

A Dominant T Cell Receptor β -Chain in Response to a Short Ragweed Allergen, Amb a 5¹

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HLA-DR α , β *1501 (and the closely related DR α , β *1502) heterodimers are the primary class II MHC restriction elements controlling T cell responses to a ragweed pollen allergen, Amb a 5 (M_r = 5000). Using a novel, quantitative, competitive PCR (QC-PCR) assay and a TCR β -gene internal standard (IS), we have examined the TCR β -gene use in Amb a 5-specific T cells. Multiple TCR V β (and V α) genes were found in polyclonal T cell lines from two unrelated subjects, and 30% of the cells were stained positive for V β 5.2/5.3 as judged by a flow cytometry analysis. An Amb a 5-specific Th2 clone (AP1.2) was found to express V β 5.2 (and V α 8) and to have a unique V-D-J junctional region sequence. To determine the relative frequency of clone AP1.2 β -gene expression in the polyclonal T cell lines and in the PBMC using a QC-PCR assay, we first created a TCR IS by duplicating the J β 1.5 gene segment of the AP1.2 β -gene. A QC-PCR assay was performed using the modified AP1.2 β -gene as a competitor and a V β 5.2 or a D β -specific primer, each paired with a C β primer. Results showed the presence of 30% of AP1.2 β transcripts in the polyclonal T cell lines, but no detectable amplified products were found in the total RNAs (200 ng) of autologous PBMC. These findings were confirmed by sequencing independent clones of PCR-amplified V β 5.2-C β fragments (from two unrelated subjects) from each cell line, which suggested that the frequency of the clone AP1.2 β sequence is quite low in the PBMC but is dominant in polyclonal Amb a 5-specific cell lines. *The Journal of Immunology*, 1995, 154: 6157–6162.

Amature CD4⁺ T cell, through its clonally derived TCR, recognizes a peptide fragment of an Ag only in the context of an MHC class II molecule (in humans, HLA-DR, -DQ, or -DP). The TCR responsible for this “dual recognition” is composed of two subunits, α and β , each generated by somatic rearrangement of multiple gene segments (V α J α C α and V β D β J β C β , respectively) (1). The rearrangement patterns are unique and are consistent for the life of the cell and its clonal descendants. The hypervariable regions of the α - and β -chains are encoded within and between the junctional regions of both VJ and VDJ gene segments, respectively. These third complementarity-determining regions (CDR3s)⁴ confer the MHC/Ag peptide specificity (2, 3). The PCR has been

used in the analysis of TCR V gene use, and several studies of the TCR β -gene repertoire in humans have demonstrated a restricted expression of the V β -gene family in both normal and diseased states (4–8). In these studies, the TCR V gene use was assessed using a large panel of primers specific for each of the V gene segments (4–6); also, a C β -C β reference template has been used in a “quantitative” PCR method to enumerate the relative frequency of TCR V gene expression in the cells (9). However, variability in the amplification efficiency of particular primers introduces uncertainty into the quantitation process, and the method is at present incapable of evaluating the CDR3 sequences directly.

Human T cells and Th2 cytokines are important in the regulation of IgE synthesis and have been implicated in the pathogenesis of a variety of human atopic diseases, including asthma (10, 11). Previously, we have studied the responses of T cells to a well characterized, short, ragweed allergen, Amb a 5, and demonstrated that the HLA-DR α , β *1501 (and the closely related DR α , β *1502) heterodimers are the class II MHC restriction elements

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⁴ Abbreviations used in this paper: CDR3, third complementarity-determining region; QC-PCR, quantitative, competitive-PCR; RT-PCR, reverse transcription-PCR; IS, internal standard.

controlling T cell responses to Amb a 5 (12). Using reverse transcription-PCR (RT-PCR) and quantitative, competitive PCR (QC-PCR) assays for cytokines, we have also demonstrated that the responses of T cells to Amb a 5 belong to the Th2 phenotype, and Amb a 5-specific T cells are able to induce specific IgE synthesis *in vitro* (13). In this report, we have analyzed the TCR β -gene uses in Amb a 5-specific T cells and demonstrated a novel quantitative analysis of an allergen-specific TCR β -gene sequence. We have created a cRNA standard by duplicating the J β region gene sequence of an Amb a 5-specific Th2-cell clone (AP1.2), followed by *in vitro* transcription. This modified gene segment was used to analyze directly the expression of Ag-specific CDR3 sequences, and the standard cRNA was coamplified with the analyzed template in the same reaction tube using a single pair of primers. This quantitative PCR was performed to analyze the relative representation of AP1.2 β -gene sequence in polyclonal T cell lines and in autologous PBMC.

