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Shared Human T Cell Receptor V_β Usage to Immunodominant Regions of Myelin Basic Protein

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Multiple sclerosis (MS) may be an autoimmune disease mediated by T cells specific for a myelin protein. Investigations have demonstrated myelin basic protein (MBP)–reactive T cells that were activated in vivo in MS patients, suggesting that MBP may be a target antigen in MS. The variable (V) region of the T cell receptor (TCR) β chain was examined among 83 T cell lines from both MS patients and healthy subjects that were reactive with the immunodominant region of human MBP (residues 84 to 102) or with a second immunodominant region of MBP (143 to 168). V_β17 and to a lesser extent V_β12 were frequently used in recognition of MBP(84–102) among different individuals. In contrast, V_β17 was very infrequent among lines reactive with MBP(143–168). These data demonstrate shared TCR V_β gene usage for the recognition of immunodominant regions of the human autoantigen MBP. Such TCR structures may be used as targets for specific immunotherapy in MS.

MULTIPLE SCLEROSIS (MS) IS A chronic inflammatory disease of the central nervous system characterized by prominent T cell and macrophage infiltrates and demyelination. The pathogenesis of MS is thought to arise from autoreactive T cells specific for a myelin protein that initiate the inflammatory process (*1, 2*). The recent demonstration of in vivo–activated myelin basic protein (MBP)–specific T cells in MS patients implicates MBP-reactive T cells in the pathogenesis of the disease in some individuals (*3*). Two regions of human MBP located between residues 84 to 102 and 143 to 168 were recently identified to be immunodominant (*4, 5*). MS patients have a higher frequency of T cells reactive with MBP(84–102) in their blood than do controls. Reactivity to MBP(84–102) is associated with DR2, whereas reactivity to MBP(143–168) is associated with DRw11 (*4*).

Experimental autoimmune encephalomyelitis (EAE) is the primary animal model for MS and can be induced by immunization with MBP in adjuvant or by passive transfer of $CD4^+$ T cells reactive with MBP (*2, 6*). T cells that induce EAE in both mice and rats are specific for immunodominant MBP peptides presented by particular major histocompatibility complex (MHC) class II molecules and recognize MBP with specific T cell receptor (TCR) variable (V) regions of the β and α chains (*7–9*). In B10.PL mice encephalitogenic T cells recognize an NH_2-terminal MBP peptide (residues 1 to 9) presented by an $H\text{-}2^u$–encoded MHC molecule (*7*). The majority of T cells recognizing this MHC-peptide complex use V_β8.2 and V_α2 or V_α4 (*8*). In Lewis rats TCR segments homologous with the mouse V_β8.2 and V_α2 genes have been found in encephalitogenic T cells specific for MBP(68–88) and I-A (*9*). Administration of a V_β8.2-specific monoclonal antibody (MAb) is effective in treating murine EAE, and immunization with V_β8.2-specific peptides ameliorates EAE in the Lewis rat (*8, 10*). Thus, we analyzed in humans which V_β regions were used among T cells recognizing immunodominant regions of MBP.

TCR V_β usage of MBP-reactive T cell lines was determined by the polymerase chain reaction (PCR) amplification of cDNA with TCR V_β1 to V_β20 primers, followed by Southern (DNA) blotting, and hybridization with an internal TCR C_β probe (*11–14*). Particular care was taken to prevent contamination of samples. Amplified and nonamplified samples were handled separately; reagents were divided into aliquots and tested for the presence of amplified material, and negative controls were included for all experimental steps (RNA isolation, cDNA synthesis, and PCR amplification).

Two series of experiments were performed to test the validity of this approach. First, we demonstrated that all primers except V_β20 were able to amplify cDNA from peripheral blood T cells (Fig. 1). Second, the specificity of PCR amplification was examined by analysis of V_β gene usage in 69 independent T cell clones previously established by single-cell cloning with mitogen (phytohemagglutinin, PHA) and interleukin-2 from a normal subject and an MS patient (*15*). Because of the high cloning efficiencies, these clones provide a representative analysis of V_β gene usage among peripheral blood T cells. TCR V_β gene usage could be determined for 65 of 69 (94.2%) of these T cell clones, indicating that a large proportion of the TCR V_β repertoire was recognized by our V_β primers. Whereas 58 of these clones (84%) were positive for a single V_β, seven clones (10.1%) were double-positive, possibly because of the presence of two rearranged and expressed TCR genes or the presence of two cell populations in the sample.

