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Genomically imposed and somatically modified human thymocyte V_β gene repertoires

(human T-cell receptor/"superantigen"/thymic selection/RNase protection assay/polymorphism)

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ABSTRACT **The effect of thymic selection on the expressed human T-cell antigen receptor β-chain variable region (V_β) gene repertoire was examined by using a multiprobe RNase protection assay. The relative abundance of transcripts for 22 V_β genes (encompassing 17 of the 20 human V_β gene subfamilies) within a thymus, and among 17 thymuses, was variable. On the basis of the presence of corresponding mRNAs, no genomic deletions were detected, but several coding region polymorphisms were identified. Analysis of mature T-cell subsets revealed the absence of complete "superantigen"-mediated V_β deletions, suggesting that this phenomenon, in contrast to mouse, is uncommon or absent in humans. However, several V_β genes were over- or underexpressed in one or both mature single-positive ($CD4^+8^-$ or $CD8^+4^-$) thymocyte subsets compared to syngeneic total, mostly immature thymocytes. Whether these changes are induced by relatively weak superantigens or conventional antigens and whether the downshifts are caused by negative selection or lack of positive selection remains to be determined.**

T-cell antigen receptor (TCR) diversity is generated by random rearrangement of multiple germline variable (V), diversity, and joining gene segments, N-region insertions, and α and β chain pairing (reviewed in refs. 1 and 2). Studies in mouse (reviewed in refs. 3 and 4) indicate that the resulting vast number of randomly generated TCR specificities is subsequently modified in the thymus by elimination of potentially harmful T cells with strongly self-reactive receptors (negative selection) and preferential retention of T cells with receptors for antigens associated with major histocompatibility complex class I or class II molecules (positive selection).

For most antigens, recognition is based on all variable portions of TCR α and β chains (5). However, a category of antigens has recently been described for which T-cell recognition is essentially based on the expressed V_β gene segment alone. Such antigens, collectively termed "superantigens" (6), include not only endogenous molecules such as minor lymphocyte stimulating (*Mls*) determinants (7–10) and other self-ligands (11–13) but also exogenous substances, such as microbial products (14). Significantly, neonatal exposure to such V_β-binding superantigens results in intrathymic deletion of nearly all T cells expressing the specific V_β gene(s). Although the phenomenon of bacterial superantigen-mediated engagement of specific V_β-bearing T-cell clones occurs in both humans and mice, endogenous superantigen-related reactions have thus far only been identified in mice.

The human genome is estimated to contain ≈60 TCR V_β genes, classified into 20 subfamilies (2, 15). To date, little is known about the relative levels of V_β gene expression in humans, the effect of coding region gene polymorphism on the expressed V_β gene repertoire, or the presence of self-superantigen-mediated V_β-specific positive and negative selection modifications. The multiprobe RNase protection assay, independently developed by us (16, 17) and Okada and Weissman (18), has successfully been used for broad analysis of the expressed TCR repertoire in mouse. To determine the role of thymic selection on V_β expression in humans, we adapted this assay to the study of human V_β genes and herein report our results with thymocyte subsets.

MATERIALS AND METHODS

Cell Preparations. Total thymocytes were prepared from 17 thymuses (HT-1 to HT-17) of children undergoing cardiovascular surgery at the Children's Hospital (Los Angeles). Donors ranged from 3 days to 10 years old and included eight males and nine females (10 Caucasians, 6 Hispanics, and 1 Asian). Double-positive ($CD4^+8^+$) and double-negative ($CD4^-8^-$) thymocytes were isolated by two-color fluorescence-activated cell sorting with appropriate antibodies (Becton Dickinson). Single-positive ($CD4^+8^-$ or $CD8^+4^-$) thymocytes were isolated from five donors (HT-13 to HT-17) by using magnetic beads conjugated with anti-CD4 or anti-CD8 antibodies (Dynal, Great Neck, NY).

RNA Probes. All probe templates were generated by the PCR method (19) on DNA isolated as described (20) from a single human thymus (HT-5, Caucasian male, 3 days old). PCR products were purified, ligated into *Sma* I-digested pGEM-7zf (Promega), and sequenced (21). Plasmids containing correct sequences were linearized (*Hin*dIII or *Eco*RI), and their transcription products were tested separately in the RNase protection assay. On the basis of the size of the protected bands, 22 linearized templates were then pooled into one of three probe sets with the following composition.

