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Repertoire of Transcribed Peripheral Blood T-Cell-Receptor Beta Chain Variable-Region Genes in Acute Rheumatic Fever

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Patients with severe group A streptococcal infections have abnormalities in the Vβ repertoire of peripheral blood T cells that are consistent with superantigen stimulation by cytoplasmic membrane proteins. The purpose of this study was to determine whether similar changes in Vβ repertoire could be found for patients with acute rheumatic fever (ARF). The mean Vβ repertoire of peripheral blood T cells in nine hospitalized ARF patients was similar to that of 34 controls and did not change during 6 months of follow-up in 6 of the ARF subjects. We were unable to detect changes in the Vβ repertoire of peripheral blood T cells from patients with ARF that could be attributed to the influence of a superantigen.

Rheumatic fever (RF) is a multisystem inflammatory disease that occurs as a sequel to pharyngeal infection by group A streptococci. There is strong evidence that activated T cells are involved in the pathogenesis of acute RF (ARF) (4, 6, 7, 10, 12, 13, 15). It is not currently known whether these T cells are activated by peptides presented by HLA molecules or whether they are responding to proteins with superantigen properties that are known to occur in group A streptococcal cytoplasmic membranes (8, 18, 19). Depletion of T cells carrying specific Vβ genes has been reported in severe group A streptococcal infections (20). The magnitude of the depletion suggests that this change is driven by superantigens rather than HLA-peptide complexes. The purpose of this study was to determine whether changes in Vβ repertoire that are consistent with a superantigen-mediated immune response occur in peripheral blood T cells of patients with ARF.

Subjects. Nine Polynesian children (mean age, 10.2 ± 0.3 years) admitted to hospital with ARF were enrolled in the study. The control subjects (mean age, 15.6 ± 0.3 years) were 34 healthy Polynesian high school pupils without any history of ARF. The University of Auckland ethics committee approved the study, and informed consent was obtained.

Protocol. Subjects with ARF (see Table 1) were hospitalized for an average of 8 ± 1 days (range 3 to 15 days) after the onset of ARF symptoms, diagnosed by using modified Jones criteria (3). All patients had negative blood cultures; negative blood serology for *Mycoplasma pneumoniae*, hepatitis B, and rubella; negative stools for *Yersinia enterocolitica*; and negative rheumatoid factor. Acute bacterial arthritis was excluded by bone scan when clinically indicated. The patients in this study were the first nine patients to be enrolled in a larger study designed to investigate the effect of intravenous immunoglobulin on RF outcome. They were randomized, in a double-blind study design, to receive either dextrose-saline or intravenous immunoglobulin (a total of 2.8 g/kg of body weight) in four doses on days 1, 2, 14, and 28. A blood sample for measurement of the Vβ repertoire in peripheral blood mononuclear cells (PBMC) was taken within 48 h of admission, before treatment was given. In subjects 1 to 6 of Table 1 (two given immunoglobulin and four given dextrose-saline), further blood samples for analysis of Vβ repertoire were taken 14 days after the fourth dose of immunoglobulin or dextrose-saline (6 weeks after admission) and at 6 months after admission, when they were asymptomatic.

Analysis of peripheral blood Vβ repertoire. Vβ repertoire in PBMC was analyzed by the method of Hudson et al. (11) with modifications (9). Briefly, double-stranded T-cell receptor (TCR) β chain cDNA was synthesized by using oligonucleotide dT15 to prime first-strand synthesis and a mixture of two Vβ-specific redundant oligonucleotides to prime second-strand synthesis. TCR Vβ sequences were amplified in an anchored PCR. The product was radiolabelled by random priming with [α-^{32}P]dCTP and hybridized to a filter dot blotted (in triplicate) with immobilized probes for the Vβ gene families Cβ1 and Cβ2 in pBluescript and pBluescript alone. The radioactivity of the DNA hybridized to each immobilized plasmid was quantified by liquid scintillation. The percentage of TCR β chain RNA transcripts in the sample containing the message for each of the Vβ families was calculated as follows: [(mean radioactivity for each Vβ family − mean pBluescript radioactivity)/(mean total Vβ radioactivity − 20 × mean pBluescript radioactivity)] × 100. Changes in the frequencies of Vβ families in PBMC of greater than 50% are usually considered to be due to a superantigen (2, 5, 17, 20). We have shown that our assay can detect differences in Vβ repertoire between samples of PBMC of this magnitude (1).

