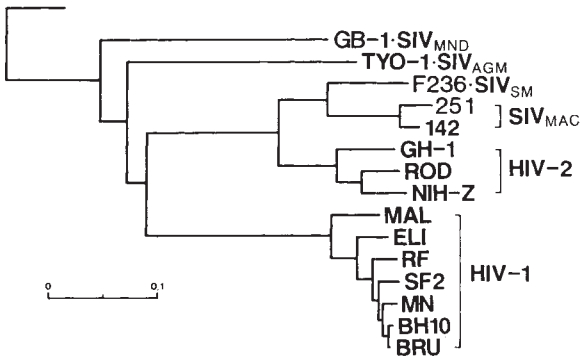

FIG. 2 Phylogenetic tree showing the evolutionary relationship of SIV$_{MND}$ and known primate lentiviruses. The tree was constructed by the neighbour-joining method[35] after alignment of the nucleotide sequences of the reverse transcriptase and the integration protein in the *pol* gene. The viral sequences used in this study were as follows: GB-1 isolate of SIV$_{MND}$ in this study; TYO-1 isolate of SIV$_{AGM}$[12]; Delta/F236 isolate of SIV$_{SM}$[4]; 251[36] and 142[2] isolates of SIV$_{MAC}$; GH-1[27], ROD[14] and NIH-Z[26] isolates of HIV-2; MAL[37], ELI[37], RF[38], SF2[39], MN[40], BH10[41] and BRU[13] isolates of HIV-1. The sequences of ovine visna lentivirus (VISNA)[42] and equine infectious anaemia virus (EIAV)[43] were used as reference species of lentiviruses in the calculation to make the position of the root in the tree clear. Horizontal distances from each branch are proportional to the number of amino-acid substitutions. The scale bar indicates 0.1 substitutions per site.

isolate can be considered as a representative strain of SIV$_{AGM}$, which is highly endemic in African green monkeys in the wild[12,28,33]. Our sequence analysis of SIV$_{MND}$ and SIV$_{AGM}$ suggests that several species of African non-human primates have harboured SIV for a long time and that these SIVs are specific to their natural hosts. □

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Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis

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T CELLS expressing the $\alpha\beta$ T-cell receptor (TCR) for antigen can elicit anti-idiotypic antibodies specific for the TCR that regulate T-cell function[1–4]. Defined sequences of the TCR, however, have not been used to elicit specific antibodies and the role of cellular immunity directed against TCR determinants has not been studied. We immunized Lewis rats with a synthetic peptide representing a hypervariable region of the TCR Vβ8 molecule. Subsequent induction of experimental autoimmune encephalomyelitis, a paralytic disease of the central nervous system mediated primarily by Vβ8$^+$ T cells specific for myelin basic protein[5,6] was prevented. T cells specific for the TCR Vβ8 peptide conferred passive protection against the disease to naive rats, apparently by shifting the predominant T-cell response away from the major encephalitogenic epitope of basic protein. This is the first report demonstrating the use of a synthetic TCR V-region peptide to induce specific regulatory immunity and has important implications for the regulation of human disease characterized by common TCR V-gene usage.

In a previous study[6], we reported the complete nucleotide and deduced amino-acid sequence for the rearranged rat TCR α- and β-chain genes (with sequence homology to the mouse Vα2 and Vβ8 families respectively) used in response to the major encephalitogenic epitope of basic protein, namely residues 72–89. Within the TCR Vβ8 region, we identified and synthesized a 21-amino acid sequence that included the second complementarity determining region (CDR2) and was predicted to be immunogenic for T cells[7,8]. A control peptide was synthesized from the corresponding region of a different TCR Vβ sequence that was homologous to the mouse Vβ14 family[9].