TCR V_β gene usage was analyzed in a total of 64 MBP(84–102)–reactive T cell lines and 19 MBP(143–168)–reactive lines established from five patients with definite early relapsing-remitting MS (*4*) and five control subjects. Thirty-one T cell lines from patient Hy reacted with MBP(84–102) (patient 1, Fig. 2). The V_β17 segment was used in 24 (77.4%) T cell lines, whereas other cell lines used V_β4, V_β7, or V_β14. Thus, V_β17 is the major TCR V_β structure used to recognize MBP(84–102) in this MS patient. V_β17 usage was also found in T cell lines from the four other patients examined (Fig. 2) and was present in 6 of 20 T cell lines. V_β12 was also used frequently and was found in 7 of 20 T cell lines reactive with MBP(84–102) (Figs. 1 and 2). This V_β is homologous to the mouse V_β8.2 (*16*), which is the predominant TCR V_β used among encephalitogenic T cells in mice and rats (*8, 9*).

We examined TCR V_β gene usage among T cell lines reactive with MBP(84–102) in five healthy individuals (Fig. 2). All five cell lines from subject Rt, who was $DR2^+$, were

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$V_\beta17^+$. Both $V_\beta17$ and $V_\beta12$ genes were amplified from one cell line from subject Cr, and one of the two cell lines from subject Hr was $V_\beta12^+$. In two other healthy controls, $V_\beta8$ was found in four of five T cell lines. These data show that $V_\beta17$ and $V_\beta12$ can be TCR recognition elements for this immunodominant region in some healthy individuals. $V_\beta17$ usage was more prominent in MS patient Hy and in DR2 control Rt, compared to the other subjects studied, possibly because of the presence of certain MHC polymorphisms affecting peptide presentation or T cell receptor recognition.

MS patient Cy expressed both the DR2 and DRw11 antigens and thus had T cells that recognized either the immunodominant MBP(84–102) or MBP(143–168) (*4*). This provided the opportunity to compare TCR V_β usage among T cells reacting to different MBP determinants (Figs. 1 and 2). Of seven lines proliferating to MBP(84–102), three expressed $V_\beta12$ (Fig. 2A) and one expressed $V_\beta17$. In contrast, six of nine T cell lines recognizing the MBP(143–168) used $V_\beta14$ (Fig. 2B) and only one line each used the TCR $V_\beta12$ and $V_\beta17$ TCR genes (Fig. 2).

In random mitogen-derived T cell clones generated by single-cell cloning from a normal individual, either $V_\beta12$ or $V_\beta17$ was individually present among 3 of 32 (9.4%) TCR V_β (*15*). In contrast, $V_\beta17$ was found in 36 of 64 (56.3%) of all T cell lines reactive with MBP(84–102). PCR analysis of the TCR V_β repertoire in the peripheral blood of three MS patients (patients Hy, Cy, and Fn) and four control subjects (Rt, Md, An, and Wg, from whom the PHA clones had been generated) was also performed. The Southern blot patterns for all these samples were very similar and no deletions of T cell receptor V_β families could be detected. In total, these results suggest that $V_\beta17$ is selectively involved in the recognition of the immunodominant MBP(84–102) region.

It was initially surprising that a single TCR V_β gene would be identified after only three cycles of in vitro stimulation with antigen. To prove that the TCR gene segment identified by PCR was the V_β gene used to recognize the MBP peptide, we cloned two $V_\beta17^+$ T cell lines (Hy.2H9 and Hy.2G5) by limiting dilution. All 11 individual clones established from these two cell lines, which were reactive with both MBP and MBP(84–102), were $V_\beta17^+$. Three of these clones were further analyzed with the complete panel of V_β primers and were all found to be negative for the other V_β segments.