Probe set A. V_β17.1 [nucleotides (nt) 59–285; ref. 22], V_β8.2 (nt 32–240; ref. 23), V_β18.1 (nt 103–282; ref. 24), V_β19.1 (nt 116–276; ref. 25), V_β8.1 (nt 89–240; ref. 23), V_β16.1 (nt 71–218; ref. 26), V_β15.1 (nt 60–196; ref. 27), V_β4.1 (nt 76–196; ref. 28), V_β11.1 (nt 70–177; ref. 28), V_β14.1 (nt 72–159; ref. 22), V_β5.1 (nt 185–265; ref. 29), and V_β5.2 (nt 202–265; ref. 29). *Probe set B.* V_β6.4 (nt 61–285; ref. 27), V_β13.2 (nt 63–282; ref. 30), V_β3.1 (nt 85–282; ref. 26), V_β1.1 (nt 51–230; ref. 28), and V_β7.1 (nt 61–237; ref. 28). *Probe set C.* V_β2.1 (nt 1–277; ref. 31), V_β6.6 (nt 61–285; ref. 27), V_β8.3 (nt 35–238; ref 23), V_β13.1 (nt 99–282; ref. 30), and V_β12.1 (nt 79–245; ref. 22).

Transcription of probe sets was performed by adding 50–100 ng of template pool to a 5-μl Riboprobe system (Promega) reaction with 75 μCi of [^{32}P]UTP at a final concentration of 15 μM. The β-chain constant region (C_β) probe (nt 348–425; ref. 32) was labeled in a separate reaction with [^{32}P]UTP at 1/20th the specific activity of the V_β probes.

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Abbreviations: TCR, T-cell antigen receptor; nt, nucleotide(s); V, variable; C, constant.

The Multiprobe RNase Protection Assay. RNA was isolated from cell pellets of total thymocytes or from single-positive cells attached to beads as described (33). Hybridization of 1–2 μg of T-cell RNA with radiolabeled C_β probe (10^5 cpm) and each of the probe sets (2×10^3 cpm per uridine) was performed at 56°C for 12–16 hr. Unhybridized probes and target RNA were digested (1 hr, 37°C) with RNase A (5 μg/ml) and RNase T1 (10 units/ml). Purified (20) "protected" probe–target mRNA duplexes were electrophoresed in standard sequencing gels and autoradiographed on Kodak XRP film. Quantitation of V_β transcript levels was performed with a radioanalytic imaging apparatus (AMBIS Systems, San Diego). The net cpm at a given V_β band was calculated by the formula [(cpm of V_β-specific band) − (cpm background around the band)]/(number of uridine residues in the specific V_β probe); this value was then expressed as the percent of total C_β transcripts.

Southern Blot, Cloning, and Sequencing. *Eco*RI- or *Bam*HI-digested genomic DNA was separated on a 0.7% agarose gel, transferred to nylon membranes, and hybridized with radiolabeled probes. Final washes were at 65°C for 30 min in 1× standard saline citrate/0.1% SDS. Sequences corresponding to V_β2.1, -3.1, -12.1, and -15.1 were obtained from first-strand cDNA as described above.

RESULTS

Multiprobe V_β RNase Protection Assay. Fig. 1 shows the results of assaying 1 and 2 μg of total thymocyte RNA from one (HT-1) of the 17 thymuses. Specific bands are identifiable for each of the 22 V_β genes, with intensities increasing proportionately.

To assess the specificity, reproducibility, and linearity of the assay, thymocytes from HT-17 were mixed with increasing numbers of Jurkat cells, known to express V_β8.1 (23), and analyzed with probe set A. The signal corresponding to V_β8.1 in a triplicate assay increased linearly over the range tested, and even 600 Jurkat cells (0.1% in the mixture) were sufficient to significantly increase (>2 SD) the counts in the V_β8.1 band over background levels obtained with thymocytes alone (data not shown). In contrast, band intensity in the other V_βs remained constant, including V_β8.2, which has only four nucleotide differences from V_β8.1.

Relative V_β mRNA Levels in Unselected Thymocytes. Fig. 2 shows the relative expression levels for the 22 V_β genes in largely unselected total thymocytes of the prototypic individual (HT-5) from which these genes were cloned. The considerable variation in relative levels for assessed V_βs (percent of C_β transcripts) was similar to that in mouse (12, 18). In this thymus, V_β12.1 was almost undetectable (see below), V_β11.1 (0.30%) was the next lowest, and V_β2.1 (5.66%) was the most abundant. When more than one member of large V_β subfamilies were analyzed, transcript levels for some genes varied considerably. The sum of transcript levels for the 22 V_β genes was ≈32% of total C_β transcripts, which roughly corresponded to the analyzed fraction (approximately one-third) of the predicted V_β genes. This estimate, however, should be considered tentative since, in the RNase protection assay, the V_β and C_β probes cannot distinguish between functional and nonfunctional (out-of-frame or truncated) transcripts.