Immunization of Lewis rats with the TCR Vβ8 peptide but not with the TCR Vβ14 peptide or saline prevented completely the induction of experimental autoimmune encephalomyelitis (EAE) (Table 1). The Vβ8-peptide-immunized rats developed both specific antibodies [0.63 optical density (OD) units to the Vβ8 peptide compared with 0.02 OD units to the control peptide at a 1:200 dilution of serum analysed by enzyme-linked immunosorbent assay (ELISA)] and delayed type hypersensitivity (DTH) responses 17/100 mm = 0.17 mm ear swelling to 50 μg TCR Vβ8 peptide compared with 0/100 mm = 0.00 mm swelling to the control peptide. The Vβ14 peptide also induced specific immunity in rats injected with the Vβ14 peptide even though it did not confer protection against EAE. Lymph node cells isolated from the protected rats responded to the TCR Vβ8 peptide as well as to guinea-pig basic protein (GPBP) and PPD (purified protein derivative of *Mycobacterium tuberculosis*) (Table 2).

T-cell lines were selected from the lymph nodes of the protected rats that responded specifically to the Vβ8 but not to the Vβ14 peptide (Table 2). The TCR Vβ8 peptide-specific T cells

TABLE 1 Immunization with TCR Vβ8-peptide prevents induction of EAE

Immunization protocol	EAE Induction* Incidence	Day of onset	Duration	Severity†
TCR Vβ8 peptide + CFA‡ GPBP + CFA after 30 days	0/10	—	—	—
TCR Vβ14 peptide + CFA§ GPBP + CFA after 30 days	4/4	14 ± 2	6 ± 2	3.1 ± 0.3
Saline + CFA GPBP + CFA after 30 days	14/14	14 ± 2	6 ± 1	3.0 ± 0.5

* EAE was induced by subcutaneous injection of 50 μg GPBP + 400 μg *Mycobacteria* in complete Freund's adjuvant (CFA).

† Value represents the mean of the maximum severity of EAE. 0, no signs; 0.5, lethargy, weight loss; 1, limp tail; 2, hind-leg weakness; 3, hind-quarter paralysis, incontinence; 4, moribund.

‡ 100 μg of the TCR peptide [DMGHGLRLIHYSYDVNSTEKG (single-letter code)] representing residues 39–59 of the rat cDNA clone Vβ510 (ref. 6) which has homology with the mouse Vβ8 family was mixed with CFA containing 100 μg *Mycobacteria* and injected subcutaneously.

§ 100 μg of TCR peptide [APGGTLQQLFYSFNVGQSELV] representing residues 39–59 of the rat cDNA clone CRTB188 (ref. 9) which has homology with the mouse Vβ14 family was mixed with CFA containing 100 μg *Mycobacteria* and injected subcutaneously.

TABLE 2 Specificity of T-cell lines from rats protected from EAE by Vβ8 peptide

Stimulant	Proliferation (c.p.m. $\times 10^{-3}$) LN	TCR Vβ8 peptide selected line	GPBP selected line	TCR Vβ8 line + GPBP line
Medium	11 ± 1	2 ± 1	1 ± 0	6 ± 1
Con A	99 ± 6	96 ± 4	80 ± 4	123 ± 5
TCR Vβ8 peptide	27 ± 3	100 ± 6	1 ± 0	140 ± 4
+ OX-6 (anti I-A)	ND	99 ± 8	ND	ND
+ OX-17 (anti I-E)	ND	100 ± 4	ND	ND
+ OX-18 (anti class I)	ND	31 ± 5	ND	ND
+ W3/25 (anti-CD4)	ND	105 ± 7	ND	ND
+ OX-8 (anti-CD8)	ND	22 ± 3	ND	ND
TCR Vβ14 peptide	10 ± 1	2 ± 0	ND	ND
GPBP	16 ± 1	3 ± 1	93 ± 6	102 ± 5
1–37	13 ± 1	ND	1 ± 1	13 ± 2
44–89	20 ± 1	ND	46 ± 12	56 ± 1
44–68	15 ± 1	1 ± 0	2 ± 0	22 ± 5
72–89	16 ± 1	1 ± 0	27 ± 3	20 ± 2
+ OX-6	ND	ND	1 ± 0	ND
+ OX-17	ND	ND	23 ± 2	ND
+ OX-18	ND	ND	27 ± 1	ND
+ W3/25	ND	ND	1 ± 0	ND
+ OX-8	ND	ND	26 ± 2	ND
87–99	12 ± 1	ND	1 ± 0	ND
90–170	13 ± 3	ND	1 ± 0	14 ± 3
GPBP + Vβ8 peptide	30 ± 2	ND	55 ± 9	149 ± 9
PPD	48 ± 3	2 ± 0	1 ± 1	ND
Rt-BP	11 ± 2	1 ± 0	9 ± 2	9 ± 1