T cell receptor $V_\beta17^+$ PCR products were cloned and sequenced by the dideoxy method (Table 1). The V_β sequences of all ten lines were homologous to the published $V_\beta17.1$ sequence (*13*), except for some nucleotide substitutions at the variable-diversity (VD) junction observed in four lines. These sequence data confirm that specific V_β segments were amplified by the use of this approach. Analysis of the V_β junctional sequence indicated that all six T cell lines generated from patient Hy and one line from subject Rt used the $J_\beta2.1$ segment, whereas three T cell lines from subjects Fn, Ns, and Cr used different J_β segments. To further determine how frequently the $J_\beta2.1$ gene segment was used by other $V_\beta17^+$ T cells from patient Hy, we amplified cDNAs from 20 cell lines with the $V_\beta17$ primer in

Table 1. Sequence analysis of TCR V_β chains of ten T cell lines reactive with MBP(84–102). Complimentary DNAs were amplified with PCR primers for the $V_\beta17$-leader segment and the TCR C_β region (*10, 19*). Amplified DNA was cloned into M13 and sequenced by the dideoxy method (three M13 plaques per T cell line) (*11, 14*). All T cell lines use the $V_\beta17.1$ gene segment, and the sequences are homologous to the published sequence (*13*). All TCR V_β chains were found to have an in-frame rearrangement. Shared TCR sequences are underlined.

Cell line	V_β	D_β	J_β	V_β-J_β usage
		MS patients		
Hy.1A8	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	ThrAspTrpSer act gac tgg agc	SerTyrAsnGluGlnPhe tcc tac aat gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Hy.2C9	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	ThrAspTrpSer act gac tgg agc	SerTyrAsnGluGlnPhe tcc tac aat gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Hy.3A10	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	ThrAspTrpSer act gac tgg agc	SerTyrAsnGluGlnPhe tcc tac aat gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Hy.2C8	TyrLeuCysAlaSerArg tat ctc tgt gcc agt agg	ThrSerGly act agc ggc	SerTyrAsnGluGlnPhe tcc tac aac gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Hy.2H9	TyrLeuCysAlaSerArg tat ctc tgt gcc agt agg	ThrSerGly act agc ggc	SerTyrAsnGluGlnPhe tcc tac aac gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Hy.2G5	TyrLeuCysAlaSerArg tat ctc tgt gcc agt agg	ThrSerGly act agc ggc	SerTyrAsnGluGlnPhe tcc tac aac gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Fn.1G6	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	IleProPro atc cct cca	SerTyrGluGlnTyrPhe tcc tac gag cag tac ttc	$V_\beta17.1$-$J_\beta2.7$
Ns.1G11	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	AlaAspArg gcg gac agg	AspGlnProGlnHisPhe gat cag ccc cag cat ttt	$V_\beta17.1$-$J_\beta1.5$
		Controls		
Rt.1A9	TyrLeuCysAlaSerSer tat ctc tgt gcc agt agt	ThrGlyGlnGlyLeuAsp acg ggt cag ggg ttg gat	GluGlnPhe gag cag ttc	$V_\beta17.1$-$J_\beta2.1$
Cr.1B12	TyrLeuCysAlaSerGly tat ctc tgt gcc agt ggg	AspAsnGlyGlyGlu gac aat ggt ggg gag	GlnTyrPheGlyProGly cag tac ttc ggg ccg ggc	$V_\beta17.1$-$J_\beta2.5$