To determine whether the expression pattern of the prototypic thymus (HT-5) was reflected in other individuals, the 16 remaining thymuses were analyzed (Fig. 3). The sum of transcript levels for the 22 V_β genes was similar among them, with extreme levels from a low of 27.5% to a high of 37.8% of the C_β levels. The most abundant were V_β2.1, -3.1, -5.1, -5.2, -8.1, -13.1, and -19.1 (>2%), and least abundant were V_β8.3, -11.1, -13.2, and -18.1 (<0.5%); the remainder had intermediate mean levels (0.5–2%). Expression levels for

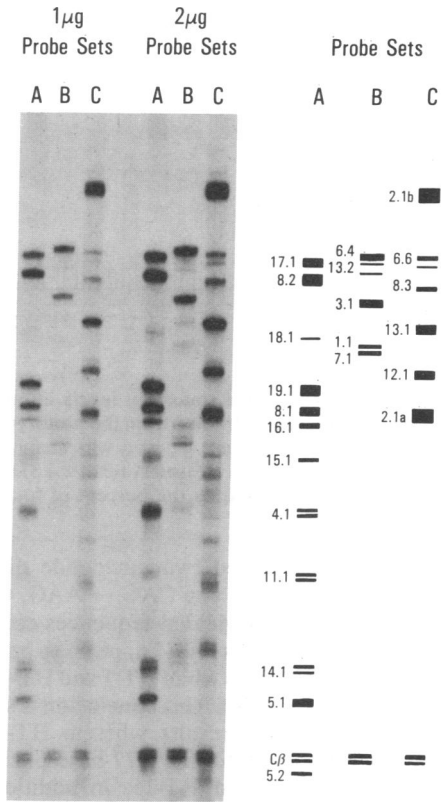

FIG. 1. Human V_β-specific multiprobe RNase protection assay. Twenty-two radiolabeled antisense V_β RNA probes, organized into three probe sets, were hybridized with 1 or 2 μg of total thymocyte RNA. After RNase digestion, protected probe–RNA duplexes were separated on a urea/polyacrylamide sequencing gel. Autoradiographic profiles and prototypic templates are shown. V_β designations are according to established nomenclature (2).

most of the V_β genes generally followed that of the prototypic thymus despite diverse ethnic origins of the individuals, but significant variations occurred in several V_βs.

No relationship between the thymus donor's age and V_β expression levels was identified (data not shown); thymuses from 3- and 9-day-old individuals (HT-5 and HT-10, respectively) expressed levels similar to a 10-year-old donor (HT-8) for all but one (V_β12 in HT-5, see below) of the 22 V_β genes.

Basis of Variability in V_β Expression Levels in Total Thymocytes from Different Individuals. Several possibilities must be considered to explain thymus-to-thymus differences in the expression of some V_β genes, including differences in T-cell subset composition, rearrangement rates, and polymorphisms that might affect gene transcription, α and β chain pairing, or RNA probe hybridization.

Although some distribution variations in single-positive subsets were noted among individual thymuses ($CD4^+8^-$ ranging from 17% to 25% and $CD8^+4^-$ ranging from 8% to 19% of total thymocytes), no correlation with V_β transcript levels was found (data not shown). Moreover, no significant differences in relative V_β transcript levels between total thymocytes and $CD4^-8^-$ or $CD4^+8^+$ cells isolated from two thymuses were noted (data not shown).

To study the role of allelic polymorphism as a cause of V_β expression variations in total thymocytes from different individuals, V_β genes from thymuses with the most striking differences were cloned and sequenced. Three were selected for V_β2.1 with expression levels of 1.87% (HT-1), 3.21% (HT-11), and 5.66% (HT-5). In HT-1 and HT-11, two types of V_β2.1 sequences were identified, of which one (termed V_β2.1a) matched the published V_β2.1 sequence (31) and the

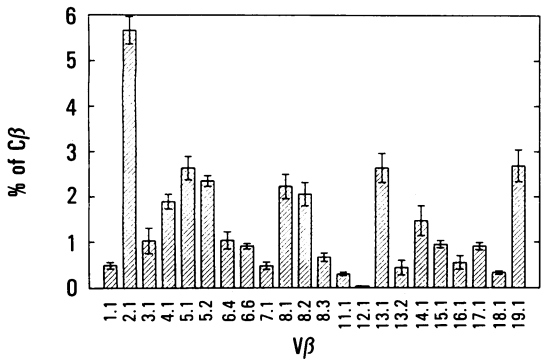

FIG. 2. Relative TCR V_β gene transcript levels in human total thymocytes. Total RNA (1 μg) extracted from the prototypic thymus (HT-5) from which the probes were derived was analyzed by the multiprobe RNase protection assay. Results (mean ± 1 SD) from four independent assays are expressed as the percent of total C_β transcripts.

