BOTH RAT AND MOUSE T CELL RECEPTORS SPECIFIC FOR THE ENCEPHALITOGENIC DETERMINANT OF MYELIN BASIC PROTEIN USE SIMILAR V α AND V β CHAIN GENES EVEN THOUGH THE MAJOR HISTOCOMPATIBILITY COMPLEX AND ENCEPHALITOGENIC DETERMINANTS BEING RECOGNIZED ARE DIFFERENT

By FRANK R. BURNS,* XIAOBIN LI,* NING SHEN,* HALINA OFFNER, YUAN K. CHOU, ARTHUR A. VANDENBARK, AND ELLEN HEBER-KATZ*

From *The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104; and the Department of Neurology, Microbiology and Immunology, Oregon Health Science University, Portland, Oregon 97207

The study of an autoimmune disease model such as experimental allergic encephalomyelitis (EAE)[1] will hopefully reveal a means to control such disease. EAE is an antigen-specific T cell–mediated disease that is considered to be a model for multiple sclerosis due to its similar pattern of central nervous system (CNS) lesions and demyelination (1, 2). This disease was first noted in patients who had developed neurological deficits after inoculation with early rabies vaccine preparations containing residual myelin from in vivo brain culture of the virus (3). One obvious target for regulation of this T cell–mediated disease would be a TCR idiotype. This would require (a) that the disease-inducing T cells respond to one (or a few) auto-antigenic determinant; and (b) that they use a restricted set of TCR genes to recognize this antigen.

That a limited number of antigenic determinants are responsible for EAE has been established in several animal models, including mouse, rat, and guinea pig. In all cases, myelin basic protein (MBP) is the target agent (4–6). Using small peptides it was further shown that, in Lewis rat, all of the encephalitogenic activity could be localized to the amino acid 68–88 fragment (MBP 68–88) (5, 7). Thus, a limited region of a single protein, and presumably, therefore, a single or limited number of antigenic determinants, is responsible for activating EAE-inducing T cells (8).

Evidence exists both for and against limited TCR repertoire usage in Lewis rat, EAE-inducing (MBP 68–88-reactive) T cells. It has been shown that protection from disease could be obtained by prior immunization with either inactivated EAE-inducing

[1] *Abbreviations used in this paper:* CNS, central nervous system; EAE, experimental allergic encephalomyelitis; GP, guinea pig; MBP, myelin basic protein.

T cell lines or clones, while no protection was derived from immunization with T cells of irrelevant antigen specificity (9), implying that a determinant present upon encephalitogenic T cells, but not the general T cell population, was inducing protection. Thus, EAE-inducing T cells might share a crossreactive idiotypic marker capable of conferring protection. This concept has been supported by recent reports of suppressor T cells capable of inhibiting EAE (10, 11) and most recently by our finding that an anti-TCR antibody could indeed modulate EAE in the Lewis rat (12, 13).

On the other hand, studies in our laboratory on MBP 68–88-specific Lewis rat × BW5147 hybrid T cells found that these cells use a diverse but limited repertoire of Vβ-Jβ rearrangements (Southern blot data obtained using β constant region probes) (14). It was also found that these T cell hybrids could be divided into three distinct antigen fine specificity patterns based on the degree of their responsiveness to MBP and MBP peptides from various species (8). Thus, our results were indicative in this case of at least some degree of diversity existing in Lewis rat MBP 68–88-specific T-cells.

To determine if MBP 68–88-specific T cells could share idiotypic markers conferred via common V region usage, we generated a cDNA library from the MBP 68–88-specific T cell hybridoma 510, sequenced the TCR genes, and made V region probes derived from this clone. We found that there was extensive sharing of V genes between T cells reactive to the encephalitogenic determinant of MBP, a finding similar to that already seen in the mouse (15–17). Furthermore, upon comparison of the rat and mouse data, we found an unexpected sharing of analogous V regions even though different encephalitogenic determinants were being recognized in association with different MHC molecules.

Materials and Methods

DNA and RNA Preparations and cDNA Library Construction.   Using 1–5 × 10⁷ cells from each of the T cell hybridomas, lines, and clones, DNA and total cell RNA were isolated by guanidine isothiocyanate CsCl gradient (18). Briefly, cells were centrifuged at 1,500 rpm for 10 min and resuspended in 3 ml of 4 M GIT buffer, then vortexed to break cells apart. The cell preparation was then added on top of a 2-ml 5.7 M CsCl cushion and ultracentrifuged for 18 h at 35,000 rpm using an SW 50.1 rotor. RNAs were pelleted to the bottom of the centrifuge tubes, and DNA was located in the layer above the RNA within CsCl cushion. DNA was recovered by ethanol precipitation and purified with proteinase K and phenol/chloroform. RNA pellets were dissolved in sterile water then precipitated with 0.3 M sodium acetate and 2.5 vol of 100% ethanol.