Rats were immunized subcutaneously (s.c.) with 400 μg TCR Vβ8 peptide in CFA containing 1 mg *M. tuberculosis*, and were challenged s.c. with 50 μg GPBP in CFA at the same time or with 100 μg encephalitogenic peptide after 30 days. Twenty days after simultaneous challenge, draining lymph nodes were removed and a single-cell suspension was tested in microtitre wells (5×10^5 cells per well) for response to antigens and mitogens. The remainder of the cells were stimulated in 6-cm diameter Petri dishes with either TCR peptide or GPBP (50 μg ml^{-1}) for 3 days and transferred into interleukin-2 (IL-2) rich medium for an additional 4 days. These cells (2×10^4 per well) were restimulated with antigens and mitogens in the presence of irradiated thymic accessory cells. Stimulation in the presence of TCR peptide or GPBP was carried out in the presence of 2 μg per well monoclonal antibodies. Underlined values indicate significant stimulation. ND, not done.

TABLE 3 Response of TCR Vβ8 peptide-specific T-cell line to attenuated Vβ8 (+) or (−) T cells

Stimulator line (specificity)	Vβ8 expression	Proliferation (c.p.m. $\times 10^{-3}$)
None		7 ± 2
GPBP (S72–89)*	+	31 ± 3
GPBP (55–74)*	−	8 ± 1

T-cell-line cells were irradiated (2,500 R) and 2×10^4 cells were mixed in the absence of additional accessory cells with 2×10^5 TCR specific T cells for 3 days, the last 18 h with [^{3}H]Thy. The cells were harvested and [^{3}H]Thy uptake assessed by liquid scintillation. Background proliferation of irradiated T cells specific for GPBP (S72–89) and GPBP (55–74) was 130 and 220 c.p.m. respectively.

* TCR V gene use for these T-cell lines was described prevously[11].

were strongly positive by immunofluorescence for the CD4 marker and weakly positive for the CD8 marker, but the response to the Vβ8 peptide was restricted only by MHC class I molecules.

A GPBP-specific T-cell line was also selected from protected rats immunized with both the TCR Vβ8 peptide and GPBP. This line had an uncharacteristically low response to the encephalitogenic 72–89 peptide relative to GPBP[10]. Once selected and activated, GPBP-specific T-cell-line cells from TCR-peptide-protected rats were encephalitogenic (hind-leg paralysis with 10 million cells in all 3 rats), indicating that Vβ8 peptide immunization did not result in the deletion of encephalitogenic T-cell precursors.

Mixing of the TCR Vβ8-specific and BP-specific T cells did not impair the response to GPBP, even in the presence of the TCR peptide (Table 2). The presence of TCR Vβ8-specific T cells, however, caused, an increased response to all of the

TABLE 4 TCR Vβ8 peptide-specific T-cells protect against EAE

Transfer dose*	Incidence	Induction of EAE (GPBP/CFA) Day of onset	Duration	Severity†	DTH (mm $\times 10^{-2}$) GPBP	PPD
None	5/5	12	6.5	3.1	32	21
3×10^7	0/5	—	—	0	24	21
1×10^7	0/3	—	—	0	ND	ND

* T-cell line cells were stimulated with TCR Vβ8 peptide plus thymic accessory cells for 3 days prior to intraperitonal transfer into naive recipient rats. The rats were challenged on the same day with GPBP/CFA.