other (termed V_β2.1b) showed two nucleotide differences (TGG → AGG, Trp-10 → Arg and CAG → AAG, Gln-42 → Lys). In contrast, HT-5 only yielded sequences corresponding to the probe (V_β2.1b). Thus, HT-5 appears to be homozygous for the V_β2.1b allele (*b/b*) while HT-1 and HT-11 appear to be V_β2.1 heterozygous (*a/b*). This conclusion is supported by RNase protection assay results wherein HT-5 gave a single band corresponding to the 277-base-pair V_β2.1b-specific probe and HT-1 and HT-11, in addition to the primary band, gave a distinct secondary band (Fig. 1) whose size (150 base pairs) corresponded to the longest homologous sequence (nt 127–277) between the two V_β2.1 alleles.

Five thymuses exhibited considerably lower V_β15.1 expression levels (<0.2%) than the remaining 12 (0.6–1%), and we therefore cloned and sequenced V_β15.1 from HT-9 (low expressor). A single nucleotide difference from the published sequence (27) was noted (CGG → CAG, Arg-44 → Gln), thereby identifying two alleles, 15.1a (homologous to the probe) and 15.1b, respectively. Thus, it is likely that HT-9 and the other four very low V_β15.1-expressing thymuses (HT-3, HT-6, HT-12, and HT-17) are either heterozygous (*a/b*) or homozygous (*b/b*) for the V_β15.1b allele, while the remaining 12 donors are most likely homozygous for the V_β15.1a allele (*a/a*).

With regard to V_β12.1, three main expression levels were identified: the prototypic HT-5 thymus had undetectable levels, eight individuals had low relative levels (0.36–0.57%), and eight had high relative levels (0.76–1.62%). To study the basis for this variation, a RNase protection assay using only the probe for V_β12.1 was performed. A protected band ≈15 base pairs shorter than expected was found with HT-5, suggesting polymorphism within the PCR primer used to generate this probe. Indeed, sequencing the entire V_β12.1 gene of this individual revealed a single conservative nucleotide substitution (TAT → TAC, Tyr-31) within the region of the 5′ oligonucleotide primer. Since all five sequenced clones contained this polymorphism, HT-5 is likely homozygous for one of the two V_β12.1 alleles, hereinafter designated 12.1b. Analysis of the remaining individuals indicated complete concordance between the presence or absence of the secondary band and V_β12.1 transcript levels; i.e., those with low levels exhibited the primary as well as the secondary band, classifying them as heterozygous (*a/b*) for the two V_β12.1 alleles, whereas those with high relative levels exhibited only the primary band, classifying them as homozygous for the 12.1a allele (*a/a*).

In contrast, polymorphisms within the probe region do not appear to account for V_β3.1 expression level variations. First, Southern blot analysis of eight thymuses (HT-2, HT-3, and HT-5 through HT-10) with expression levels from 0.73% to 5.74% showed a single V_β3.1 band of identical size (data not shown), suggesting that all have the same number of V_β3.1 genes. Second, the portion of V_β3.1 corresponding to the prototypic probe was cloned from these eight thymuses and yielded sequences identical to the probe. Third, secondary bands were not observed when low or high expressors were analyzed by the protection assay using this probe.

Thus, although low V_β gene expression levels are sometimes caused by polymorphisms leading to inadequate probe hybridization, other variations appear to be caused by as yet undefined genomic or somatic mechanisms.

V_β Repertoire Selection in Mature Thymocyte Subsets. In mouse, the expressed V_β repertoire in mature single-positive medullary and peripheral T cells is highly modified from the randomly created repertoire of immature double-positive cortical thymocytes (reviewed in refs. 3 and 4). To determine whether such TCR V_β repertoire changes can be detected in humans, we compared the relative quantity of each of the 22 human V_β transcripts in primarily immature total thymocytes vs. single-positive ($CD4^+8^-$ or $CD8^+4^-$) mature thymocytes from five randomly selected donors (2 Hispanics, 1 Asian, and 2 Caucasians; 11 months to 4 years of age). Percent relative changes are diagrammatically depicted in Fig. 4. For

