cut behind the eye; and the ipsilateral map was recorded.
14. We did not include data from those sites at which we recorded only contralateral input or from those sites that received input from the monocular region of the contralateral eye's visual field. No statistically significant difference was observed between the control tecta with Elvax implants and those without. The data are therefore presented together. Data for azimuth alone rather than angular separation were analyzed because of slight tipping of the head in some frogs when the optic nerve was cut after mapping the contralateral projection.
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Predominant Expression of T Cell Receptor $V_\alpha 7$ in Tumor-Infiltrating Lymphocytes of Uveal Melanoma

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Expression of T cell receptor (TCR) V_α genes in tumor-infiltrating lymphocytes (TILs) within intraocular melanoma was studied. Primers for 18 different human TCR V_α families were used to analyze TCR V_α-C_α gene rearrangements in TIL in these melanomas obtained at surgery. A limited number of TCR V_α genes were expressed and rearranged in these tumors, and TILs expressing $V_\alpha 7$ were found in seven of eight of these uveal melanomas. TCR gene usage is also restricted in experimental autoimmune disease, in T cells within organs like skin and other epithelial tissues, and in the brain of patients with multiple sclerosis (MS). The restricted usage of TCR genes in TIL may indicate that a specific antigen in these melanomas is targeted.

IDENTIFICATION OF THE SPECIFICITY of T cells involved in the local immune response to malignant tumors is an important step in the development of cancer immunotherapy. The use of expanded populations of TILs for therapy appears promising in animal models of solid tumor and in human clinical trials of melanoma (*1*), although the TCR genes expressed in TILs are not yet known. Recent evidence from studies in autoimmunity (*2, 3*) and allograft rejection (*4*) indicates that effector cells may utilize a very limited range of TCR genes. We asked whether there was restricted heterogeneity of TCR expression in TILs.

We chose to study melanoma of the uveal tract, which includes the pigmented portion of the eye, the iris, ciliary body, and choroid, because circumstantial evidence suggests that this tumor elicits an immune response that may protect the host (*5, 6*). In humans with uveal melanoma and in murine models of ocular melanoma, antitumor immunity to melanoma antigens is inducible (*7*). Melanoma of the uveal tract are often fatal, with nearly half of the patients dying within 10 to 15 years of enucleation (*7*). The primary lesions of uveal melanoma, like those of its cutaneous counterpart, may remain localized for long periods, metastatic disease may ensue only decades later, and spontaneous remissions occur (*6*).

To determine the extent of TCR gene usage by TILs, we examined the diversity of V_α genes in transcripts of rearranged TCR α-chain genes in eight uveal melanoma specimens obtained at surgery (*8*). Various methods could have been used to address this question, including immunohistochemical staining for TCRs, Northern blotting of mRNA from tissues, or extended in vitro culture of TILs (*9*). Unfortunately, a wide panel of monoclonal antibodies (MAbs) specific for TCR V_α regions in humans is unavailable. Also mRNA or DNA from TIL in surgical specimens is often scarce. Long-term culture of TILs in vitro may bias analysis of the TCR repertoire. Therefore, we decided to exploit gene amplification by polymerase chain reaction (PCR). The PCR method allows enzymatic amplification of a target DNA sequence. By selection of the appropriate oligonucleotides, the method is sensitive enough to detect a DNA sequence from one TIL in a population of 10^5 cells within a solid tumor mass (*10*).

To analyze the usage of V_α genes, we analyzed the cDNA reverse transcribed from mRNA isolated from uveal melanoma specimens. Eighteen different V_α-specific oligo-