† Value represents the mean of the maximum severity of EAE. See Table 1 legend.

peptides of GPBP except the encephalitogenic 72–89 sequence. The TCR peptide-specific T cells therefore seemed to alter the peptide recognition pattern of GPBP reactive T cells and indicated the possibility of cell–cell interactions.

Direct interactions could also be demonstrated. The addition of irradiated $V\beta8^+$ target cells induced significant proliferation by the TCR-specific T-cell line in the absence of additional accessory cells, whereas $V\beta8^-$ T cells[11] did not (Table 3), indicating the direct recognition of the TCR sequence on the target T-cell surface. The TCR peptide-specific T cells, however, were not cytotoxic for the BP-reactive target cells (data not shown).

The protective ability of Vβ8 peptide-specific T cells was established by adoptive transfer. Rats injected with as few as 10 million Vβ8 peptide-specific T cells did not develop EAE (Table 4). The transferred protection appeared to be T-cell mediated, as we were unable to detect Vβ8 peptide-specific antibodies in the serum of protected rats. DTH results (Table 4) indicated that the adoptively transferred T cells could prevent the induction of EAE without compromising T-cell recognition of other antigens.

The ability of Vβ8-peptide-specific T cells to alter the response patterns of GPBP specific T-cell lines *in vitro* and to protect naive rats from EAE and reduce DTH reactions *in vivo* indicated that the pattern of response to BP epitopes might be altered in rats protected by TCR-peptide-specific T cells. As shown in Table 5, lymph-node cells (LNC) from the EAE-protected animals responded well to the TCR peptide, but poorly to GPBP and the encephalitogenic 72–89 peptide of GPBP compared to LNC from the control group. By contrast, LNC from the protected group showed a significant response to the 87–99 peptide of BP, whereas LNC from the control group had no response to this peptide (Table 5). The selection of a TCR-Vβ8-peptide-specific T-cell line from the lymph nodes of adoptively protected rats (Table 5) indicated that TCR-peptide-specific T cells had migrated to and persisted in the lymph nodes that drained the site of GPBP injection.

These results demonstrate for the first time the use of a synthetic peptide from the CDR2 region of the TCR to induce Vβ8-specific regulatory T cells that prevent the induction of EAE. Computer modelling of ternary interactions among TCR chains, antigenic peptides, and MHC restriction molecules is consistent with CDR involvement in peptide/MHC binding when the TCR is folded in an energetically favourable conformation[12,13]. The indisputable regulatory effects of T cells specific for CDR2 support the notion that this region has biological importance.

It seems unlikely that the responder T cell is interacting directly with the functional TCR Vβ8 molecule on the target T-cell surface. Indeed, it is conceivable that endogenous TCR peptide could be 'processed' and expressed preferentially on the T-cell surface in association with class I molecules[14]. If a natural form of the TCR peptide is associated with the MHC on the T-cell surface, the interacting TCR-specific T cell could interfere with normal activation by BP.

Vaccination with attenuated T cells has provided indirect evidence that protection against autoimmunity may include T-cell recognition of TCR[15–20]. These data indicated that protective immunity was induced against target structures shared by different T-cell clones specific for the same disease-inducing epitope, but did not implicate the TCR directly. The immunogenicity and immunoregulatory activity demonstrated here of a defined region of the TCR Vβ8 chain expressed by encephalitogenic T cells is an important step forward in understanding anti-idiotypic regulation, and provides a clear explanation for the protective effects of the vaccination approach.

The induction of anti-idiotypic antibodies directed against TCR determinants expressed on the surface of intact T cells has played a crucial role in the identification and function of the TCR itself[1–4]. Two of the TCR Vβ8 specific monoclonal antibodies, F23.1 and KJ16, can inhibit EAE in the mouse, which also uses the TCR Vβ8 gene in response to BP[21,22]. Our approach using a synthetic peptide to induce TCR-peptide-specific antibodies may be of value in producing a variety of highly specific antibodies for assessing sequences important in TCR function. The potential regulatory properties of antibodies raised to the Vβ8 peptide will be addressed in a separate study.