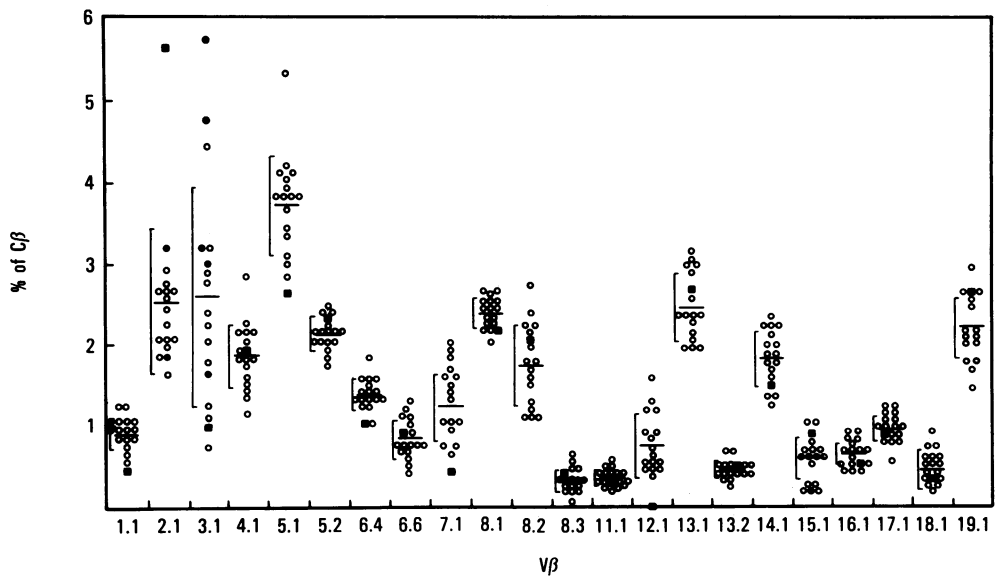

FIG. 3. Relative TCR V_β gene transcript levels in unselected total thymocytes from 17 individuals. Each dot represents the mean value for a given thymus assessed in two to nine independent RNase protection assays and expressed as a percent of total C_β transcripts. For each V_β, the mean ± 1 SD for all 17 individuals is also shown. Solid squares depict values with the prototypic thymus (HT-5) from which probes were derived, whereas solid dots indicate individuals whose genes were cloned and sequenced.

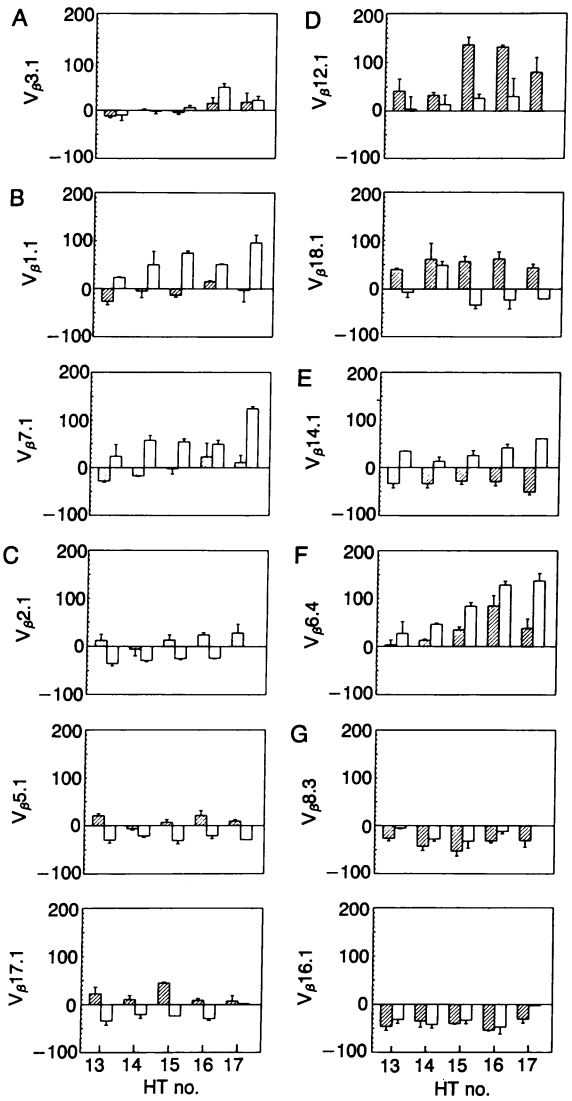

FIG. 4. V_β repertoire modifications in five human thymuses. Percent relative changes in V_β gene transcript levels from total, mostly immature thymocytes to syngeneic mature single-positive $CD4^+8^-$ (hatched bars) or $CD8^+4^-$ (open bars) thymocytes derived from five individuals were calculated by using the formula: {[($CD4^+8^-$ or $CD8^+4^-$ value) − (total thymocyte value)]/(total thymocyte value)} × 100. Examples of insignificant change (*A*) and significant ($P < 0.05$) over- (*B*) or underexpression (*C*) in the $CD8^+4^-$ subset, over- (*D*) or underexpression (*E*) in the $CD4^+8^-$ subset, and over- (*F*) or underexpression (*G*) in both subsets compared to total thymocytes are depicted.