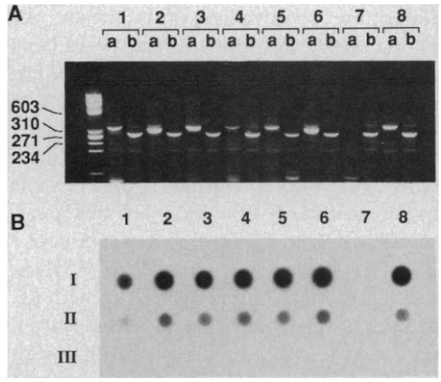

Fig. 1. (**A**) Amplified products from eight uveal melanoma tissues are shown after 35 cycles of PCR with 5′-sense primer of $V_\alpha 7$ and 3′-primer of C_α as shown in Table 1 (lanes a) and primers for melanotransferrin (L-MEL 1; 5′-TAC CTG GTG GAG AGC GGC CGC CTC-3′, R-MEL 2; 5′-AGC GTC TTC CCA TCA GTG T-3′) (lanes b). The PCR conditions were the same as in Table 2. The size of amplified products obtained with melanotransferrin primers was 286 bp. The leftmost lane shows the marker sizes. Lane number indicates the case numbers of melanoma samples. (**B**) Dot blot hybridization of amplified cDNA from eight melanoma tissues. Row I (10 μl) and row II (1 μl) of each amplified product obtained with $V_\alpha 7$ and C_α primers as described in the legend to (A) were hybridized with [γ-^{32}P]-labeled $V_\alpha 7$ oligonucleotide probe (*17*). As a negative control, the PCR product of melanotransferrin primers was also blotted (row III, 10 μl per sample). Case numbers were the same in Fig. 1 and Table 2. After each sample of PCR-amplified product was denatured with 0.4 M NaOH for 10 min at room temperature, they were applied to nitrocellulose paper (GeneScreen Plus, Du Pont Biotechnology Systems). The paper was fixed with ultraviolet (UV) light for 10 min, then prehybridized in 5× SSPE (0.18 M NaCl, 10 mM phosphate (pH 7.4), and 1 mM EDTA), 5× Denhardt's solution, 0.1% SDS containing salmon sperm DNA at 42°C for 3 hours. Thereafter samples were hybridized for 12 to 16 hours with 5×10^6 to 8×10^6 cpm of ^{32}P-labeled $V_\alpha 7$ oligonucleotide probe at 42°C. After hybridization, blots were washed twice in 1× SSPE and 0.1% SDS at room temperature for 10 min and placed under x-ray film (X-O mat AR; Kodak) for 30 min to 2 hours to develop (*18*).

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nucleotides representing the major human TCR V_α families were used for the 5′-primers and a C_α sequence was used for the 3′-primer (Table 1). Total RNA was extracted from each of eight uveal melanoma samples and was reverse transcribed (*11*). The resulting cDNA was amplified by means of individual sets of V_α-C_α primers or with primers for melanotransferrin, a specific marker for melanoma (*12*). Each V_α primer yielded a band of 300 to 400 bp when the PCR product was stained with ethidium bromide.

The amplified products obtained with the $V_\alpha 7$ primer were further identified by hybridization with $V_\alpha 7$- and C_α-specific oligonucleotide probes. In all cases where the $V_\alpha 7$ rearranged product was visualized on agarose gel electrophoresis with ethidium bromide staining (Fig. 1A), a positive hybridization was observed on dot blotting to the $V_\alpha 7$ oligonucleotide probe (5′-CTG GAG CTC CTG TAG AAG GAG-3′) (Fig. 1B). Amplified melanotransferrin did not hybridize with this probe at all (Fig. 1B). In addition, the $V_\alpha 7$-C_α–amplified product hybridized to a C_α oligonucleotide probe (5′-CAG AAC CCT GAC CCT GCC GTG TAC-3′) but not with $V_\alpha 1$- and $V_\alpha 4$-specific oligonucleotide probes.

Analysis of V_α expression in TILs from melanoma specimens is shown in Table 2. Among seven of eight cases only one to three V_α were detected. In sample 8, five V_α genes ($V_\alpha 7$, 8, 9, 12, and 14) were detected.

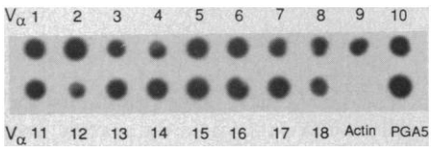

Fig. 2. Detection of PCR-amplified products by dot blot hybridization with a C_α probe on peripheral blood lymphocytes stimulated with PHA. Complementary DNA was prepared from 1 μg of mRNA isolated from a pool of peripheral blood lymphocytes from five different individuals, stimulated with PHA (3 μg/ml). The amplified product (10 μl) was dot-blotted onto transfer membranes (GeneScreen Plus, Du Pont Biotechnology Systems) after denaturation with 0.4 M NaOH and 25 mM Na_2EDTA. Actin PCR products served as negative controls and PGA 5 as the positive control. Filters were fixed under UV light and prehybridized for 3 hours at 42°C in 5× SSPE, 5× Denhardt's solution, salmon sperm DNA (100 μg/ml), and 0.1% SDS and hybridized overnight at 42°C with 1×10^6 cpm per milliliter of ^{32}P-labeled probe (5′-AATATCCA-GAACCCTGACCCT-3′) (*17*). Filters were washed in 1× SSPE and 0.1% SDS at 45°C twice for 10 min. Kodak XAR-5 film with Lightning-Plus intensity screens (Du Pont) were used for autoradiography at −70°C for 30 min. Signals correlate with the presence of a specific rearranged band in the ethidium bromide–stained agarose gel.