TCR peptide vaccination may have application in human autoimmune or malignant conditions that are characterized by common TCR V-gene usage. This approach would seem to have clear advantages over other approaches such as tolerance induced with potentially pathogenic target autoantigens, or whole-cell vaccination which is 'individual' specific and complicated by the presence of extraneous cell-surface antigens. □

TABLE 5 Specificity of T-cell lines from rats protected from EAE by Vβ8 peptide-specific T cells.

Protocol	Stimulant	Proliferation (c.p.m. $\times 10^{-3}$) LN	TCR peptide selected line	GPBP selected line
3×10^7 Vβ8 peptide-specific T cells GPBP+CFA	Medium	7±1	2±1	5±1
	Con A	125±14	46±14	39±1
	Vβ8 peptide	90±6	52±3	6±1
	GPBP	8±0	0±0	16±4
	1–37	7±2	ND	6±1
	44–89	12±2	ND	17±2
	44–68	9±1	4±3	9±1
	72–89	12±3	3±1	11±1
	87–99	41±7	4±2	9±1
	90–170	12±2	ND	8±1
Saline, GPBP+CFA	Medium	12±1	Not selected	6±1
	Con A	38±4		88±10
	Vβ8 peptide	8±1		6±2
	GPBP	32±5		65±2
	1–37	12±0		6±1
	44–89	34±6		91±5
	44–68	19±3		24±3
	72–89	29±2		64±5
	87–99	11±1		6±0
	90–170	11±2		7±1

Lymph-node cells were collected and tested 20 days after simultaneous injection of TCR Vβ8 peptide-specific T line cells (or saline) and GPBP/CFA. T cell lines were selected from the LNC as described in Table 2. Underlined values indicate significant stimulation.

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Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*

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In antibodies, a heavy and a light chain variable domain, VH and VL, respectively, pack together and the hypervariable loops on each domain contribute to binding antigen[1–4]. We find, however, that isolated VH domains with good antigen-binding affinities can also be prepared. Using the polymerase chain reaction[5], diverse libraries of VH genes were cloned from the spleen genomic DNA of mice immunized with either lysozyme or keyhole-limpet haemocyanin. From these libraries, VH domains were expressed and secreted from *Escherichia coli*. Binding activities were detected against both antigens, and two VH domains were characterized with affinities for lysozyme in the 20 nM range. Isolated variable domains may offer an alternative to monoclonal antibodies and serve as the key to building high-affinity human antibodies. We suggest the name 'single domain antibodies (dAbs)' for these antigen binding demands.

We have analysed the interactions with antigen of individual domains of the anti-lysozyme antibody, D1.3 (ref. 1). The VH domain was expressed in *E. coli* and secreted into the periplasm[6,7], alone or in association with the Vκ domain (fig. 1). Analysis of culture medium, by passage through a lysozyme–Sepharose affinity column[8], followed by SDS-PAGE[9] revealed that both the isolated VH domain, or the associated Fv fragment, could bind to lysozyme, and could be purified to homogeneity in a single step, with yields of ~200 μg l^{-1} and 10 mg l^{-1}, respectively. The VH domain appears to be monomeric by FPLC (Pharmacia, Superose 12 column). The N-terminal sequences of both domains were checked by gas-phase protein sequencing[10,11].

As shown in Table 1, the affinity of Fv fragment for lysozyme and the stoichiometry of binding of the VH domain to lysozyme were determined by titration using fluorescence quench techniques. The affinity of VH domain for lysozyme was determined from the kinetics of binding. The affinity of the Fv fragment (3 nM) is similar to the parent antibody (2 nM). The VH domain binds lysozyme tightly in an equimolar complex with an affinity for lysozyme (19 nM) which is only 10-fold weaker. Separated heavy and light chains have previously been identified with antigen[12] or hapten binding activities[13] although the affinities were poor, with no evidence for binding by single chains[13,14] rather than dimers[15].