Poly(A)⁺ RNA was selected for by two passages over oligo-dt-cellulose (19). cDNA synthesis was performed using a cDNA synthesis kit (Amersham Corp., Arlington Heights, IL) as per manufacturer's instructions. Eco RI methylase was used to protect internal sites. cDNAs were ligated to phosphorylated Eco RI linkers (New England Biolabs, Beverly, MA). Excess linkers were removed by Eco RI digestion. The cDNAs were cloned into the Eco RI site of λgt11. Viral DNA was then packaged (Packagene; Promega Biotech, Madison, WI). Murine C region probes (provided by S. Hedrick, UCSD, La Jolla, CA) were used to isolate the rat TCR cDNA clones. The cDNAs and appropriate restriction fragments were subcloned into M13 and sequenced in both directions by dideoxy chain termination. Sequence analysis was carried out using University of Wisconsin (Madison, WI) software (20).

T Cell Cultures and Testing of Specificity.   Lewis rats were challenged subcutaneously with emulsions of Freund's complete adjuvant containing GP-MBP. T lymphocyte lines, BP20 and BP29, were selected from suspensions of lymph node cells after immunization. Cells were stimulated once with GP-MBP together with x-irradiated thymocytes as APC. Clones 3–6 were selected from the BP20 T cell line and clones 11–17 were selected from the BP29

line after stimulation. All the clones were restimulated once with antigen and thymocytes as mentioned above. Cell lines and clones were shown to be of the T helper phenotype, using W3/25 mAbs. T cell proliferation was observed by incubating cell lines and clones with MBP for 72 h, followed by 0.5 mCi [^{3}H]thymidine for 18 h. Labeled thymidine uptake was recorded. Activation of T cell lines and clones for EAE transfer was carried out using 5 × 10^6 cells, 5 × 10^6 X-irradiated APC, and 125 mg/ml antigen in 10 ml of stimulation medium, and then was cultured for 3 d. The blasts were counted and 10^6 cells were injected intraperitoneally into naive Lewis rats. The clinical signs of disease were recorded and scored. Delayed-type hypersensitivity was also recorded as a change in ear thickness (21).

Vα 510 and Vβ 510 Probes. Vα 510 and Vβ 510 probes were prepared from isolated Lewis rat × BW5174 hybridoma 510 TCR α and β chain cDNA clones. The Vα probe is 136 bp with Eco RI and Pst I restriction enzyme treatment and the Vβ probe is 240 bp with Eco RI and Alu I treatment. The Vβ probe includes 6 bp of D region sequence (Fig. 2).

RNA Blots. Northern blot hybridizations were done using 20 μg of total RNA from each T cell line and clone; RNAs were fractionated on a 1.0% agarose gel with 1 × MOPS buffer and 2% formaldehyde was used (18). RNAs were blotted to Nytran and then hybridized with ^{32}P-labeled Vα 510 and Vβ 510 probes in 50% formamide 5× SSC, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 1 × Denhardt's solution at 42°C for 12 h (Nytran protocol; Schleicher & Schuell, Inc., Keene, NH). The Northern blots were then washed in 0.1 × SSC and 0.1% SDS at 52°C for 2 h with two changes of washing buffer.

Results

The 510 α Chain. The variable region of the 510 α chain was sequenced (Fig. 1) and the closest match was found to be the mouse TA39, a member of the Tα2 family (22), with a 77% homology at the amino acid level.

The joining region also bore greatest homology to the J region of TA39. All of the matches, however, fell towards the COOH-terminal end of the joining region (9 of 13 amino acids identical between residues 101 and 113, inclusive), while there was no demonstrable homology between amino acid residues 94 and 100, inclusive, and any published J region.

Constant region nucleotide sequence comparisons with a recently published rat TCR α chain of the AO rat strain (23) revealed 100% homology within the coding region, but slight differences in the 3' untranslated region, which we attribute to inter-strain polymorphism. In addition, our clone contained an additional 164 bases of 3' untranslated region.

The 510 β Chain. The Vβ510 sequence (Fig. 2) showed a maximum homology of 79% at the amino acid level with Vβ4 of mouse (24). This Vβ was later designated 8.2 by others (25). The β J and D regions were most homologous with the Jβ1.1 and Dβ1 of the mouse; the J region being 94% homologous at the nucleic acid level, while a D segment of 5 bp matched exactly with a portion of the mouse Dβ1 region. The β chain constant region was the Cβ1 homologue and has not been previously reported for rat.