In seven of eight cases $V_\alpha 7$ was expressed and rearranged. Melanotransferrin was amplified in all cases. Amplification artifacts due to contaminating DNA were excluded by performing controls in which no amplification was observed without cDNA samples or with genomic DNA. Identical results were obtained after a further experiment with different aliquots of each patient's tumor.

We have demonstrated that all 18 TCR V_α primers work efficiently. Thus all 18 TCR 5′-primers amplify V_α-C_α rearrangements in pooled T cells stimulated by phytohemagglutinin (PHA) (Fig. 2) (*3*). Moreover, T cell clones responsive to pertussis toxin, alloantigens, or *Borrelia bergdorfei* were analyzed. Each T cell clone expressed only one TCR V_α gene. Individual clones expressed V_α 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 15, or 16.

Additional characterization of the $V_\alpha 7$-amplified products was obtained by restriction mapping with the endonucleases, Dde I, Kpn I, and Hinf I. The restriction pattern was consistent with the known map of $V_\alpha 7$ (*13*). Taken together, these results confirm that $V_\alpha 7$ is predominantly expressed in T cells infiltrating into uveal melanomas.

Restricted TCR gene expression has been demonstrated in encephalitogenic T cells mediating the prototypic autoimmune dis-

Table 1. Sequence of T cell receptor primers. Nucleotide sequence of primers used for PCR. The size of amplified products (V_α bands) with V_α and 3′ C_α primers ranged from about 300 to 400 bp. The sequences of V_α and C_α are from previously published reports (*14*). Oligonucleotides were synthesized and column purified by Operon Technologies, Inc. (Alameda, California).

Primer	Clone	5′ → 3′ sequence	Family members
$V_\alpha 1$	HAP 10	TTGCCCTGAGAGATGCCAGAG	1.1, 1.2, 1.3
$V_\alpha 2$	HAP 26	GTGTTCCCAGAGGGAGCCATTGCC	2.1, 2.2
$V_\alpha 3$	HAP 05	GGTGAACAGTCAACAGGGAGA	3.1
$V_\alpha 4$	HAP 08	ACAAGCATTACTGTACTCCTA	4.1
$V_\alpha 5$	HAP 35	GGCCCTGAACATTCAGGA	5.1
$V_\alpha 6$	HAP 01	GTCACTTTCTAGCCTGCTGA	6.1
$V_\alpha 7$	HAP 21	AGGAGCCATTGTCCAGATAAA	7.1, 7.2
$V_\alpha 8$	HAP 41	GGAGAGAATGTGGAGCAGCATC	8.1, 8.2
$V_\alpha 9$	HAP 36	ATCTCAGTGCTTGTGATAATA	9.1
$V_\alpha 10$	HAP 58	ACCCAGCTGGTGGAGCAGAGCCCT	10.1
$V_\alpha 11$	HAP 02	AGAAAGCAAGGACCAAGTGTT	11.1
$V_\alpha 12$	PGA 5	CAGAAGGTAACTCAAGCGCAGACT	12.1
$V_\alpha 13$	AB 11	GCTTATGAGAAACACTGCGT	13.1
$V_\alpha 14$	AB 21	GCAGCTTCCCTTCCAGCAAT	14.1
$V_\alpha 15$	AC 24	AGAACCTGACTGCCCAGGAA	15.1
$V_\alpha 16$	AE 212	CATCTCCATGGACTCATATGA	16.1
$V_\alpha 17$	AF 211	GACTATACTAACAGCATGT	17.1
$V_\alpha 18$	AC 9	TGTCAGGCAATGACAAGG	18.1
C_α	PGA 5	AATAGGTCGAGACACTTGTCACTGGA	C_α

Table 2. Usage of TCR V_α gene in uveal melanomas. All TCR 5′-primers amplify TCR sequences from germline DNA by means of a specific 3′-primer from V_α for each family. We have detected V_α-C_α rearrangements of all TCR V gene members in a variety of activated T cells. These include single rearrangements of specific V_α members in T cell clones reactive to pertussis toxin, *B. bergdorfei*, and alloantigens, as well as rearrangements of all V_α members in pooled T cells stimulated by PHA (*3*). TCR V_α families expressed in human uveal melanoma are shown. A single-stranded cDNA sample was amplified with a V_α-specific primer and with a C_α primer at a final concentration of 1 μM in each reaction. The amplification was performed with 2.5 units of *Taq* polymerase (Ampli Taq; Perkin-Elmer Cetus) on a DNA thermal cycler (Perkin-Elmer Cetus). The PCR cycle profile was 95°C denaturation for 1 min, annealing of primers at 55°C for 1 min, and extension of primers at 72°C for 1 min for 35 cycles. PCR products were separated on 1% regular agarose and 3% NuSieve agarose gels (FMC Corporation) and expression of V_α families was considered positive when a rearranged band (300 to 400 bp) was visualized with ethidium bromide staining. Experiments were repeated three times per sample. Results were identical with a different aliquot of each sample.