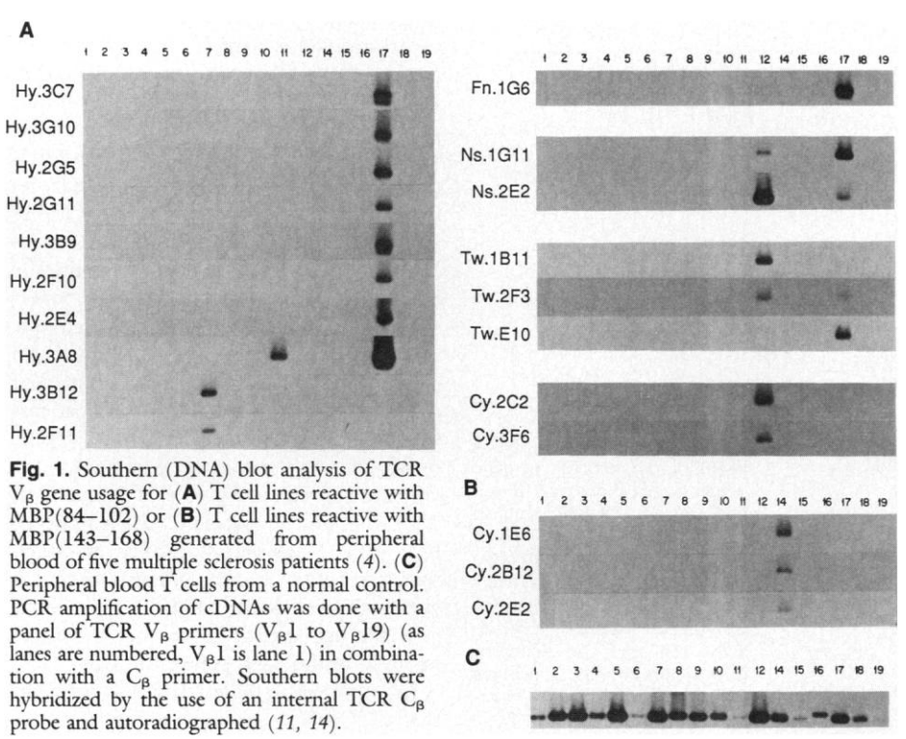

Fig. 1. Southern (DNA) blot analysis of TCR V_β gene usage for (**A**) T cell lines reactive with MBP(84–102) or (**B**) T cell lines reactive with MBP(143–168) generated from peripheral blood of five multiple sclerosis patients (*4*). (**C**) Peripheral blood T cells from a normal control. PCR amplification of cDNAs was done with a panel of TCR V_β primers ($V_\beta1$ to $V_\beta19$) (as lanes are numbered, $V_\beta1$ is lane 1) in combination with a C_β primer. Southern blots were hybridized by the use of an internal TCR C_β probe and autoradiographed (*11, 14*).

combination with a C_β primer or a $J_\beta 2.1$ primer (Fig. 3). All of these lines were positive for $V_\beta 17$ as well as $J_\beta 2.1$ gene segments, whereas the negative controls (RNA extracted from all cell lines and not converted to cDNA, and reagents used for cDNA synthesis and amplification) were negative by PCR and Southern blotting. This indicates that $V_\beta 17$ and $J_\beta 2.1$ are the major TCR elements used for recognition of MBP(84–102) in this patient.

Comparison of the VDJ sequences (J, joining) of the six T cell lines from subject Hy demonstrated only two V_β sequences, though each line was originally derived from separate cultures. These could be common sequences because of selection of particular TCR structures by MBP(84–102) or because of oligoclonal expansion of MBP-reactive T cells after in vivo antigen exposure.

Four of five patients studied were positive for the disease-associated DR2 antigen, whereas patient Tw was HLA-DR3,DR4. Nevertheless, three cell lines that used $V_\beta 12$ or $V_\beta 17$ were present among four lines analyzed from this MS patient (Fig. 2), indicating that shared MHC class II antigens may not be mandatory for shared TCR V_β gene usage in recognition of MBP(84–102).

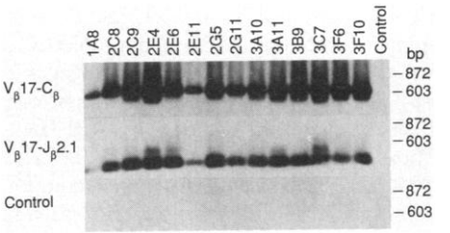

Fig. 3. PCR amplification of cDNA from 14 T cell lines reactive with MBP(84–102) generated from MS patient Hy. Amplification was performed with a combination of $V_\beta 17$-C_β primers, $V_\beta 17$-$J_\beta 2.1$ primers, and $V_\beta 17$-C_β primers with RNA from T cell lines as a negative control (*11*). Rightmost lane shows negative control reaction (no cDNA). Southern (DNA) blots demonstrate a shared usage of $V_\beta 17$ and $J_\beta 2.1$ gene segments among these T cell lines.