MBP 68-88-specific Autoreactive Hybridomas and T Cell Clones. To determine the V gene usage by the MBP-specific T cell repertoire, T cells of two types were examined. The first were antigen-specific Lewis rat × BW5147 T cell hybridomas that were generated from 10 different fusions of primary cell cultures as described in Table I (8). These hybridomas had previously been shown to display different β chain rearrangements on Southern blot analysis using C region probes (14). The second population was composed of encephalitogenic T cell clones derived from two T cell lines, BP20 and BP29, which were specific for the MBP 68-88 encephalitogenic deter-

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                  . . . L ──▶
  1  caagatcctgacagcatcgtttttactcctaggccttcacctggctggggtgagtggcca    60
     K  I  L  T  A  S  F  L  L  L  G  L  H  L  A  G  V  S  G  Q
                              | V ──▶
 61  gcaggagagacgtgaccagcagcaggtgaaacaaagtcccatatctctgacagtctggga   120
     Q  E  R  R  D  Q  Q  V  K  Q  S  P  I  S  L  T  V  W  E

121  aggaggacccacagttctgagctgcagttatgaggacagcgcttttgactacttcccatg   180
     G  G  P  T  V  L  S  C  S  Y  E  D  S  A  F  D  Y  F  P  W

181  gtatcagcagttccctggggaaggccctgtactcctgatagccatacgatcagtgtccaa   240
     Y  Q  Q  F  P  G  E  G  P  V  L  L  I  A  I  R  S  V  S  N

241  taaaaagaaagatggacgattcacagtcttcctcaggaaaagtgagaaacagctctcctt   300
     K  K  K  D  G  R  F  T  V  F  L  R  K  S  E  K  Q  L  S  L
                                                              |
301  gcacatcgaagactctcagcctggagactcagccacctacttctgtgcagcaagccacaa   360
     H  I  E  D  S  Q  P  G  D  S  A  T  Y  F  C  A  A  S  H  N
          ?            | J ──▶                    |
361  taacaacaatgccccacgatttggagcaggaaccagactaacagtcaaaccaaatgtcac   420
     N  N  N  A  P  R  F  G  A  G  T  R  L  T  V  K  P  N  V  T
     C ──▶
421  agacccagagcctgctgtgtaccagctgaaagatccccagtctgataacatcaccctctg   480
     D  P  E  P  A  V  Y  Q  L  K  D  P  Q  S  D  N  I  T  L  C

481  cctgttcaccgactttgattctcaaaccaatgtgtcccagagcgcaagttccgaagcgtt   540
     L  F  T  D  F  D  S  Q  T  N  V  S  Q  S  A  S  S  E  A  F

541  tgtctctggcaatactgtgatggacatgaaggctatggattccaagagcaacggggccat   600
     V  S  G  N  T  V  M  D  M  K  A  M  D  S  K  S  N  G  A  I

601  tgcctggagtaaccaaacaagcttcagctgcaaagacgccttcaagaagaacgcctccca   660
     A  W  S  N  Q  T  S  F  S  C  K  D  A  F  K  K  N  A  S  H

661  ccccacttcagatgtgccctgtgatgccaagttggtcgagaaaagctttgaaacagatat   720
     P  T  S  D  V  P  C  D  A  K  L  V  E  K  S  F  E  T  D  M

721  gaacctaaactttcaaaacctgtcagtcatgggactccgaatcctcctgctgaaagtggc   780
     N  L  N  F  Q  N  L  S  V  M  G  L  R  I  L  L  L  K  V  A
                                              | 3' UT ──▶
781  cggatttaacctgctcatgacgctaaggctgtggtccagttgagattgaaagagcgtgac   840
     G  F  N  L  L  M  T  L  R  L  W  S  S

841  tccaaagctccttcctcctcctcatcctccgctccctcttcaagccaaaaggagccctcc   900

901  cacctgtaaagaaggctgtctgaggactgactggatctccccgatctgtgaagaaggttg   960

961  ctggagagctaagctcagctgtacccactcagcccccttgctgctgctgaccattcacag   1020

1021 agacgcaggggctgggctctcctggatctgaagacctcctctcccatgatagatttccca   1080

1081 gcagaatctcttagagaatgttgtaaagaatatgttttttgtgtggtttttttttttttgt   1140

1141 atttttaaagcatccataaagaagtacatattactctttcatcaaggtgtagaaattatc   1200

1201 tcattgtctagacgctccccgctactgtgtgtattgagccacattgtatattattctgct   1260

1261 gccgatgacgtcattaaaggtgattcagaaaaaaaa  1296
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FIGURE 1. Nucleotide sequence and amino acid translation of 510 rat α chain cDNA. Leader (L), variable (V), joining (J), constant (C), and 3' untranslated (UT) regions are denoted. These designations are made based on homology with previously reported TCR genes (32) and are discussed in the text. The 510 α chain cDNA, 1,296 bp, contains an open reading frame that codes for a peptide of 273 amino acids as well as 457 bases of 3' untranslated region. By analogy to mouse and human TCR α chains, the encoded peptide includes 25 amino acids of leader sequence as well as the entire V, J, C, and 3' untranslated region. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00803.

