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Immunology

T-cell receptor (TCR) usage in Lewis rat experimental autoimmune encephalomyelitis: TCR β-chain-variable-region Vβ8.2-positive T cells are not essential for induction and course of disease

(myelin basic protein/multiple sclerosis)

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ABSTRACT **Predominant usage of Vβ8.2 gene segments, encoding a T-cell receptor (TCR) β chain variable region, has been reported for pathogenic Lewis rat T cells reactive to myelin basic protein (MBP). However, up to 75% of the α/β T cells in a panel of MBP-specific T-cell lines did not display TCR Vβ8.2, Vβ8.5, Vβ10, or Vβ16 elements. To further investigate TCR usage, we sorted the T-cell lines for Vβ8.2- and Vβ10-positive T cells or depleted the lines of cells with these TCRs. Vβ8.2-positive T cells and one of the depleted T-cell lines strongly reacted against the MBP peptide MBP-(68–88). The depleted T-cell line caused marked experimental autoimmune encephalomyelitis (EAE) even in Lewis rats in which endogenous Vβ8.2-positive T cells had been eliminated by neonatal treatment with anti-Vβ8.2 monoclonal antibodies. T-cell hybridomas generated from this line predominantly used Vβ3 TCR genes coexpressed with TCR Vα2 transcripts, which were also used by Vβ8.2-positive T cells. Furthermore, Vβ10-positive T cells reactive to MBP-(44–67) were encephalitogenic when injected immediately after positive selection. After induction of EAE by sorted Vβ8.2- or Vβ10-positive T-cell lines, immunocytochemical analysis of the spinal cord tissue showed a predominance of the injected TCR or of nontypable α/β T cells after injection of the depleted line. Our results demonstrate heterogeneity of TCR β-chain usage even for a single autoantigen in an inbred strain. Moreover, Vβ8.2-positive T cells are not essential for the induction and progression of adoptive-transfer EAE.**

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system mediated by major histocompatibility complex class II-restricted CD4-positive T cells. Myelin basic protein (MBP) is a dominant autoantigen in the Lewis rat, and most of the encephalitogenic MBP-reactive T lymphocytes recognize a peptide spanning aa 68–88 of guinea pig MBP [MBP-(68–88)] (1, 2) presented by RT1.B^{l} major histocompatibility complex molecules. These T cells have been reported to use a highly restricted set of T-cell-receptor (TCR) α/β chains, variable regions Vα2 (3, 4) and Vβ8.2 (3–5). In addition, a marked conservation of the first 2 aa (Asp-Ser) in the junctional regions of Vβ8.2 receptors has been described with only a few or no non-germ-line-encoded nucleotide additions (5, 6).

The pathogenic importance of T cells expressing these peculiar TCRs was substantiated by the efficacy of Vβ8.2 TCR-directed therapy of EAE (7–9) (T. Hünig, personal communication) and by studies showing a strong bias for Vβ8.2 T cells infiltrating the spinal cord (10–12). However, a broader spectrum of TCRs expressed in EAE lesions was observed by reverse transcriptase (RT)–PCR performed on tissue taken during the early invasive process (13) and in late stages of EAE (10, 14). Although the latter findings could be explained by a preferential attraction of bystander inflammatory cells to the lesion (15), an active role of non-Vβ8.2 T cells in the pathogenesis of Lewis rat EAE has been proven by the encephalitogenicity of Vβ6- and Vβ14-positive T cells reactive to the MBP epitope at aa 87–99 (16, 17). Diverse TCR β-chain usage by rat encephalitogenic T cells reactive to MBP residues 68–88 has been reported (18). However, a possible contribution of host-derived Vβ8.2 cells was not excluded in a previous investigation (17) where EAE was induced by transfer of Vβ8.2-negative T-cell lines.

To analyze the contribution of defined T-cell populations in adoptive-transfer EAE, we sorted cells from MBP-specific encephalitogenic T-cell lines by their TCR-Vβ-region gene usage and typed the immunoinflammatory infiltrate in spinal cord lesion.‡ Our data demonstrate that Vβ8.2-negative T cells that are reactive to aa 68–88 of MBP, the main encephalitogenic epitope, are capable of inducing adoptive-transfer EAE even in Lewis rats depleted of Vβ8.2 T cells.