A **Multiple sclerosis**

Cell line	TCR V_β	Cell line	TCR V_β	Cell line	TCR V_β
Patient 1 (DR2, DR7)		Patient 1 (cont.)		Patient 3 (DR2, DRw11)	
Hy.1B12	**$V_\beta 17$**	Hy.2C12	**$V_\beta 17$, $V_\beta 1$**	Cy.2C2	**$V_\beta 12$**
Hy.1G9	**$V_\beta 17$**	Hy.2E2	**$V_\beta 17$, $V_\beta 1$**	Cy.3F6	**$V_\beta 12$**
Hy.1H7	**$V_\beta 17$**	Hy.2E11	**$V_\beta 17$, $V_\beta 2$**	Cy.4C1	**$V_\beta 12$**
Hy.2C9	**$V_\beta 17$**	Hy.3A11	**$V_\beta 17$, $V_\beta 2$**	Cy.2G5	**$V_\beta 17$**
Hy.2E4	**$V_\beta 17$**	Hy.2C8	**$V_\beta 17$, $V_\beta 11$**	Cy.2H11	$V_\beta 1$, $V_\beta 7$
Hy.2E6	**$V_\beta 17$**	Hy.3A8	**$V_\beta 17$, $V_\beta 11$**	Cy.3D2	$V_\beta 1$, $V_\beta 7$
Hy.2F10	**$V_\beta 17$**	Hy.3C3	$V_\beta 4$	Cy.2C6	$V_\beta 2$
Hy.2G5	**$V_\beta 17$**	Hy.3C6	$V_\beta 4$	Patient 4 (DR2, DR4)	
Hy.2G11	**$V_\beta 17$**	Hy.3B7	$V_\beta 4$	Ns.1G11	**$V_\beta 12$,$V_\beta 17$**
Hy.2H9	**$V_\beta 17$**	Hy.2F11	$V_\beta 7$	Ns.2E2	**$V_\beta 12$,$V_\beta 17$**
Hy.3A10	**$V_\beta 17$**	Hy.3B12	$V_\beta 7$	Ns.2A5	$V_\beta 1$
Hy.3B9	**$V_\beta 17$**	Hy.1H3	$V_\beta 14$	Ns.2C10	$V_\beta 3$, $V_\beta 14$
Hy.3C7	**$V_\beta 17$**	Hy.2B2	$V_\beta 14$	Ns.2D11	$V_\beta 5$, $V_\beta 7$
Hy.3G10	**$V_\beta 17$**	Patient 2 (DR2, DR 7)		Patient 5 (DR3, DR4)	
Hy.3F6	**$V_\beta 17$**	Fn.1G6	**$V_\beta 17$**	Tw.B11	**$V_\beta 12$**
Hy.3F7	**$V_\beta 17$**	Fn.1M7	$V_\beta 4$	Tw.2F3	**$V_\beta 12$,$V_\beta 17$**
Hy.3F10	**$V_\beta 17$**	Fn.3E17	$V_\beta 3$, $V_\beta 5$	Tw.E10	**$V_\beta 17$**
Hy.1A8	**$V_\beta 17$**	Fn.1E6	$V_\beta 6$, $V_\beta 8$	Tw.2E2	$V_\beta 14$

Controls

Cell line	TCR V_β	Cell line	TCR V_β	Cell line	TCR V_β
Control 1 (DR2)		Control 2 (DR2)		Control 4 (DR7, DRw11)	
Rt.1A9	**$V_\beta 17$**	Hr.1B7	**$V_\beta 12$**	An.3E1	$V_\beta 8$, $V_\beta 1$
Rt.3C1	**$V_\beta 17$**	Hr.1C9	$V_\beta 5$	An.3H3	$V_\beta 8$
Rt.3G11	**$V_\beta 17$**	Control 3 (DR2)		An.3C12	$V_\beta 2$
Rt.3A3	**$V_\beta 17$**,$V_\beta 14$	Md.2A4	$V_\beta 8$, $V_\beta 6$	Control 5 (DR1, DR9)	
Rt.3F1	**$V_\beta 17$**,$V_\beta 14$	Md.2F1	$V_\beta 8$, $V_\beta 18$	Cr.1B12	**$V_\beta 17$,$V_\beta 12$**