minant and were generated from lymph nodes of guinea pig (GP) MBP-primed Lewis rats as described (20). BP20 and BP29 were isolated independently, with four T cell clones (No. 3–6) generated from the BP20 line and 7 clones (No. 11–17) selected from the BP29 line. Both lines and all of the clones were CD4⁺, OX22⁺, caused delayed-type hypersensitivity and when injected into naive Lewis rats, caused severe CNS disease (21). All clones reacted strongly to GP-MBP and to the synthetic peptide sequence 72–84 of rat MBP and GP-MBP. Finally, Southern blot data from

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     . . V —→
  1 tccttgagttgtaagcagaataataatcacaacaacatgtactggtatcggcaggacatg   60
     S  L  S  C  K  Q  N  N  N  H  N  N  M  Y  W  Y  R  Q  D  M

 61 gggcatggtctgaggctaatccattactcatatgatgttaacagtactgaaaaaggagat  120
     G  H  G  L  R  L  I  H  Y  S  Y  D  V  N  S  T  E  K  G  D

121 gtccccaatgggtacaaagtctccagaccaagccaaggggatttcttcctcacgctagag  180
     V  P  N  G  Y  K  V  S  R  P  S  Q  G  D  F  F  L  T  L  E
                                            |  D  | J —→
181 tcggcttcccctctcagacatctgtgtacttctgtgccagcagtgacagctcaaacaca   240
     S  A  S  P  S  Q  T  S  V  Y  F  C  A  S  S  D  S  S  N  T
                                    |  C —→
241 gaagttttctttggtaaaggaacgagactgacagttgtagaggatctgaaaacggtgact  300
     E  V  F  F  G  K  G  T  R  L  T  V  V  E  D  L  K  T  V  T

301 ccacccaaggtctccttgtttgagccatcagaagcagagatcgcagacaaacaaaaggct  360
     P  P  K  V  S  L  F  E  P  S  E  A  E  I  A  D  K  Q  K  A

361 accctcgtgtgcttggccaggggcttcttccctgaccatgtggagctgagctggtgggtg  420
     T  L  V  C  L  A  R  G  F  F  P  D  H  V  E  L  S  W  W  V

421 aatgggaaggagatccgcaatggggtcagcacagaccctcaggcctacaaggaaagcaac  480
     N  G  K  E  I  R  N  G  V  S  T  D  P  Q  A  Y  K  E  S  N

481 aatatcacctactgcctgagcagccgcctgagggtttctgctaccttctggcacaatcct  540
     N  I  T  Y  C  L  S  S  R  L  R  V  S  A  T  F  W  H  N  P

541 cgcaaccacttccgctgccaagtgcagttctatgggcttacagaggaagacaactggtca  600
     R  N  H  F  R  C  Q  V  Q  F  Y  G  L  T  E  E  D  N  W  S

601 gaggactcacccaaacctgtcacacagaacatcagtgcgggagcctggggccgagcagat  660
     E  D  S  P  K  P  V  T  Q  N  I  S  A  G  A  W  G  R  A  D

661 tgtgggattacctcagcatcctatcaacaaggggtcttgtctgccaccatcctttatgag  720
     C  G  I  T  S  A  S  Y  Q  Q  G  V  L  S  A  T  I  L  Y  E

721 atcctgataggaaaagccaccctgtatgctgtgcttgtcagtacgctggtagtgatggct  780
     I  L  I  G  K  A  T  L  Y  A  V  L  V  S  T  L  V  V  M  A
            |  3' UT —→
781 atggtcaaaagaaagagttcctgaagccagacctgaaggtgaatccaagagcttacaacc  840
     M  V  K  R  K  S  S

841 cattgtggtgttgatacagattttcttctaccggcgcttctgaagagcctattctccttt  900

901 gtgaatcccaaagtctgcctagtcatgtatatttgtgtttgtattgtttgaaatccttgt  960

961 ggcctggagagtgcctagcacacttacaattagtaaccataaccaataaaacatgttcta  1020

1021 caatggcc  1028
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FIGURE 2. Nucleotide sequence and amino acid translation of the 510 rat β chain cDNA. Variable (V), diversity (D), joining (J), constant (C), and 3' untranslated (UT) regions are denoted. These designations are made based on homology with previously reported TCR genes (32). The 510 β chain cDNA clone is 1,057 bp long and contains an open reading frame encoding 267 amino acids. By analogy to mouse and human TCR genes, the clone is slightly truncated at the 5' end, lacking the codons for the leader and the 5' proximal portion of the mature Vβ. The clone contains a continuous open reading frame extending for 267 amino acids encompassing 75 amino acids of V region, a J region of 15 amino acids, a D region of 3 amino acids, and a constant region of 174 amino acids. In addition, there is a 3' untranslated region of 228 bp. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00803.

the clones using the Vα510 and Vβ510 probes provided evidence that none of them were clonally related (data not shown).