Statistics. Data are presented as the means ± standard errors of the means (SEMs). Analysis of variance was used to calculate the significance of differences between the means of groups of subjects. The significance of changes in Vβ repertoire with time in 6 RF patients was calculated by paired *t* tests. Comparisons of variance were performed by using an *F* statistic. The criterion for statistical significance was $p < 0.0026$

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TABLE 1. Clinical features of the patients with ARF[a]

Patient	Sex[b]	Age (yr)	Major criteria		Minor criteria				Streptococcal infection by:	
			Carditis[c]	Polyarthritis	ESR[d]	Temp ≥ 38°C	Arthralgia	PR interval	Serology[e]	Culture
1	M	13	Yes	Yes	120	Yes	+	Normal	+	+
2	F	9	Yes	No	115	No	+	Normal	+	−
3	M	12	No	Yes	79	No	+	Prolonged	+	−
4	M	8	No	Yes	74	Yes	+	Normal	+	−
5	F	8	No	Yes	124	No	+	Prolonged	+	−
6	M	11	No	Yes	85	No	+	Prolonged	+	−
7	M	9	Yes	Yes	120	Yes	+	Normal	+	+
8	F	11	Yes	Yes	106	Yes	+	Prolonged	+	−
9	F	11	Yes	No	131	No	+	Prolonged	+	+

[a] The Vβ repertoire of patients 1 to 6 was monitored for 6 months after hospitalization.
[b] M, male; F, female.
[c] Carditis was diagnosed on the basis of clinical findings and color Doppler echocardiography.
[d] ESR, erythrocyte sedimentation rate (millimeters per hour).
[e] Elevated or rising anti-streptolysin O, DNase B, and antihyaluronidase titers.

because of multiple testing. Statistics were calculated using the SAS software program (SAS Institute Inc., Cary, N.C.).

Results. (i) Comparison of RF patients and controls. The mean percentages of PBMC TCR Vβ transcripts in the baseline samples from the ARF patients and control subjects are shown in Fig. 1. There were no differences in the mean percentages of any of the 19 Vβ families for the two groups. The variances of the percentage of each of the 19 Vβ families were also similar in the two groups (data not shown).

(ii) Changes in Vβ repertoire with time in RF patients. There were no differences between the Vβ repertoires of the immunoglobulin- and placebo-treated subjects at any time point, and the two groups were combined for the analysis of changes in Vβ repertoire at 6 weeks and 6 months after admission. The mean lymphocyte counts (10^9 cells per liter) of the ARF patients at admission, 6 weeks postadmission, and 6 months postadmission were 2.5 ± 0.3, 2.6 ± 0.2, and 2.5 ± 0.3, respectively. The mean values for each of the 19 Vβ families at baseline and at the two follow-up time points are shown in Fig. 2. There were no significant changes in the mean Vβ repertoire of PBMC at any time point. In addition, there were no significant changes in the variances of the percentage of each Vβ family with time in these six patients.

Discussion. Changes in the in vivo Vβ profile of peripheral blood T cells have been recorded in a number of inflammatory diseases. Severe group A streptococcal infections have been associated with a 50 to 70% reduction in the Vβ1.1, Vβ5.1, and Vβ12 gene families (20). Kawasaki disease is associated with a two- to threefold increase in the mean frequency of Vβ2 and Vβ8.1 gene families that returns to normal during convalescence (2), and the mean frequency of the Vβ2.1 gene family is approximately doubled in microscopic polyarteritis (17) and toxic shock syndrome (5). These changes in Vβ repertoire are always accompanied by an increase in variance.

We did not find any differences in either the mean or variance of the frequency of 19 Vβ gene families between PBMC from ARF patients and controls. The effective sample size for the study was 14 subjects per group. With this sample size, there was an 85% chance of detecting a 50% difference in frequency of a Vβ gene family with a standard deviation of