V_β3.1, -4.1, -5.2, -6.6, -8.1, -8.2, -11.1, -13.1, -13.2, -15.1, and -19.1, there were no significant changes in transcript levels in most thymuses between the primarily immature total thymocytes and the syngeneic single-positive mature thymocytes (shown for V_β3.1, Fig. 4*A*). Importantly, however, significant ($P < 0.05$, Student's *t* test) changes of over- or underexpression in single-positive thymocyte subsets compared to corresponding total thymocytes were observed for the remaining V_βs. Thus, V_β1.1 and V_β7.1 (Fig. 4*B*) showed a comparative $CD8^+4^-$ subset-bias increased expression, and V_β2.1, -5.1, and -17.1 (Fig. 4*C*) showed a comparative $CD8^+4^-$ subset-bias decreased expression in relation to syngeneic total thymocytes. Conversely, V_β12.1 and V_β18.1 (Fig. 4*D*) were comparatively overexpressed in the $CD4^+8^-$ subset, whereas V_β14.1 (Fig. 4*E*) was underexpressed in the $CD4^+8^-$ subset (and overexpressed in the $CD8^+4^-$ subset). Moreover, V_β6.4 (Fig. 4*F*) was overexpressed and V_β8.3 and V_β16.1 (Fig. 4*G*) were underexpressed in both single-positive subsets. Relative increases or decreases varied considerably, with significant upward shifts from 11% (V_β6.4 in HT-14) to 137% (V_β6.4 in HT-17) and downward shifts from 21% (V_β17.1 in HT-14) to 54% (V_β16.1 in HT-16). Overall, these findings suggest that V_β repertoire changes occur in humans upon thymocyte transition to the mature single-positive stage. However, the observed decreases in V_β expression levels were never of the same magnitude as those conferred in mice by neonatal exposure to self or environmental superantigens.

DISCUSSION

We describe herein the analysis of V_β expression in thymocytes of several individuals by using a human V_β multiprobe RNase protection assay. Application to human thymocyte populations allowed us to address the qualitative and quantitative characteristics of genomically imposed V_β repertoires among diverse genetic backgrounds and to determine the extent to which human V_β repertoires might be affected by positive and negative somatic selection.

On the basis of the presence of corresponding mRNAs, no genomic deletions were detected, confirming the rarity of V_β genomic deletions reported earlier by Concannon *et al.* (34). Thus, humans appear to differ from mice in that extensive genomic deletions have been identified encompassing approximately one-half of the total mouse V_β genes in at least five different inbred strains (35–37) and in wild mice from different geographic locations (38–40).

The relative abundance of each of the 22 V_β transcripts within a thymus was highly variable over a range of ≈20-fold, a value similar to that in murine total thymocytes (18). However, expression levels do not necessarily correspond to the degree of homology between the V_β genes of these two species (2). The reasons for this unequal V_β gene expression are not yet clear. While correlation of the degree or frequency of expression for immunoglobulin V_H genes with chromosomal location has been described (41, 42), no such correlation exists for TCR genes (43). Therefore, other factors, such as recombinase signal sequences, transcriptional rates, and preferential pairing of V_β and V_α, may contribute to these differences.

Although the overall patterns of V_β gene expression levels were relatively similar among the 17 thymuses, considerable variations for some V_βs were observed. Some of these variations (i.e., V_β3.1) may be explained on the same basis as variations within a given thymus, while others (i.e., V_β2.1, -12.1, and -15.1) may be caused by polymorphisms affecting the degree of hybridization with our RNA probes. Previous studies have identified coding region polymorphisms for V_β1.1 and V_β6.7 (44, 45). Although each V_β riboprobe used in the present study covered only a portion of the coding sequence (from 18% to 98%), polymorphisms were identified for three other V_β genes (V_β2.1, -12.1, and -15.1). In separate studies with human peripheral blood T cells (R.B., D.H.K., R.S.B., and A.N.T., unpublished results) and with human thymic cDNA libraries (A. Plaza, D.H.K., and A.N.T., unpublished results), polymorphisms for eight additional V_β genes were revealed. On the basis of these findings, V_β gene coding region polymorphisms are probably extensive and may contribute to overall human TCR repertoire diversification.