V Gene Usage by 68–88-specific Lewis T Cell Hybridomas. V region–specific fragments were isolated from both the 510 α and β chain cDNA clones, labeled with ^{32}P, and used as hybridization probes of Northern blots of the panel of T cell hybridoma mRNAs from cells specific for MBP 68–88. These cells were derived from seven separate fusions and exhibited three different antigenic fine specificity patterns (Table I) (8).

A strong correlation between specificity for the encephalitogenic determinant of

TABLE I

Northern Blot Data Using T Cell Hybridomas

Cell line	Fusion No.	Specificity	Immunogen	Probe 510 Vα	Probe 510 Vβ
510.2	1	MBP 68-88.1	GP-14	+	+
MI 1.2	2	MBP 68-88.1	GP MBP	−	+
M 2.53	3	MBP 68-88.1	GP MBP	+	+
M 4.5	4	MBP 68-88.1	GP MBP	+	+
M 4.6	4	MBP 68-88.1	GP MBP	+	+
BP14.3.1	5	MBP 68-88.1	GP MBP	−	+
19 B8.10	6	MBP 68-88.1	GP-19$	−	+
M 3.29	7	MBP 68-88.2	GP MBP	+	+
M 2.55	3	MBP 68-88.2	GP MBP	+	+
BP14.3.21	5	MBP 68-88.3	GP MBP	+	+
16B4 2.28	8	MBP 68-88*	GP-16	−	−
M 3.34	7	MBP non-68-88	GP MBP	−	−
M 3.35	7	MBP non-68-88	GP MBP	−	−
M 4.20	4	MBP non-68-88	GP MBP	+	−
MH 12.1	9	MBP non-68-88	GP MBP	−	−
M 3.22	7	MBP non-68-88	GP MBP	−	−
B6F	10	OVA	OVA	−	−
B4	10	OVA	OVA	−	−
Con A-blasted splenocyte			−	− (<10%)	− (<10%)
Total MBP 68-88 specific‡				7 of 10	10 of 10
Non-MBP 68-88 specific				1 of 8	0 of 8

* 16B4 2.28 responds to peptide only, and not to any whole MBP, and was not included in the total count.
‡ The fine specificity of the T cell hybridomas was as previously described (8). Briefly, the specificity patterns are defined as 68-88.1: GP MBP = 68-88 = rat MBP; 68-88.2: GP MBP = 68-88 ≫ rat MBP; and 68-88.3: GP MBP ≫ 68-88 = rat MBP. BW5147 × Lewis rat T cell hybridomas were derived from a primary fusion and not subject to long-term in vitro selection. They were tested for antigen specificity by a secondary IL-2 assay using the antigens as previously described (8).
$ The guinea pig sequence 68-88 of MBP or GP-19 is G-S-L-P-Q-K-S-Q-R-T-Q-D-E-N-P-V-V-H-F.

MBP and use of both Vα510 and Vβ510 hybridizing TCR mRNAs was observed among the T cell hybridomas (Fig. 3, Table I). The α chain V region probe hybridized to 7 of 10 of the RNAs derived from MBP 68-88-specific hybridomas. In contrast, this Vα probe hybridized to only one of five mRNAs from hybridomas of MBP non-68-88 specificity, zero of two OVA-specific hybrids, and zero of one 68-88 non-MBP-specific hybrid. In addition, no hybridization to RNA from Con A-stimulated normal Lewis splenocytes was detected, indicating that Vα510 hybridizing mRNAs constitute <10% of normal α chain message in splenic T cells. Thus, use of 510 Vα correlates highly with specificity of the T cells for the encephalitogenic determinant of MBP. Hybridomas of all three reported antigenic fine specificity phenotypes were among the 510 Vα+ cells (Fig. 3; Table I).