TABLE 1 Affinities of Fv fragment and VH domains for hen egg lysozyme

	Stoichiometry	Affinity (nM)	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	k_{off}/k_{on} (nM)
Fv-D1.3	ND	3	1.9×10^6	ND	ND
VH-D1.3	1.2	ND	3.8×10^6	0.075	19
VH3	ND	ND	2.9×10^6	0.036	12
VH8	ND	ND	3.3×10^6	0.088	27

Cultures of 500 ml were grown and induced (see Fig. 1 methods), and the supernatant passed through a 0.45 μm filter (Nalgene), then through a 5 ml lysozyme-Sepharose affinity column. After washing with phosphate buffered saline (PBS), the Fv fragment or VH domains were eluted with 50 mM diethylamine, and analysed for purity by SDS-PAGE[9]. The proteins were titrated with lysozyme at 25 °C using fluorescence quench (Perkin Elmer LS 5B Luminescence Spectrometer)[27] to determine the number of active binding sites, to measure the affinity of the Fv fragment and the stoichiometry of binding of the VH domain (mole lysozyme per mole domain). The concentration of the VH domain of the D1.3 antibody was determined by hydrolysis followed by quantitative amino-acid analysis. The kinetics of lysozyme binding were determined by stopped-flow (HI Tech Stop Flow SHU) at 20 °C under pseudo-first order conditions with binding sites in five to ten fold excess over lysozyme[28]. For the kinetics, the concentration of binding sites, not protein, was measured ND, not determined. k_{on} is the second order rate constant for association, and k_{off} is the first order rate constant for dissociation.

In the D1.3 antibody, lysozyme interacts extensively with both domains, and forms three hydrogen bonds to the Vκ domain, and nine hydrogen bonds to the VH domain. Binding of lysozyme buries ~300 Å^2 of Vκ domain away from solvent, and 400 Å^2 of the VH domain[1]. Our results show, however, that the Vκ domain makes only a small net contribution to the energetics of binding. This is surprising as the removal of a single hydrogen bond[16] or a single van der Waals contact[17] can lead to tenfold loss in affinity. The VH domain presumably binds to lysozyme in a similar way to the antibody and this is consistent with inhibition of binding of the Fv fragment by the VH domain (data not shown, but see Fig. 1 legend). It is possible that the whole surface of interaction might reorientate slightly, perhaps by rocking on side chains, to create a new set of contacts[18].

The result prompted us to obtain VH domains with antigen-binding activities from antibody-producing cells. Previously we have demonstrated the cloning of immunoglobulin variable regions from hybridoma mRNA for expression of chimaeric antibodies, using the polymerase chain reaction (PCR)[5,19]. We now used PCR to amplify the rearranged VH genes from the spleen DNA of a mouse immunized with lysozyme (Fig. 2). The amplified DNA was cloned into the vector M13VHPCR1 (ref. 19) for sequencing. The complete sequences of 48 VH gene clones were determined (data not shown). All but two of the mouse VH gene families[20] were represented, with frequencies of: VA (1), IIIC (1), IIIB (8), IIIA (3), IIB (17), IIA (2), IB (12) and IA (4). In 30 clones the D segments could be assigned to families SP2 (14), FL16 (11) and Q52 (5), and in 38 clones the JH minigenes to families JH1 (3), JH2 (7), JH3 (14) and JH4 (14). The different sequences of CDR3 marked each of the 48 clones as unique. Nine pseudogenes and 16 unproductive rearrangements were identified; of the clones sequenced, 27 have open reading frames. Clearly we can generate a diverse repertoire of VH genes using PCR, but cannot rule out a systematic bias due to our choice of primers and hybridization conditions. VH gene libraries have also been generated using PCR

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