Materials and Methods

Ag and preparation of cells

Amb a 5 ($M_r = 5000$) was purified from short ragweed pollen as described previously (14). The Amb a 5-specific T cell line (APL) and clone AP1.2 were derived from the PBMC of an Amb a 5-positive, ragweed-allergic, asthmatic individual (AP; African American) after periodic Amb a 5 and rIL-2 stimulation and by the limiting dilution cloning method as described (12). An Amb a 5-specific T cell line (DSL) was isolated from an additional Amb a 5-positive, ragweed-allergic, asthmatic subject (DS; Caucasian), following the same protocol. The responses of both cell lines and clone AP1.2 were specific for Amb a 5 and were restricted by DR α , β *1501, the class II specificity shared by both cell donors (12, 15). The bronchoalveolar lavage cells of an asthmatic patient (#617) who is ragweed-allergic, but shows no serum Abs to Amb a 5, were included for comparison.

Preparation of RNA from cells

Total RNAs were prepared from various cell cultures stimulated with Ag (3 μ g/ml) *in vitro* in the presence of irradiated autologous PBMC for 14 days. At this time, only T cells could be detected in the cultures of either T cell lines or clone AP1.2 as judged by flow cytometry analysis of anti-CD3 mAb-stained cells. RNAs were isolated from the cells by RNazo1[®] (Tel-Test Inc., TX) containing guanidinium isothiocyanate as described (16). The RNA was quantitated by spectrophotometry, and the integrity of the RNA sample was assessed by running an aliquot of the RNA (usually 0.5–1 μ g) on a 2% agarose gel containing ethidium bromide and by observing the 28S and 18S of ribosomal RNA bands; also, RT-PCR analyses of a housekeeping gene, β -actin, were performed on each sample as controls (17).

PCR amplification of TCR genes

To determine the specific TCR V gene uses and sequences, the PCR technique was used to amplify the α and β cDNA (VaJaC α and V β D β J β C β , respectively) of the T cell lines, APL and DSL, and clone AP1.2. The protocol for RT and PCR was the same as described (18). An aliquot of total cellular RNA (200 ng or otherwise indicated) was reverse-transcribed to cDNA in the presence of avian myeloblastosis virus reverse transcriptase (2.5 U/ μ l), 1 mM each dNTPs (dATP, dCTP, dGTP, and dTTP), RNase inhibitor (1 U/ μ l), oligo(dT)₁₆ (2.5 μ M), 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl; pH 8.3), and MgCl₂ (5 mM) to a total volume of 20 μ l. The RT-mix was incubated at 42°C for 20 min (conducted in the Perkin-Elmer-Cetus thermocycler; Perkin-Elmer Cetus, Norwalk, CT) for RT, followed by 99°C for 5 min to inactivate the reverse transcriptase. An aliquot of the RT product was directly subjected

to PCR in the presence of a "master mix" containing PCR buffer, MgCl₂ (final concentration, 2 mM), dNTPs (final concentration, 0.4 mM each), AmpliTaq polymerase (Perkin-Elmer-Cetus; 1 U/50 μ l reaction), and paired primers for TCR genes (0.5 μ M each primer) to a total volume of 50 μ l. The TCR gene-specific primers were synthesized based on the published sequences (18) at the Johns Hopkins DNA Synthesis Laboratory. The RT product was amplified sequentially with two C β and C α oligoprimers and each of the V β 1–20 (including V β 5.1 and V β 5.2/5.3) and V α 1–18 primers, respectively.

PCR was conducted for 45 cycles or otherwise indicated under the following conditions: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Final extension was at 72°C for 10 min. The reaction product was visualized on 3% agarose (2% Nusieve GTG, FMC, Rockland, ME; and 1% agarose, Sigma Chemical Co., St. Louis, MO) electrophoresis in 1X tris borate-EDTA buffer containing 0.5 μ g/ml ethidium bromide. As a negative control, an aliquot of each RNA sample was subjected to PCR amplification without the RT step. Verification of the specificity of amplification was established by Southern blotting using an internal C β or C α probe or by sequencing using Sequenase and dideoxy chain termination (see below).

Flow cytometry analysis

Amb a 5-specific T cell lines and PBMC were isolated from the cultures, suspended in PBS/1% heat-inactivated FCS, and incubated with mouse anti-human TCR V β 5.2/5.3 (mAb 1C1, T Cell Diagnostic, Cambridge, MA) followed by staining with fluorescein-conjugated goat anti-mouse Ab (Becton Dickinson, Mountain View, CA). Samples were extensively washed and analyzed on a Coulter Epics Profile Analyzer (Coulter, Hialeah, FL).