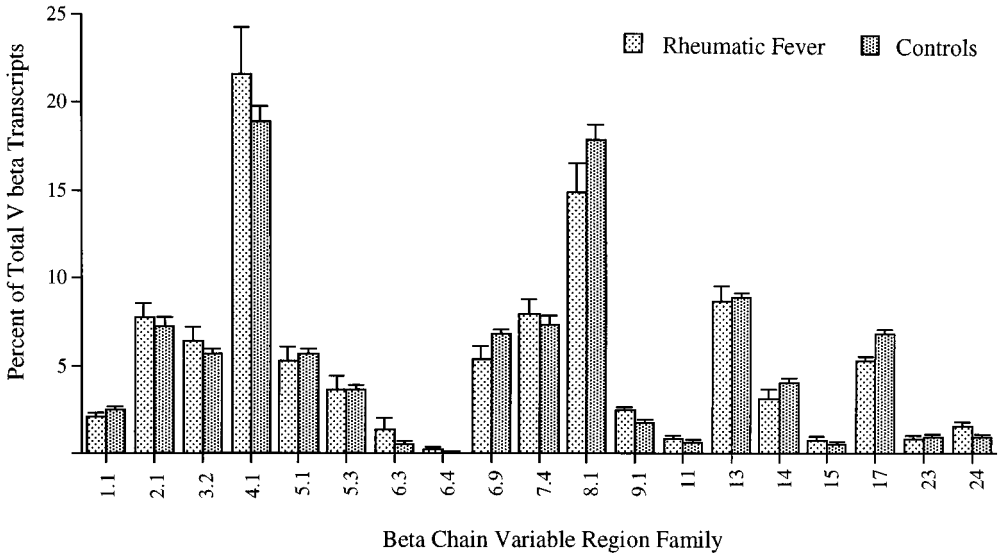

FIG. 1. Comparison of the Vβ repertoires of patients with ARF (n = 9) and healthy controls (n = 34). Each bar shows the mean ± SEM for a Vβ family.

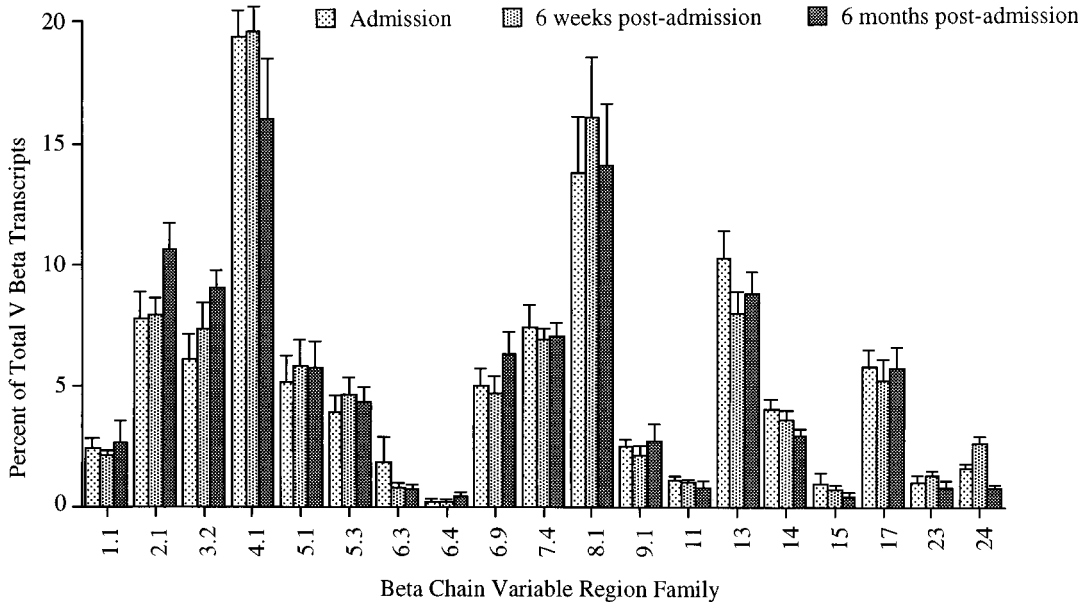

FIG. 2. Comparison of the Vβ repertoires of six patients with ARF at admission, at 6 weeks after admission, and at 6 months after admission. Each bar shows the mean ± SEM for a Vβ family.

33% at a significance level of 0.0025. Thus, our sample size is adequate to exclude changes in Vβ repertoire of a magnitude reported in other acute inflammatory diseases. In addition, there were no differences in the distributions of the 19 Vβ families between PBMC taken at the time of active ARF and PBMC taken at convalescence in six patients.

It is possible that superantigen-like changes in Vβ repertoire were present at the time of the original streptococcal infection, which generally occurs 10 to 21 days before the onset of ARF symptoms. These changes may have resolved by the time the patients were admitted to the hospital. It is also possible that changes in Vβ repertoire do not occur in peripheral blood in ARF and are present only in synovium or cardiac tissue. Tissue-specific changes in Vβ repertoire that are not found in peripheral blood have been reported for patients with psoriasis (14) and Crohn's disease (16). It is also possible that changes in Vβ repertoire occur but differ between individual patients. This would not result in changes in the mean Vβ repertoire. However, an increased variance might be expected, and this did not occur.

In summary, we could not detect changes in the Vβ repertoire of peripheral blood T cells from ARF patients that could be attributed to the influence of a superantigen.

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