An important component of our study was the search for evidence of positive and negative selection in humans, particularly those mediated by superantigens. Our data document significant differences in transcript level values for several V_β genes upon transition of T cells from immature to mature single-positive subsets. These presumed selection changes, manifested as over- or underexpression, usually affected only one or the other single-positive subset, although

sometimes both were affected. Interpretation of these findings with regard to positive and negative selection is difficult at present. If transcript levels are higher in one or both single-positive thymocyte subsets compared to mostly immature total thymocytes, then preferential positive selection can be invoked. On the other hand, a low comparative value in one or both single-positive subsets may be interpreted as negative selection, or absence of positive selection. In the mouse, such dual interpretations can be resolved by appropriate genetic studies. In humans, family and other studies will be required for final interpretation of the results.

Our study of five thymuses suggests that complete superantigen-imposed V_β deletions are unusual or even absent in humans. Additional studies of peripheral blood T cells from 38 normal individuals concur with this conclusion (R.B., D.H.K., R.S.B., and A.N.T., unpublished results). Previous studies of peripheral blood from three subjects by quantitative PCR (46) have also failed to document complete V_β clonal deletions. Although not all human V_β genes have been analyzed in this regard, considering the extent of superantigen-mediated clonal deletions in the mouse (25–50% of V_β genes are somatically deleted in several strains), it can be concluded with considerable confidence that more extensive studies will probably not significantly alter this interpretation. The "subset-biased" V_β repertoire modifications described here may be due to the effects of "weak" self-superantigens or simply be a consequence of selection imposed by conventional peptide ligands. The nature of murine self-superantigens has not been fully recognized. By examining recombinant inbred mouse strains, Woodland *et al.* (13) reported that one of the genes encoding the $V_\beta 5.2$-deleting cotolerogens was mapped to chromosome 12 and linked to the endogenous mammary tumor virus-9. However, if mammary tumor viruses are proven to be the self-superantigens in mouse, it should be noted that related retroviral genes are not known to be functional in humans (J. M. Coffin, personal communication).

Some V_β clonotypes susceptible to self-superantigen-mediated clonal deletions cross-react with exogenous superantigens (i.e., bacterial toxins; ref. 14). Thus, it has been hypothesized (14) that eliminating these cells early in ontogeny avoids adverse effects that might be induced later in life if such toxins are encountered. The lack of somatically imposed complete V_β deletions in humans suggests this concept may not be applicable to all species.

Note Added in Proof. Further documentation of the above cited linkage of mammary tumor viral gene products with V_β deletions in the mouse has recently been provided (47–50).