Quantitative, competitive PCR analysis of Amb a 5-specific TCR genes

Similar to our studies of human cytokine-gene expression using QC-PCR assay and internal standards (IS) for cytokines (17), we have constructed an IS for the analysis of Amb a 5-specific TCR gene expression. In this procedure, we first ligated the PCR-amplified TCR V β 5.2-C β fragments (with *Bam*HI sites at both ends) of clone AP1.2 with a *Bam*HI-treated pSP64-poly(A) vector (Promega, Madison, WI). The native AP1.2 β -gene contains a *Csp*I site in the D region. This site was cleaved with *Csp*I, treated with alkaline phosphatase, and then ligated with the AP1.2 TCR J β fragment. The resultant 52-bp longer fragment served as the β -gene cRNA IS.

After purification, the plasmids were linearized using *Eco*RI and were transcribed *in vitro* to cRNA using SP6 polymerase according to the manufacturer's protocol (Promega). After amplification, the products of the competitor and wild-type β -genes can be differentiated on the agarose gel by their different fragment sizes. In our initial experiments, we optimized the conditions for this QC-PCR assay to determine the linearity, reproducibility, and range of exponential amplification of the assay (17). We chose 45 cycles of amplification to ensure that the PCR products were detectable either by incorporating radioactivity or by scanning ethidium bromide-containing gels (see below). The exponential phase of amplification for the IS was below 10^{-9} μ g of the input IS cRNAs under this condition.

To determine the relative representation of AP1.2 TCR β -gene expression, varying amounts of the IS cRNAs were mixed with 200 ng of total RNAs from various cell cultures and were subjected to RT and PCR in the presence of a C β and a V β or a D β primer. The amplified products were then separated on agarose gel containing ethidium bromide, the intensities of the amplified fragments were scanned on a flat-bed scanner (Molecular Dynamics, Sunnyvale, CA), and the digital data were analyzed with the aid of the computer software provided, as described previously (19, 20). The two-dimensional band was compressed to a one-dimensional plot (plot profile option) by averaging the horizontal pixels across a band, and the background was subtracted. The intensities of each band were plotted against varying amounts of input cRNAs. At the exponential phase of amplification, the quantity of the test sample was determined by calculating the ratio of the intensities between the standard and template and by extrapolating from a linear regression curve.

Table I. TCR α - and β -gene usages in Amb a 5-specific T cell lines detected by RT-PCR

Line	Specificity	V α	V β
APL	Amb a 5/DR α , β *1501	2, 5, 8	5.2, 9, 10, 11, 12, 14, 16, 17, 18
DSL	Amb a 5/DR α , β *1501	2, 5, 8	5.2, —, —, 11, 12, 14, 16, 17, 18

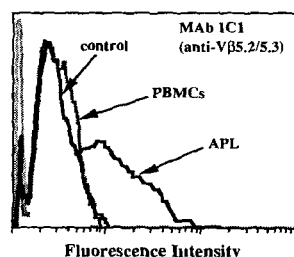


FIGURE 1. Surface expression of TCR V β 5.2/5.3. Isolated cells were suspended in PBS/1% heat-inactivated FCS and were incubated with mouse anti-human TCR V β 5.2/5.3 (mAb 1C1), followed by staining with fluoresceinated goat anti-mouse IgG Ab.

M13 and "TA" cloning and dideoxy-sequencing

About 10% of the amplified product was allowed to incubate for 2 h at 37°C with 40 U of an appropriate enzyme (e.g., *Bam*HI). Digested DNA was electroeluted and purified. The digested DNA was then ligated into the M13 mp10 vector for 16 h at 15°C in the presence of T4 ligase. Recombinant vectors were transfected into the *Escherichia coli* host strain, JM103. The single-stranded phage DNAs containing the insert were isolated and sequenced by the dideoxy primer extension method using the Sequenase kit, as suggested by the manufacturer (United States Biochemical, Cleveland, OH). In some cases, the PCR-amplified products were cloned directly into a "TA" vector according to the manufacturer's instructions (Invitrogen, San Diego, CA). The PCR-amplified products from clone AP1.2 were sequenced directly using the Applied Biosystem DNA Sequencer.