B **Multiple sclerosis**

Cell line	TCR V_β	Cell line	TCR V_β
Patient 3 (DR2, DRw11)		Patient 4 (DR2, DR4)	
Cy.1E6	**$V_\beta 14$**	Ns.2D6	$V_\beta 3$
Cy.2B12	**$V_\beta 14$**	Patient 2 (DR2, DR7)	
Cy.2E2	**$V_\beta 14$**	Fn.1H5	$V_\beta 4$
Cy.3G10	**$V_\beta 14$**	Fn.2A10	$V_\beta 4$
Cy.3H10	**$V_\beta 14$, $V_\beta 8$**	Fn.2A5	$V_\beta 2$
Cy.4C10	**$V_\beta 14$, $V_\beta 17$**	Patient 5 (DR3, DR4)	
Cy.1C12	$V_\beta 12$	Tw.2C9	$V_\beta 12$
Cy.1E9	$V_\beta 7$		
Cy.3F9	$V_\beta 1$		

Controls

Cell line	TCR V_β	Cell line	TCR V_β
Control 2 (DR2)		Control 6 (DR1, DR7)	
Hr.2E10	$V_\beta 3$, $V_\beta 5$	Bn.2G1	$V_\beta 12$
Hr.3E9	$V_\beta 7$	Bn.3D6	$V_\beta 12$
		Bn.3C10	$V_\beta 5$, $V_\beta 8$

Fig. 2. T cell receptor V_β gene usage in MBP-reactive T cell lines. (**A**) T cell lines reactive with MBP(84–102), (**B**) T cell lines reactive with MBP(143–168). V_β gene usage was determined by PCR amplification of cDNAs derived from MBP-reactive cell lines and Southern (DNA) blotting. PCR primers covered all published V_β families ($V_\beta 1$ to $V_\beta 20$) (*11, 14*).

In mice, shared V segment usage has been reported among cytochrome c–reactive T cell clones restricted by different MHC molecules (*17*). Four cytochrome c–specific T cell clones derived from four strains of mice with different MHC haplotypes were found to use the same $V_\alpha 11.2$ gene segment but different J_α gene segments. These results are consistent with our observation of shared TCR gene usage among different individuals, as MHC polymorphisms may result in shared V_β but different J_β gene usage in response to a specific peptide.

Genetic studies showed a linkage of disease susceptibility to the T cell receptor V_β locus and the MHC DR2 allele (*18*). Our observation of shared T cell receptor V_β gene usage in response to an immunodominant DR2-associated MBP peptide (*4*) may provide a connection between these observations. Determination of T cell receptor V gene usage may also provide a specific target for therapeutic intervention. Both MAbs and synthetic TCR peptides specific for the $V_\beta 8.2$ TCR have been used therapeutically in EAE (*8–10*). A potential obstacle to designing TCR V segment–based therapies is the genetic heterogeneity of individuals, in particular at the MHC locus. Our data provide evidence that $DR2^+$ MS patients share antigen reactivity and TCR V_β gene usage. This may prove to be of therapeutic value, as approximately 60% of MS patients share this disease-associated MHC phenotype (*18*). Nonetheless, further investigations will be required to demonstrate that in vivo–activated MBP(84–102)–reactive T cells bearing specific T cell receptors are involved in the pathogenesis of MS.

In summary, these data demonstrate shared TCR V_β gene usage in humans to an

immunodominant region of the autoantigen MBP. This may provide insight into the molecular mechanisms of MS and help in the design of new specific therapeutic approaches.