An even more striking correlation was seen in Vβ gene usage. All 10 MBP 68-88-specific T cell hybridomas expressed Vβ510 hybridizing RNA. In contrast, none of the hybridomas with specificity for other determinants showed hybridiza-
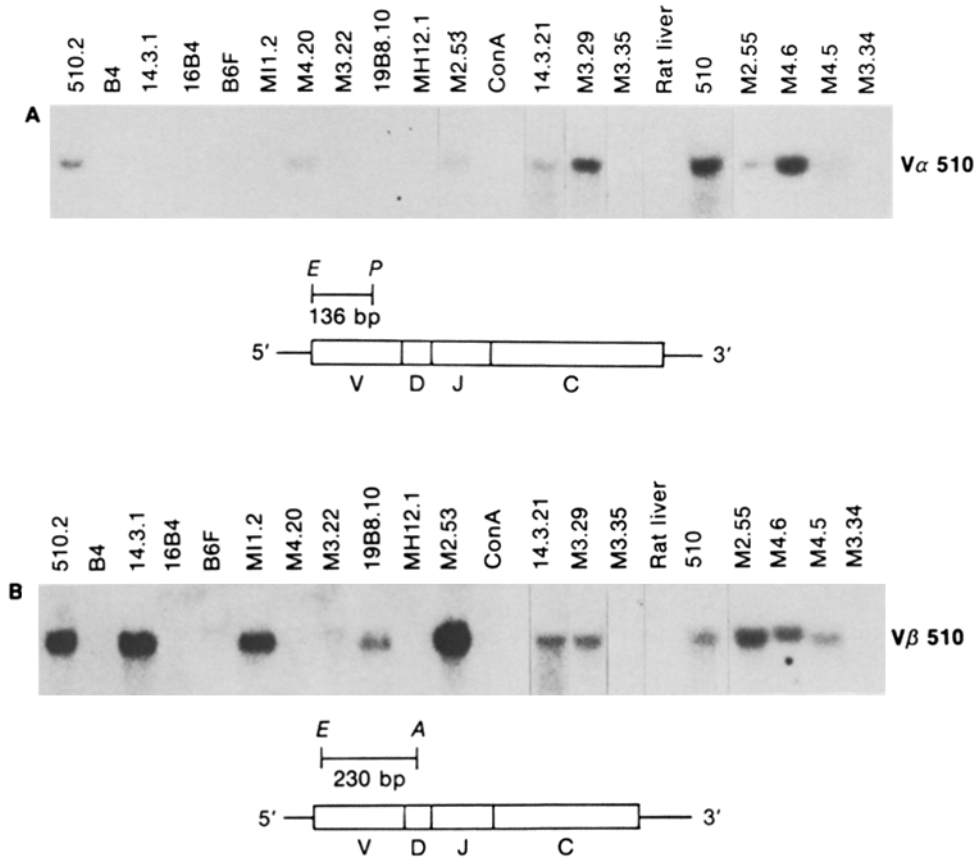
FIGURE 3. 510 V α and β usage in GP-MBP 68-88-specific hybridomas. Cells were grown in tissue culture and whole cell RNA was prepared as in Fig. 1. 20 mg/lane of whole cell RNA was fractionated by electrophoresis through a 1.0% agarose/formaldehyde gel. RNA was blotted to nytran membranes (Schleicher & Schuell, Inc.) and probed with a ^{32}P-labeled 510 Vα-specific fragment (A) or a 510 Vβ-specific fragment (B). The highest stringency wash was at 52°C in 0.1 × SSC 0.1% SDS for 1 h. The same blots were then rehybridized with rat α (A) or β (B) constant region probe (not shown) to assure presence of similar levels of total α and β chain mRNA. Vα510 and Vβ510 probes (as seen) were generated from rat × mouse 510 α and β chain cDNA clones. E, Eco RI; P, Pst I; A, Alu I.

tion. Again, evidence for the relatedness of antigen specificity and Vβ510 gene usage was provided by the lack of significant hybridization of the Vβ510 probe to RNA isolated from Con A-stimulated normal spleen cells.

V Gene Usage by Encephalitogenic T Cell Clones. The T cell hybridomas represent a population minimally selected by in vitro culture and, thus, should be representative of the MBP-specific T cell repertoire. On the other hand, these cells were not testable for their ability to induce EAE. Therefore, we examined cloned Lewis rat T cells that were all encephalitogenic in vivo. These cells, in contrast to the T cell hybrids, were more likely to be under in vitro selection pressures.

As seen in Fig. 4 and Table II, both T cell lines, BP20 and BP29, and 8 of 11 T cell clones were found to be Vα510 hybridization positive. Both T cell lines and
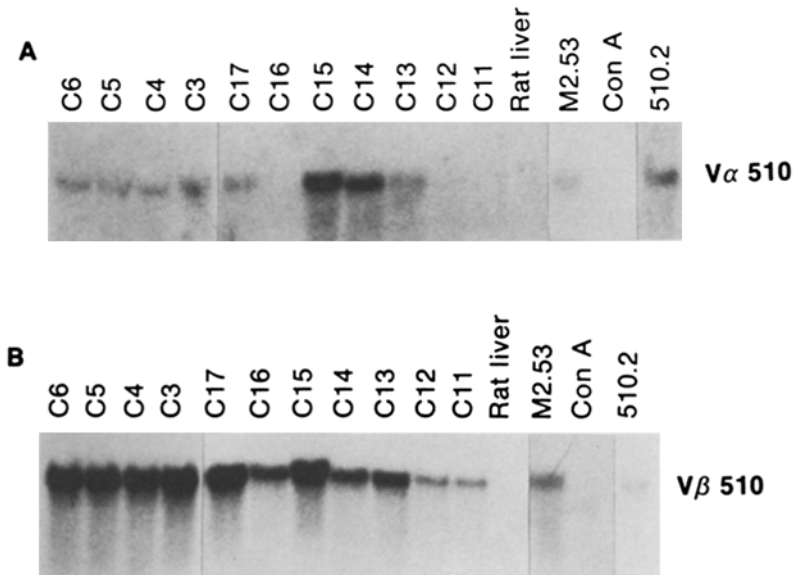
FIGURE 4.   510 Vα and Vβ gene usage in Lewis rat encephalitogenic T cell clones. Northern blot hybridization with ^{32}P-labeled Vα and Vβ probes showed hybridizing bands at ∼1.4 kb with 80% of the encephalitogenic clones using Vα510 and 100% of them using Vβ510.