Results

Using the RT-PCR technique, we first analyzed the expression of TCR genes from two polyclonal Amb a 5-specific T cell lines (APL and DSL) of two atopic subjects. RNAs from the pooled human PBMC were used as a positive control for each paired V β -C β or V α -C α primers. Table I summarizes the data from experiments using RNAs isolated from the cells. Although specific amplification for the pooled PMBCs samples was obtained for each primer pair, a set of nine V β and three V α -genes was found in a polyclonal T cell line (APL) of patient AP. A similar profile of TCR α - and β -gene use (including V β 5.2/5.3) was also found in an additional Amb a 5-specific T cell line (DSL) of patient DS, except that the V β 9 and V β 10 genes were not found in this cell line in three independent experiments. However, V β 9-C β - and V β 9-C β -amplified fragments could be found in the PBMC of subject DS. This finding suggests some variation in the Ag-specific T cell repertoire in the two study subjects.

(A) Clone AP1.2

D β
V β 5.2 — CCCCTTGTGGCGGACCGAAT — J β 1.5 — C β 1 —
V α 8 — GTGACA — J α — C α —

(B)

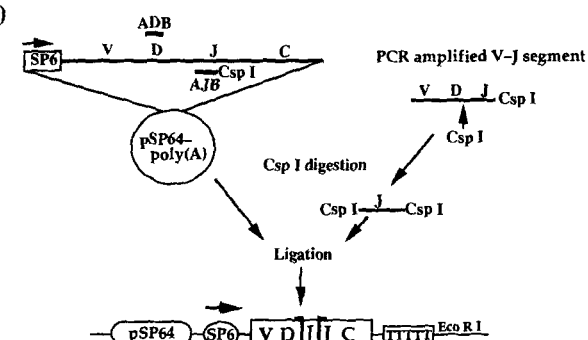


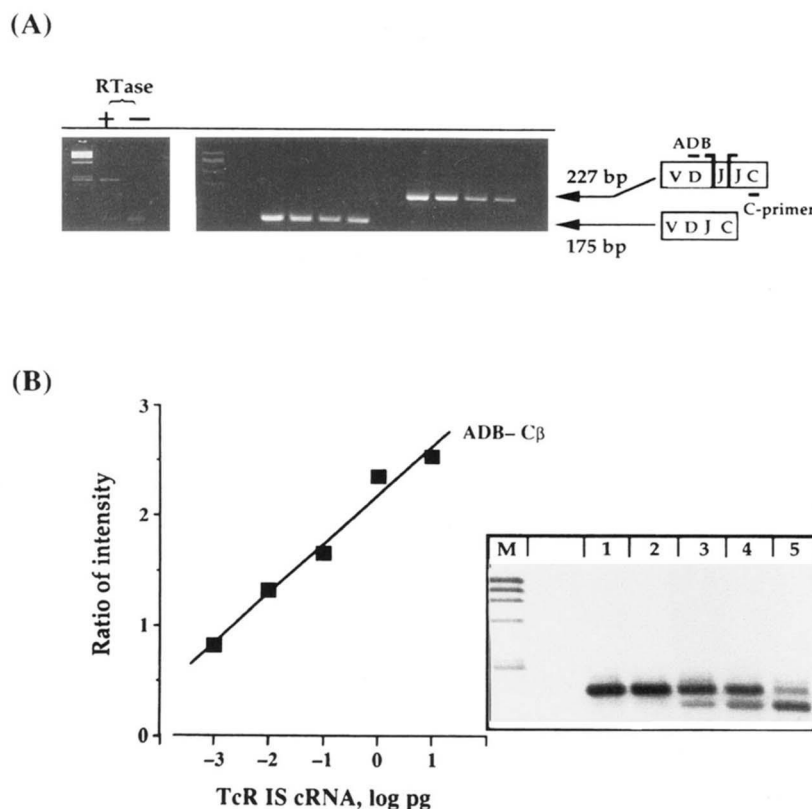
FIGURE 2. (A) Junctional sequences of TCR α - and β -genes of an Amb a 5-specific T cell clone, AP1.2. The amplified AP1.2 gene segments were electroeluted from the gel and sequenced using an internal C or V region oligoprimers. The sequencing results (accession number L38449) have been reproduced in five independent experiments using RNAs isolated from cells on different occasions; (B) cloning of AP1.2 TCR β -gene fragment into pSP64-polyA vector; see *Materials and Methods* for detailed methodology.

Flow cytometry analysis of the AP line with MAb 1C1 demonstrated that 30% of the cells in the AP line express V β 5.2/5.3 (Fig. 1). Sequencing of the amplified fragments showed only V β 5.2 gene sequences identical to those already published (see below) (8, 21).