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5. Sequences of MBP peptides: MBP(84–102): DENPVVHFFKNIVTPRTPP MBP(143–168): FKGVDAQGTLSKIFKLGGRD. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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11. Myelin basic protein–specific T cell lines were grown from peripheral blood mononuclear cells at 200,000 cells per well in the presence of human MBP (10 μg/ml). Under these conditions 1 to 20% of the wells were positive for MBP; therefore, most lines are likely to have been generated from a single MBP-reactive T cell. Cells were stimulated two times with MBP and tested for their peptide specificity by use of a panel of 13 overlapping synthetic MBP peptides. All cell lines analyzed reacted specifically with one of the 13 synthetic MBP peptides (*4*). After a third stimulation with the specific MBP peptide, RNA was extracted from cell culture pellets (20,000 to 50,000 cells) by extraction with guanidinium isothiocyanate/phenol chloroform and isopropanol precipitation in the presence of carrier tRNA. Single-stranded cDNAs were synthesized with oligo-dT and avian myeloblastosis virus reverse transcriptase. PCR amplification was done with a panel of 19 oligonucleotides corresponding to the CDR2 region of the TCR β chain and a C_β primer. Amplifications were done for 30 cycles (94°C, 1 min; 55°C, 2 min; and 72°C, 3 min) with 1 μg of each primer in 50-μl reactions. Amplified products were separated in 1% agarose gels, transferred to nitrocellulose, and hybridized with an internal oligonucleotide probe. Probes were end-labeled with [γ-^{32}P]ATP (adenosine triphosphate) and T4 polynucleotide kinase to a specific activity of 10^8 cpm/μg and hybridized. Blots were washed at a final stringency of 6× SSC (saline sodium citrate) at 70°C and autoradiographed for 2 to 18 hours. T cell lines that were positive for more than two V_β segments were considered not to be derived from a single MBP-reactive T cell and were therefore excluded from analysis. For sequencing, amplification was performed with a V_β17 primer specific for the leader segment, which contained an internal Pst I restriction site. Amplified DNA was treated with proteinase K, extracted with phenol chloroform, precipitated with ethanol, and digested with restriction endonucleases Bgl II and Pst I. Gel-purified DNA was ligated into M13mp19, and single-stranded DNA was sequenced by the dideoxy method. Negative controls were included during the procedure to test for possible contamination of RNA samples or reagents used for cDNA synthesis and amplification.
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A Receptor for Tumor Necrosis Factor Defines an Unusual Family of Cellular and Viral Proteins

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Tumor necrosis factor α and β (TNF-α and TNF-β) bind surface receptors on a variety of cell types to mediate a wide range of immunological responses, inflammatory reactions, and anti-tumor effects. A cDNA clone encoding an integral membrane protein of 461 amino acids was isolated from a human lung fibroblast library by direct expression screening with radiolabeled TNF-α. The encoded receptor was also able to bind TNF-β. The predicted cysteine-rich extracellular domain has extensive sequence similarity with five proteins, including nerve growth factor receptor and a transcriptionally active open reading frame from Shope fibroma virus, and thus defines a family of receptors.

TUMOR NECROSIS FACTOR α (TNF-α, cachectin) and β (TNF-β, lymphotoxin) are structurally and functionally homologous proteins secreted by activated macrophages and lymphocytes, respectively (*1*). These cytokines have pleiotropic activities in vitro and in vivo, including cytotoxic effects against tumors and virus-infected cells, stimulation of interleukin-1 secretion, stimulation of prostaglandin E2 and collagen production, inhibition of lipogenic gene expression in adipocytes, and stimulation of various immune effector cells (*2*). Clinical interest has focused on TNF because it appears to be a common mediator of inflammation, endotoxin-induced shock (*1*), and the wasting syndrome commonly observed in chronic infections and neoplastic disease (*3*). TNF receptors appear on virtually all somatic cells (*1*), and generally the ligands cross-compete for binding (*4*), suggesting they share a common receptor. As an aid to studying the TNF system in molecular detail, we isolated a cDNA clone of the receptor.

The SV40-transformed human lung fibroblast cell line WI26-VA4 was used as a source of mRNA for construction of a cDNA library. This cell line binds both TNF-α and -β and displays multiple affinity classes; approximately 23,000 binding sites per cell (N) were detected with ^{125}I–TNF-α that could be fit to two affinity classes, low (K_{a1} = 0.16 ± 0.10 nM^{-1}, N_1 = 19,700 ±

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