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1. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334,** 395–402.
2. Wilson, R. K., Lai, E., Concannon, P., Barth, R. K. & Hood, L. E. (1988) *Immunol. Rev.* **101,** 149–172.
3. Blackman, M., Kappler, J. & Marrack, P. (1990) *Science* **248,** 1335–1341.
4. von Boehmer, H. & Kisielow, P. (1990) *Science* **248,** 1369–1372.
5. Fink, P., Matis, L., McElliott, D., Bookman, M. & Hedrick, S. (1986) *Nature (London)* **321,** 219–226.
6. White, J., Herman, A., Pullen, A. M., Kubo, R., Kappler, J. W. & Marrack, P. (1989) *Cell* **56,** 27–35.
7. Kappler, J. W., Staerz, U., White, J. & Marrack, P. C. (1988) *Nature (London)* **332,** 35–39.
8. MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festenstein, H., Zinkernagel, R. M. & Hengartner, H. (1988) *Nature (London)* **332,** 40–45.
9. Pullen, A. M., Marrack, P. & Kappler, J. W. (1988) *Nature (London)* **335,** 796–801.
10. Abe, R., Vacchio, M. S., Fox, B. & Hodes, R. J. (1988) *Nature (London)* **335,** 35–39.
11. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49,** 273–280.
12. Vacchio, M. S. & Hodes, R. J. (1989) *J. Exp. Med.* **170,** 1335–1346.
13. Woodland, D., Happ, M. P., Bill, J. & Palmer, E. (1990) *Science* **247,** 964–967.
14. Marrack, P. & Kappler, J. (1990) *Science* **248,** 705–711.
15. Toyonaga, B. & Mak, T. W. (1987) *Annu. Rev. Immunol.* **5,** 585–620.
16. Singer, P. A., Balderas, R. S., McEvilly, R. J., Bobardt, M. & Theofilopoulos, A. N. (1989) *J. Exp. Med.* **170,** 1869–1877.
17. Singer, P. A., Balderas, R. S. & Theofilopoulos, A. N. (1990) *EMBO J.* **9,** 3641–3648.
18. Okada, C. Y. & Weissman, I. L. (1989) *J. Exp. Med.* **169,** 1703–1719.
19. Saiki, R. K., Scharf, S., Faloona, S., Mullis, K. B., Horn, G. T., Erlich, H. A. & Kazazian, H. (1985) *Science* **230,** 1350–1354.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Sanger, F., Nicklen, S. & Coulson, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Tillinghast, J. P., Behlke, M. A. & Loh, D. Y. (1986) *Science* **233,** 879–883.
23. Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S. P., Aleksander, I. & Mak, T. W. (1984) *Nature (London)* **308,** 145–149.
24. Leiden, J. M. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4456–4460.
25. Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R. -P. & Mak, T. W. (1987) *Eur. J. Immunol.* **198,** 375–383.
26. Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debre, P., Minden, M. D. & Mak, T. W. (1986) *J. Exp. Med.* **164,** 739–750.
27. Ikuta, K., Ogura, T., Shimizu, A. & Honjo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7701–7705.
28. Concannon, P., Pickering, L. A., Kung, P. & Hood, L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6598–6602.
29. Yoshikai, Y., Antoniou, D., Clark, S. P., Yanagi, Y., Sangster, R., Van den Elsen, P., Terhorst, C. & Mak, T. W. (1984) *Nature (London)* **312,** 521–524.
30. Duby, A. D. & Seidman, J. G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4890–4894.
31. Tunnacliffe, A., Kefford, R., Milstein, C., Forster, A. & Rabbitts, T. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5068–5072.
32. Toyonaga, B., Yoshikai, Y., Vadasz, V., Chin, B. & Mak, T. W. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8624–8628.
33. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
34. Concannon, P., Gatti, R. A. & Hood, L. E. (1987) *J. Exp. Med.* **165,** 1130–1140.
35. Behlke, M. A., Chou, H. S., Huppi, K. & Loh, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 767–771.
36. Haqqi, T. M., Banerjee, S., Anderson, G. D. & David, C. S. (1989) *J. Exp. Med.* **169,** 1903–1909.
37. Haqqi, T. M., Banerjee, S., Jones, W. L., Anderson, G., Behlke, M. A., Loh, D. Y., Luthra, H. S. & David, C. S. (1989) *Immunogenetics* **29,** 180–185.
38. Huppi, K., D'Hoostelaere, B., Mock, M., Jouvin-Marche, E., Behlke, M., Chou, H., Berry, R. & Loh, D. (1988) *Immunogenetics* **27,** 51–56.
39. Jouvin-Marche, E., Trede, N. S., Bandeira, A., Tomas, A., Loh, D. Y. & Cazenave, P.-A. (1989) *Eur. J. Immunol.* **19,** 1921–1926.
40. Pullen, A. M., Potts, W., Wakelands, E. K., Kappler, J. & Marrack, P. (1990) *J. Exp. Med.* **171,** 49–62.
41. Yancopoulos, G. D., Desiderio, S. V., Paskind, M., Kearney, J. F., Baltimore, D. & Alt, F. W. (1984) *Nature (London)* **311,** 727–733.
42. Schroeder, H. W., Jr., Hillson, J. L. & Perlmutter, R. M. (1987) *Science* **238,** 791–793.
43. Lai, E., Concannon, P. & Hood, L. (1988) *Nature (London)* **331,** 543–546.
44. Robinson, M. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9422–9426.
45. Li, Y., Szabo, P., Robinson, M. A., Dong, B. & Posnett, D. N. (1990) *J. Exp. Med.* **171,** 221–230.
46. Choi, Y., Kotzin, B., Herron, L., Callahan, J., Marrack, P. & Kappler, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8941–8945.
47. Woodland, D., Happ, M. P., Gollob, K. J. & Palmer, E. (1991) *Nature (London)* **349,** 529–530.
48. Marrack, P., Kushnir, E. & Kappler, J. (1991) *Nature (London)* **349,** 524–526.
49. Frankel, W. N., Rudy, C., Coffin, J. M. & Huber, B.T. (1991) *Nature (London)* **349,** 526–528.
50. Dyson, P. J., Knight, A. M., Fairchild, S., Simpson, E. & Tomonari, K. (1991) *Nature (London)* **349,** 531–532.