Clone AP1.2 was one of the first generated from the APL cells (12). It is restricted by DR α , β *1501, expresses a Th2-cytokine profile, and is able to induce Amb a 5-specific IgE synthesis (13). In a total of five independent RT-PCR experiments, analysis of the TCR gene use showed that clone AP1.2 expresses V α 8 and V β 5.2 genes (Fig. 2A) and is also stained positive for MAb 1C1 (data not shown). Analysis of the sequences of the amplified TCR α - and β -gene fragments from clone AP1.2, using either internal C or V region oligoprimers, showed that clone AP1.2 has unique CDR3 sequences in both TCR α - and β -genes (Fig. 2A).

Because of the low frequency of Amb a 5-specific T cells in the peripheral blood of subjects (12), the representation of the AP1.2 β -gene in the PBMC and a polyclonal Amb a 5-specific T cell line of patient AP was analyzed. For this purpose, we designed a QC-PCR assay to analyze directly the AP1.2-specific sequence in the cells. We created a cRNA standard by duplicating the J β region gene sequence of the TCR β -gene in clone AP1.2. Figure 2B schematically illustrates our strategy, and detailed methodology is described in *Materials and Methods*. This modified gene segment served as a competitor in the QC-PCR assay. Results from a representative RT-PCR analysis using a D region primer (ADB) paired with a C β

FIGURE 3. (A) RT-PCR amplifications of a series of four threefold dilutions of RNAs from either the modified AP1.2 gene (with a duplication of the J β region segment; the IS) or clone AP1.2 using the AP1.2 D β region-specific primer, ADB, and a C β primer; the RT-PCR step for the IS was also performed either with or without the reverse transcriptase (RTase) and in the presence of the V β 5.2 and C β primers; (B) competitive PCR analysis of AP1.2 TCR β -genes. A series of five 10-fold dilutions (starting from 10 pg; inset, lanes 1–5; M, λ phage *Hae*III-DNA size marker) of the IS cRNAs were each mixed with 200 ng of RNAs from clone AP1.2 and were subjected to RT and PCR in the presence of a C β and a D β (ADB) primer. The amplified products were then separated by electrophoresis on an agarose gel containing ethidium bromide, and the intensities of the amplified fragments were scanned on a flat-bed scanner and the digital data were analyzed with the aid of ImageQuant software.



primer are shown in Figure 3A. First, analysis of the IS without the addition of the reverse transcriptase showed no amplified products, demonstrating no plasmid contamination. Second, specific amplification of a 279-bp fragment was observed when using V β 5.2 and C β primers. Third, using the D β region primer for the PCR, a larger amplified fragment (227 bp) from the IS can be seen on an agarose gel when compared with the product (175 bp) amplified from the wild-type template.

To optimize the QC-PCR assay, we investigated the linearity, reproducibility, and range of exponential amplification of the assay (17). A series of five 10-fold dilutions of IS cRNAs (starting from 10 pg) were mixed with varying amounts (ranging from 50 to 1 μ g) of clone AP1.2 RNA and were subjected to RT-PCR at varying cycle numbers in the presence of either a V β 5.2 or an AP1.2 D β -specific primer (ADB), each paired with a C β primer. The intensities of the amplified products were measured by densitometry, and the ratios of the relative intensities (IS/AP1.2; corrected for different m.w.) were plotted against varying amounts of input IS cRNAs. Figure 3B shows representative results under optimal conditions. A linear competitive amplification (45 cycles) was obtained from analysis of IS cRNA mixed with 100 ng of clone AP1.2 RNA. Similar results were also obtained when a QC-PCR assay was performed using a V β 5.2 and a C β primer.

Using the AP1.2 IS as a competitor and using the QC-PCR assay, the relative levels of expression of the AP1.2 sequence in autologous PBMC and a T cell line, APL,

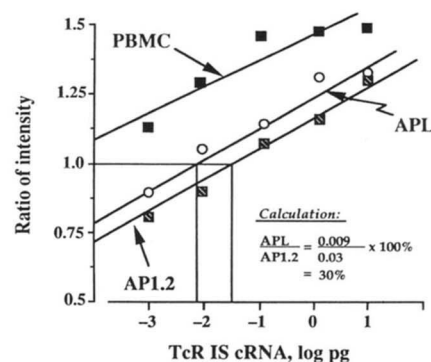


FIGURE 4. Competitive PCR analysis of AP1.2 TCR β -genes in the PBMC and a polyclonal T cell line, APL. Varying amounts of the IS cRNAs were mixed with 200 ng of total RNAs from cell cultures and were subjected to RT and PCR in the presence of C β and D β primers. The amplified products were then separated on an agarose gel; the intensities of the amplified fragments were scanned, and the digital data were analyzed.