all of the T cell clones were found to be Vβ510 hybridization positive. Thus, the two clonal populations examined, both the hybridomas and the clones, were identical in Vα and Vβ510 usage and correlated highly with not only antigen specificity but encephalitogenicity as well.

Discussion

The attempt to modulate an autoimmune disease via idiotypic regulation of autoreactive antigen-specific T cells was first shown in the rat EAE model system (9), where inactivated EAE-inducing T cells could both immunize and protect rats from EAE upon challenge with MBP. The basis of this protection was thought to be specific regulation of autoreactive T cells by recognition of idiotypic determinants on their TCRs.

To determine the molecular basis of such protection and regulation, we have investigated the relationship between TCR gene usage and fine specificity and function of Lewis rat T cell hybridomas and encephalitogenic Lewis T cell clones that are specific for encephalitogenic residues 68–88 of MBP.

TCR-α/β are disulfide-linked heterodimers that recognize antigen in the context of appropriate MHC molecules (26). Generation of receptor diversity necessary for proper immune function has been shown, in mouse and man (19, 27), to involve the rearrangement of germline V, D, and J elements upstream of one of two β constant regions and the rearrangement of V and J segments upstream of a single α constant region. This is followed by independent assortment of the two polypeptide chains. The manner in which these rearrangements occur is similar to that seen in Ig genes and has been reviewed elsewhere (25). The relationship between the struc-

TABLE II
Va510 and Vβ510 Usage in Encephalitogenic T Cells

Clone	Line	Reactivity (MBP 72-84)	EAE inducing	Probe Va510	Probe Vβ510
3	BP20	+	+	+	+
4	BP20	+	+	+	+
5	BP20	+	+	+	+
6	BP20	+	+	+	+
11	BP29	+	+	−	+
12	BP29	+	+	−	+
13	BP29	+	+	+	+
14	BP29	+	+	+	+
15	BP29	+	+	+	+
16	BP29	+	+	−	+
17	BP29	+	+	+	+
510.2	rat × mouse hybrid.	+	+	+	+
Con A-blasted splenocyte		−	−	− (<10%)	− (<10%)
Total MBP 68-88				8 of 11	11 of 11

* Splenocytes were blasted with Con A without any specific antigen.

tural elements of the TCR and antigen specificity has proven to be elusive, even when antigens are small and are studied within a single MHC context.

Reports have indicated that in particular systems: (a) responses may be highly degenerate, using a wide variety of V, D, and J elements on both chains in response to a given antigen within a single MHC context (28). (b) Responses can be somewhat delimited with half of the antigen-specific clones using the same Va and Vβ genes within a given MHC context (29). (c) Responses to mixed lymphocyte stimulation and MHC alloreactivity can be highly correlated to Vβ usage (30-32). (d) Responses may be highly restricted with the α chain determining antigen specificity and the β chain determining MHC restriction (33-35).

We have shown here that a highly restricted set of TCR Va510 and Vβ510 genes are used by Lewis rat T cells specific for the dominant encephalitogenic T cell determinant of GP MBP, amino acid residues 68-88. A survey of previously reported Vβ genes revealed that the Vβ510, used by 100% of MBP 66-88-specific T cells, is most closely related to the Vβ8.2 of mouse at the amino acid level, sharing 79% homology. At the nucleotide level, Vβ510 is slightly more homologous to Vβ8.1. The Va510, used by 70% of the Lewis rat MBP 68-88-specific T cells, is most closely related to Va2, sharing 77% amino acid homology.