Case no.	V_α families
1	$V_\alpha 2$ $V_\alpha 7$
2	$V_\alpha 6$ $V_\alpha 7$ $V_\alpha 13$
3	$V_\alpha 7$ $V_\alpha 13$
4	$V_\alpha 7$ $V_\alpha 13$ $V_\alpha 14$
5	$V_\alpha 7$
6	$V_\alpha 7$ $V_\alpha 10$
7	$V_\alpha 12$
8	$V_\alpha 7$ $V_\alpha 8$ $V_\alpha 9$ $V_\alpha 12$ $V_\alpha 14$

ease, experimental autoimmune encephalomyelitis (*2*), in T cells in demyelinating plaques from brains of patients with multiple sclerosis (*3*), and on T cells along the body's surface like skin, the tongue, and vagina (*14*). A recent study indicates oligoclonality of TCR expression in bulk cultures of TILs analyzed by Southern (DNA) blots (*15*). Also some alloreactive and virus-specific cytotoxic T cells use restricted TCR genes (*16*). Our data indicate that TILs may have a restricted TCR repertoire. If the TILs expressing $V_\alpha 7$ are involved in an antitumor response, specific culture of such cells could be advantageous in tumor therapy. Moreover, the technology should be useful for analysis of TILs within other types of solid tumor.

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Regional Variation of Extracellular Space in the Hippocampus

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The factors responsible for the unusual susceptibility of the hippocampus to seizures and ischemic cell damage are not well understood. The CA1 pyramidal subfield of the hippocampus is particularly vulnerable to seizure activity and damage after ischemia. The possibility was examined that regional differences exist in extracellular volume, which might influence neuronal excitability and response to injury in the hippocampus. CA1 stratum pyramidale exhibited an exceptionally low extracellular volume fraction (EVF) of 0.12, whereas the EVFs of CA3 and dentate were considerably higher—0.18 and 0.15, respectively. The EVF of CA1 stratum pyramidale was reversibly reduced by 30 percent when the extracellular potassium concentration was raised from 3.5 to 8.5 mM, a procedure that induced spontaneous electrographic seizures in CA1. Thus there are regional variations in the properties of the extracellular space in the hippocampus that might underlie the propensity of the CA1 region to develop seizures and to suffer damage after ischemia.

THE EXTRACELLULAR VOLUME FRACTION and tortuous diffusion pathways set fundamental constraints on the movement of ions and other substances within the interstices of the mammalian brain (*1*). Most estimates of EVF, made with a variety of techniques in several species and brain regions, fall within a narrow range of 0.17 to 0.22 (*1, 2*). Conditions that result in shrinkage or expansion of extracellular space have long been suspected of influencing neuronal activity (*3–5*). For example, shrinkage of extracellular space could, if large enough, enhance neuronal synchronization as a result of stronger electric field interactions and by a volume effect amplify the transient elevations in the extracellular potassium concentration ($[K^+]_o$) and transmitter concentrations that develop during neuronal activity (*4, 6–8*). The aim of this study was to make quantitative measurements of EVF and tortuosity (*9*) within the various layers of the hippocampal slice under both physiological and pathological conditions. We calculated the volume fraction and tortuosity of extracellular space in rat hippocampal slices (*10*) by the method of Nicholson and Phillips (*1*). Briefly, an ion-selective electrode was used to measure in real time the diffusion profile of an iontophoretically applied ion, tetramethylammonium (TMA^+), that is restricted to the extracellular compartment (Fig. 1A). The time-dependent rise and fall of the extracellular concentration of TMA^+ (Fig. 1B) is then fitted to a radial diffusion equation modified to account for EVF and tortuosity (*11*). The movement of TMA^+ within all regions of the hippocampus that we tested was well fit by the diffusion equation, irrespective of iontophoretic current intensity (Fig. 1B) or electrode spacing (*12*). Both EVF and tortuosity showed considerable regional variation (Table 1). The EVF of CA3 stratum pyramidale (0.18) was within the usual range (*1, 2*), but EVF of CA1 st. pyramidale (0.12) was 33% smaller (Fig. 1C

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