were analyzed. We varied the amount of IS by serial 10-fold dilutions starting from 10 pg, keeping the total RNA from the cells constant (200 ng). A linear relationship between the input target template and output densitometric readings of the amplified products was obtained for analysis of cells from APL line and clone AP1.2, and the linear regression curves for both samples were parallel (Fig. 4); but, the RT-PCR products of PBMC reached saturation level when the input of the IS was over 10^{-8} μ g. When the

Table II. Amino acid sequences derived from sequencing of PCR-amplified fragments

	V β 5.2	D β	J β	C β
AP1.2	--SALYLCASS	PLVGGPN	QPQHFQDGTSLIL (1.5)	EDLNKVFPP-- C β 1
APL (9/11)	--SALYLCASS	PLVGGPN	QPQHFQDGTSLIL (1.5)	EDLNKVFPP-- C β 1
APL (2/11)	--SALYLCASS	PLVGGPN	QPQHFQDGTSLIL	EDLNKVFPP-- C β 1
DSL (7/13)	--SALYLCASS	PLVGGPN	QPQHFQDGTSLIL (1.5)	EDLNKVFPP-- C β 1
617 ^a (2/3)	--SALYLCASS	YGQAS	TDTQYFGLGTRTLVL (2.3)	EDLNKVFPP-- C β 2
617 ^a (1/3)	--SALYLCASS	YVNRP	AQ --	

^a From lung lavage cells of an asthmatic, Amb a 5-negative patient after short ragweed allergen challenge.

ratios in the samples equaled 1, the amount of AP1.2 β -gene transcript was at least three logs higher in clone AP1.2 than in the PBMC, and 30% of AP1.2 β transcripts were found in the polyclonal T cell line (APL). Furthermore, when a V β 5.2 upstream primer was used instead of a D β primer, 30% of the transcripts of the APL line were AP1.2 β -genes (data not shown). These results suggest that the frequency of AP1.2-like cells is low in the PBMC but is dominant in a polyclonal cell line. Similar results were obtained from the measurement of the ratio of the radioactivities (cpm) of each amplified fragments when a trace amount of [³²P]dCTP was included in the assay. We also analyzed the Amb a 5-specific, DR α , β 1*1501-restricted T cell line from a ragweed allergic subject, DS. The ADB-specific sequence was also found in this cell line, as confirmed by sequencing of the amplified V-C fragment (see below).

To confirm the data from competitive PCR analysis, the PCR-amplified V β 5.2-C β fragments from clone AP1.2 and two T cell lines (APL and DSL) were cloned and sequenced. In parallel experiments, we also cloned V β 5.2-C β fragments from bronchoalveolar lavage cells of an asthmatic patient (#617; who is ragweed-allergic but has no serum Abs to Amb a 5) after challenge with ragweed allergen extract as control. Results show that out of 11 clones sequenced from the APL line, 9 clones showed identical AP1.2 β -gene sequences, including the CDR3 region (Table II). Similarly, in the DSL line, 7 out of 13 clones were found to have the identical sequence. Furthermore, cloning and sequencing of three amplified V β 5.2-C β fragments of a control sample, #617, showed unique D β and J β sequences. Taken together, these results suggest that the AP1.2 β -gene sequence is a major β -gene component in a polyclonal Amb a 5-specific T cell line. These data are not only consistent with our PCR results but also suggest that the majority of the V β 5.2 sequences are identical to those of clone AP1.2.

Discussion

We have demonstrated the use of a competitive template to quantitate Ag-specific TCR β -gene sequences in two polyclonal T cell lines and in PBMC. These results suggest that a great diversity of Amb a 5-specific TCR gene use exists in polyclonal T cell lines of two atopic subjects having DR2.2 phenotypes. However, the use of clone AP1.2 β -gene is dom-

inant, and the AP1.2 β -gene sequence is also seen in an additional T cell line from an unrelated allergic patient. A great diversity of TCR gene uses in Ag-specific T cells that share the same Ag/MHC specificity has been observed (22), and a highly nonrandom occurrence of particular amino acid residues in the CDR3 region has also been found (23). In our study of the V β sequences in the Amb a 5-specific cell lines, however, no apparent similarity could be identified between each of the V β -amplified fragments. It is not clear whether similar or identical sequences can be found in the junctional regions of the V α -genes. Work is in progress to examine this issue.

It is of interest, however, that a dominant and identical Amb a 5-specific TCR V β sequence can be found in two unrelated subjects of different race, suggesting a strong sequence selectivity in the responses of T cells to Amb a 5. It will be of interest to examine whether other Amb a 5-sensitive, DR2⁺ subjects show similar dominant and identical TCR β gene sequences. As a corollary, several studies have demonstrated identical TCR V β sequences in the PBMC and brain tissues of patients with multiple sclerosis (5, 24–26). Of interest, the uses of both V β 5.2 and V α 8 appear to be over-represented. In our study, the finding that Amb a 5-specific T cells share similar V gene uses, although they differ in their CDR3 region sequences, may reflect the same DR2 restriction specificity as those of myelin basic protein-specific T cells in patients with multiple sclerosis (24).

