactions with exogenous antigens but, in this instance, the antigens were not cleared as efficiently because of some feature of the disease. This leads to heightened expansion of $CD4^+$ and $CD8^+$ T cells utilizing disparate Vβ gene families, and an exaggerated difference in $CD4^+/CD8^+$ Vβ gene family usage. The fact that, with one possible exception, the increased $CD4^+/CD8^+$ T-cell Vβ gene usage differences involved different Vβ gene families in the various patients is expected, even if they are exposed to similar organisms, as the patients differ in their MHC types. Finally, it is important to mention that the fact that $CD8^+$ T cells are preferentially expanded in $CD8^{hi}$ CVI does not necessarily imply that increased $CD4^+/CD8^+$ T-cell Vβ gene family usage differences in this disease are due solely to an abnormality in $CD8^+$ T-cell Vβ gene expression. The fact is that exogenous antigens may stimulate $CD4^+$ T cells expressing particular Vβ gene families without affecting the overall $CD4^+$ T-cell number; these $CD4^+$ T cells could then provide the cytokines necessary to support the expansion of $CD8^+$ T cells responding to other antigens in a manner that does not greatly perturb the normal $CD8^+$ T-cell Vβ gene family usage profile.

Another finding in this study was that the magnitude of the difference in $CD4^+/CD8^+$ Vβ gene family usage between control individuals and $CD8^{hi}$ CVI patients fell into a (log) Gaussian distribution with a common mean, suggesting that no clear-cut subsets were present, at least in this small group of patients. Thus the consistent increase in $CD4^+/CD8^+$ Vβ gene family usage differences was the impressive finding, rather than the differences affecting a particular Vβ family. The one exception to this was inherent in the observation of $CD4^+/CD8^+$ T-cell differences in control individuals and $CD8^{hi}$ CVI patients with respect to the Vβ12 gene family. This difference was a significant outlier with respect to the aforementioned Gaussian curve, and suggested that a particular determinant was present that was interacting with a particular subset of T cells in all patients. Several possibilities can be put forward to explain this observation. One is that $CD8^{hi}$ CVI patients are exposed to a particular viral agent with a predilection for infecting $V\beta12^+$ $CD4^+$ or $CD8^+$ T cells, analogous with the fact that human immunodeficiency virus (HIV) manifests preferential replication in $V\beta12^+$ T cells.[27] The reason for such preferential T cell usage is unclear but would presumably relate to factors involved in viral entry into cells rather than immune stimulation of the latter.

Another possible explanation of the Vβ12 expression abnormality is that it represents stimulation of T cells present in the Vβ12 gene family subset that is not, in fact, dependent on the MHC restriction element with respect to either the $CD4^+$ or $CD8^+$ T cell, thus accounting for the differential abnormality in the Vβ gene family in all patients. One obvious reason for such preferential selection is the presence of an organism expressing a superantigen whose interaction with T cells is (by

definition) MHC restriction element independent and Vβ gene family specific. A final possible explanation for preferential selection is that CVI patients share a common MHC restriction element that leads to selection of a particular Vβ gene family usage pattern in the face of exposure to particular antigens. In fact, common MHC haplotypes have been reported in CVI involving both MHC class I, II and III antigens.[28]

Two implications of the findings reported here are worthy of mention, one relating to the study of Vβ gene family usage in disease in general, and one to such usage in $CD8^{hi}$ CVI in particular. With regard to the first implication, the point should be made that, in seeking out Vβ gene abnormalities in various diseases, it may be more useful to look for $CD4^+$/$CD8^+$ T-cell Vβ gene family usage differences rather than for overall changes in Vβ gene family expression. This follows from the fact that, except in the case of superantigen effects, Vβ gene usage changes will be subject to MHC selection elements, and study of such changes in groups of individuals with a variety of MHC types is necessarily obscured. On the other hand, increases in $CD4^+/CD8^+$ T-cell Vβ gene usage differences will be discerned even in groups of individuals with a variety of MHC types. In addition, assuming that some Vβ family selection is relatively non-MHC element dependent for $CD4^+$ but not $CD8^+$ T cells (or vice versa), one may see $CD4^+/CD8^+$ Vβ gene usage differentials relating to a particular Vβ family. In the future, such questions may be addressed more directly using the growing panel of anti-TCR antibodies.

With regard to the second implication, relating directly to $CD8^{hi}$ CVI, the fact that the data reported here reveal increased $CD4^+/CD8^+$ T-cell Vβ gene usage differences and, in addition, reveal a discrepancy in Vβ12 $CD4^+/CD8^+$ Vβ gene family expression supports the idea that $CD8^{hi}$ CVI is a disease associated with a particular infectious agent, presumably an intracellular viral agent that is not easily detected by other means. In line with the points covered above, one would postulate that the organism involved gives rise to several antigenic determinants, some of which select T cells bearing Vβ gene families only within the context of MHC. These give rise to a variety of $CD4^+/CD8^+$ T-cell Vβ gene family differences, some (or one) of which select T cells bearing Vβ families without regard to MHC context for either $CD4^+$ or $CD8^+$ T cells, giving rise to the Vβ12 difference.

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