It has been recently shown that the murine homologue of rat Vβ510, Vβ8.2, has been implicated in EAE-inducing T cells of the PL/J, (PL × SJL)E1, and B10.PL mice (15-17). Of the clones examined in the murine systems, ~75% of the clones used Vβ8.2 compared with 100% usage of Vβ8.2 in the rat. One major difference between mouse and rat in terms of Vβ8.2 usage is that, in the mouse, Vβ8 is a commonly used three member gene family seen in normal peripheral T cell populations (36), whereas in the rat, it is uncommon and not seen in unprimed populations. There appear to be three members of the Vβ510 family as well (data not shown).

It has also been shown that 65% of encephalitogenic T cells from the same B10.PL mice (17) use Vα2, again, the same as used by Lewis rat encephalitogenic T cells. Furthermore, these α chains from B10.PL T cells use the J region of TA 39, the same one used by the rat 510 α chain. This is extraordinary considering the fact the B10.PL mice recognize the NH_2-terminal 1-9 residues of MBP as the encephalitogenic determinant, whereas the Lewis rat recognizes residues 68-88 of MBP as encephalitogenic. Not only this, but the mouse and rat also use two different class II restricting elements, I-A^u and RT-1^l. The two peptides in fact do share four amino acids, two pairs of sequential SQ (ser gln) residues. However, we have tested whether Lewis rat 68-88-specific T cells can respond to the 1-11 peptide of MBP using either Lewis, PL/J, or B10.PL APC; and they do not respond (data not shown; peptide 1-11 kindly provided by Dr. Acha-Orbea, Stanford University, Stanford, CA). Therefore, we must conclude that antigenically these molecules are different.

The high correlation between Vα510 and Vβ510 usage and MBP 68-88 reactivity suggests that anti-idiotypic therapy/regulation in EAE might be directed against determinants specific for Vβ510 V regions, for Vα510 V region determinants, or perhaps a complex determinant composed of specific elements from both Vα and Vβ. We have generated a monoclonal anti-idiotypic antibody (mAb 10.18) against the 5.10 MBP-specific T cell hybridoma TCR that does in fact modulate EAE (12, 13). This suggests that the 5.10 idiotype is the sole or predominant EAE-inducing idiotype in the Lewis rat. In the mouse (15-17) an anti-Vβ8 mAb has been shown to suppress EAE, again supporting idiotype regulation of an autoimmune disease, and another striking parallel with the rat.

In conclusion, we have demonstrated a specific and very high level of correlation between both 510Vα and 510Vβ gene usage, reactivity for the encephalitogenic determinant of MBP, and disease induction. At this level of analysis, that is, the use of the same or closely related V regions, a distinction between T cell hybridomas showing three different fine specificity patterns was not observed. These fine specificity differences may lie in the use of different members of the Vα and Vβ region families, different D or J segments, or in junctional diversity (37). However, the finding of shared V region usage among T cells specific for the encephalitogenic determinant of MBP substantiate the argument for idiotypic regulation of Lewis rat EAE. This represents a clinically significant instance in which an autoantigen is a unique epitope, the response to which is of limited TCR V gene usage and limited idiotypic diversity.

Finally, is seems extraordinary that divergent species such as the rat and mouse use similar TCR V genes and possibly the same Jα regions as well. Thus, the same combination of Vα and Vβ genes is chosen by two different species, the mouse and the rat, which respond to two very different encephalitogenic peptide determinants on MBP, residues 1-9 for the B10.PL mouse and residues 68-88 for the Lewis rat, and recognize these antigens in th᷏ ᷏᷏ntext of different MHC molecules. Considering the extreme unlikelihood of this being a random event, we believe that this finding is significant. It is difficult to rationalize this in the context of what we currently know about TCR antigen-Ia recognition or regulation.

Summary

Prospects for specific immune intervention in T cell–mediated autoimmune disease via anti-idiotypic regulation depend on the degree of diversity of the responder

cell antigen receptor repertoire. A highly heterogenous response against self epitopes offers little chance for such regulation. We report here that the Lewis rat autoimmune disease experimental allergic encephalomyelitis, generally considered to be a model of human multiple sclerosis, is caused by T cells that use a limited set of TCR V genes. We have cloned the rat TCR α and β chain cDNAs from the Lewis rat × mouse T cell hybridoma 510, which retains the rat specificity for the encephalitogenic determinant of myelin basic protein (MBP). Using Northern blot analysis of T cell RNA with the cloned V region probes, we have found a specific, and near perfect, correlation between expression of TCR message hybridizing to the Vα510 and VB510 probes and specificity for the encephalitogenic determinant of MBP in both T cell hybridomas and encephalitogenic T cell clones. This restricted V gene usage provides a basis for observed idiotypic regulation of auto-reactive T cells, and possible therapy for autoimmune disease. A curious and unexplained observation is that the Lewis rat Vα/Vβ combination that dominates the encephalitogenic response to the 68–88 peptide of MBP is precisely the same Vα/Vβ combination used by the B10.PL mouse response to the encephalitogenic response to the 1–9 peptide of MBP.

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