Current techniques assessing TCR V gene use and sequence use a large panel of V β and V α primers, and each, together with a C region primer (4–6) or “anchored-PCR” (27) and “inverse-PCR” (6), have been used in a variety of experimental situations and disease states. More recently, Choi et al. (9) have used a C β -C β reference template in a “quantitative” PCR method to enumerate the relative frequency of TCR V gene expression in cells or tissues. These methods, although informative, require subsequent multiple cloning and sequencing of each sample in each experiment. Furthermore, variability in the efficiency of particular primers introduces uncertainty into the quantitation process, and the methods are unable to evaluate the CDR3 sequences directly. We (17) and others (28, 29) have previously developed a QC-PCR assay, in which an exogenous cytokine RNA standard is amplified with the analyzed template in the same reaction tube using one pair of primers. Our study shows that a similar competitive PCR assay can be used in analysis of

specific TCR gene expression. We demonstrate that the PCR amplification is linear and reproducible. After the products are subjected to gel electrophoresis, a numerical value for the PCR products can be obtained either by densitometry of the bands or by scintillation counting of incorporated [^{32} P]dCTP of the excised bands. Our data suggest that the technique of quantitative PCR, using a competitive template as the IS, can provide an efficient tool to measure TCR gene expression without the need to use multiple cloning and sequencing steps.

Selective CD4⁺ Th2 cell infiltration has been demonstrated in the skin and lung after allergen challenge (10, 11). It is, however, unclear whether the specific T cells are activated in situ by allergen, and the clonality of specific T cells in the lesion or the allergen-challenged sites remains to be determined. We believe that quantitative PCR amplification of allergen-specific TCR sequences will provide an important tool to study the allergen-specific T cells in this situation. In addition, various inflammatory lesions associated with multiple sclerosis, rheumatoid arthritis, and atopic and other diseases could potentially be studied in this manner.

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References

- Davis, M. M., and P. J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature* 334:395.
- Engel, I., and S. M. Hedrick. 1988. Site-directed mutations in the VDJ junctional region of a T cell receptor β chain cause changes in antigenic peptide recognition. *Cell* 54:473.
- Dembic, Z., W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer, and M. Steinmetz. 1986. Transfer of specificity by murine α and β T-cell receptor genes. *Nature* 320:232.
- Dunn, D. A., A.-S. Gadenne, S. Smitha, E. A. Lerner, M. Bigby, and P. A. Bleicher. 1993. T-cell receptor V β expression in normal human skin. *Proc. Natl. Acad. Sci. USA* 90:1267.
- Wucherpfennig, K., K. Ota, N. Endo, J. G. Seidman, A. Rosenzweig, H. L. Weiner, and D. A. Hafler. 1990. Shared human T cell receptor V β usage to immunodominant regions of myelin basic protein. *Science* 248:1016.
- Uematsu, Y., H. Wege, A. Straus, M. Ott, W. Bannwarth, J. Lanchbury, G. Panayi, and M. Steinmetz. 1991. The T-cell-receptor repertoire in the synovial fluid of a patient with rheumatoid arthritis is polyclonal. *Proc. Natl. Acad. Sci. USA* 88:8534.
- Ferradini, L., A. MacKensen, C. Genevec, J. Bosq, P. Duvillard, M.-F. Avril, and T. Hercend. 1993. Analysis of T cell receptor variability in tumor-infiltrating lymphocytes from a human regressive melanoma: evidence for in situ T cell clonal expansion. *J. Clin. Invest.* 91:1183.
- Weyand, C. M., J. Schonberger, U. Oppitz, N. N. H. Hunder, K. C. Hicok, and J. J. Goronzy. 1994. Distinct vascular lesions in giant cell arteritis share identical T cell clonotypes. *J. Exp. Med.* 179:951.
- Choi, Y., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler. 1989. Interaction of *Staphylococcus aureus* toxin "superantigen" with human T cells. *Proc. Natl. Acad. Sci. USA* 86:8941.
- Sager, N., A. Feldmann, G. Schilling, P. Kreitsch, and C. Neumann. 1992. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: frequency and lymphokine profile in the allergen patch test. *J. Allergy Clin. Immunol.* 89:801.
- Robinson, D. S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326:298.
- Huang, S. K., P. Zwollo, and D. G. Marsh. 1991. Class II MHC restriction specificity of human T-cell responses to a short ragweed pollen allergen, Amb a V. *Eur. J. Immunol.* 21:1469.
- Shinomyia, N., M. Kumai, D. G. Marsh, and S. K. Huang. 1993. Detection of specific IgE-secreting cells using an enzyme-linked immunospot assay. *J. Allergy Clin. Immunol.* 92:479.
- Mole, L. E., L. Goodfriend, C. B. Lapkoff, J. M. Kehoe, J. D. Capra. 1975. The amino acid sequence of allergen Ra5. *Biochemistry* 14:1216.
- Kumai, M., G. Krishnaswamy, D. G. Marsh, and S. K. Huang. 1993. Modulating effect of human monocyte Fc receptor on allergen-induced T-cell responses. *Immunology* 79:174.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162, 156.
- Huang, S. K., D. Essayan, G. Krishnaswamy, M. Yi, M. Kumai, H.-Q. Xiao, S.-N. Su, L. M. Lichtenstein, and M. C. Liu. 1994. Detection of allergen- and mitogen-induced human cytokine transcripts using a competitive polymerase chain reaction. *J. Immunol. Methods* 168:167.
- Panzara, M. A., E. Gussoni, L. Steinman, and J. R. Oksenberg. 1992. Analysis of the T cell receptor using the PCR and specific oligonucleotide primers. *Biotechniques* 12:728.
- MacGlashan Jr., D., J. M. White, S. K. Huang, S. J. Ono, and L. M. Lichtenstein. 1994. Secretion of interleukin-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J. Immunol.* 152:3006.
- Krishnaswamy, G., M. Kumai, M. C. Liu, S.-N. Su, B. Stealey, D. G. Marsh, and S. K. Huang. 1993. Detection of cytokine transcripts in the bronchoalveolar lavage cells of patients with asthma. *Am. J. Respir. Cell Mol. Biol.* 9:279.
- Kimura, N., B. Toyonaga, Y. Yoshikai, R. P. Du, and T. W. Mak. 1987. Sequences and repertoire of the human T cell receptor α and β chain variable region genes in thymocytes. *Eur. J. Immunol.* 17:375.
- Giegerich, G., M. Pette, E. Meinel, J. T. Epplen, H. Wekerle, and A. Hinkkanen. 1992. Diversity of T cell receptor α and β chain genes expressed by human T cells specific for similar myelin basic protein peptide/major histocompatibility complexes. *Eur. J. Immunol.* 22:753.
- Dwyer, E., S. Itescu, and R. Winchester. 1993. Characterization of the primary structure of T cell receptor β chains in cells infiltrating the salivary gland in the sicca syndrome of HIV-1 infection. Evidence of antigen-driven clonal selection suggested restricted combinations of V β J β gene segment usage and shared somatically encoded amino acid residues. *J. Clin. Invest.* 92:495.
- Chou, Y. K., P. Henderikx, M. Vainiene, R. Whitham, D. Bourdette, C. H.-J. Chou, G. Hashim, H. Offner, and A. A. Vandenbark. 1991. Specificity of human T cell clones reactive to immunodominant epitopes of myelin basic protein. *J. Neurosci. Res.* 28:280.
- Oksenberg, J. R., M. A. Panzara, A. B. Begovich, D. Mitchell, H. A. Erlich, R. S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C. C. A. Bernard, and L. Steinman. 1993. Selection of T-cell receptor V β -D β -J β gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature* 362:68.
- Oksenberg, J. R., S. Stuart, A. B. Begovich, R. B. Bell, H. A. Erlich, L. Steinman, and C. C. A. Bernard. 1990. Limited heterogeneity of rearranged T-cell V alpha transcripts in brains of multiple sclerosis patients. *Nature* 345:344.
- Loh, E. Y., J. F. Elliott, S. Cwirla, L. L. Lanier, and M. M. Davis. 1989. Polymerase chain reaction with single-sided specificity: analysis of T cell receptor delta chain. *Science* 243:217.
- Gilliland, G., S. Perrin, K. Blanchard, and H. F. Bunn. 1990. Analysis of cytokine mRNA and DNA. Detection and quantitation by competitive polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* 87:2725.
- Feldman, A. M., P. E. Ray, C. M. Silan, J. A. Mercer, W. Minobe, and M. R. Bristow. 1991. Selective gene expression in failing human heart: quantitation of steady-state levels of messenger RNA in endocardial biopsies using the polymerase chain reaction. *J. Clin. Invest.* 83:1866.