MATERIALS AND METHODS

Animals and EAE Scoring. EAE was induced in 6- to 8-week-old Lewis rats (Zentralinstitut für Versuchstierzucht, Hannover, Germany) by intravenous injection of 5×10^6 freshly activated MBP-specific T-cell blasts from sorted T-cell lines. Rats were weighed daily. Disease severity was assessed clinically by using a scale from 0 to 5 (19). To eliminate Vβ8.2 T cells, neonatal rats received two i.p. injections of 50 μg of anti-Vβ8.2 monoclonal antibodies (mAbs) per week until week 4 and thereafter 100 μg twice per week until week 6. Controls were untreated animals from the same litter.

Reagents and Antibodies. All tissue culture supplements were from GIBCO, except bovine serum albumin fraction V (Roth, Karlsruhe, Germany) and concanavalin A (Sigma). MBP was prepared as described (20). Guinea pig MBP peptides MBP-(68–88) and -(87–99) (18) were synthesized by

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Abbreviations: TCR, T-cell receptor; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; mAb, monoclonal antibody; RT, reverse transcriptase; FACS, fluorescence-activated cell sorter; V, variable; J, joining; D, diversity.

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‡The sequences reported in this paper have been deposited in the GenBank data base [accession nos. X80528 (Vβ8.2), X80515 (Vβ10), X80524 (Vβ3.3), X80525 (Vβ6), X80522 (Vβ12), X80523 (Vβ14), X80527 (Vα2 coexpressed with Vβ8.2), X80526 (Vα2 coexpressed with Vβ3.3), and X81301 (Vβα19)].

Kem-En-Tek (Copenhagen); MBP-(45–67) was from Med-Probe (Oslo).

Mouse anti-rat mAb W3/13 staining pan T cells was from Serotec. mAb R73 recognizing the α/β-TCR (21) was purified from supernatants of hybridoma cells. TCR Vβ gene products were identified with the mAbs R78 specific for Vβ8.2 TCR, B73 specific for Vβ8.5 TCR, and G101 recognizing Vβ10 TCR (22) (kindly provided by T. Hünig, University of Würzburg). mAb HIS42 detecting Vβ16 TCR was from Dianova (Hamburg, Germany).

Cells and Culture. MBP-specific CD4-positive rat T-cell lines were established from lymph nodes of female Lewis rats immunized in the hind footpad with 100 μg of MBP emulsified in complete Freund's adjuvant (Difco) (23, 24). For proliferation studies, resting T cells of the sorted T-cell lines were added at 2×10^4 cells per well with 10^6 irradiated thymic antigen-presenting cells and antigen in 100 μl of medium to wells of 96-well round-bottom microtiter plates (Nunc). Triplicate cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air for 24 h and harvested after a 16-h pulse with [^{3}H]thymidine at 0.2 μCi per well (Amersham–Buchler; 1 Ci = 37 GBq). Cells were collected on glassfiber filters by using a Betaplate 96-well harvester (Pharmacia-LKB). Radioactivity associated with the dried filter was quantified with a 96-well Betaplate liquid scintillation counter (Pharmacia-LKB).

Cells expressing TCR Vβ genes were enriched or depleted by using the magnetic cell sorting MACS system (Miltenyi Biotech, Bergisch-Gladbach, Germany) (25). Sorted T cells were then propagated in interleukin 2-containing medium. If necessary they were subjected to repeated sorting cycles.

T-cell hybridomas were generated by fusion of activated T-cell blasts from sorted T-cell lines with a variant of the mouse thymoma BW5147 (BW1100.129.237) (22) and screened for expression of TCR by fluorescence-activated cell sorter (FACS) analysis. TCR usage of positive clones was further characterized by PCR and DNA sequencing as described below.

Immunocytochemical Studies. FACS analysis of TCR expression was performed as described (24). Immunofluorescence of 5000 living cells as determined by propidium iodide exclusion was measured on a FACScan (Becton Dickinson).

For histological analysis, various segments of spinal cord and brain were removed and processed as described (23). Cellular infiltrates were characterized in serial sections by incubation with mAb R73, recognizing the α/β-TCR (21), or mAbs specific for the TCR Vβ region (22) at 4°C overnight and visualized as described (23).

Cross-sections from two segments of the lumbar spinal cord of each animal were examined. The number of T cells was counted in serial sections in 10 0.8-mm^2 fields at a primary magnification of ×200.

Fluorescent Cell Trafficking Studies. The PKH26 fluorescent labeling kit (26) (Sigma) was used at a final dye concentration of 10 μM to label encephalitogenic T-cell blasts by the supplier's instructions.

TCR Sequence Analysis. Total RNA was extracted by using Roti-Quick-Kits (Roth). Approximately 30 μg of total RNA was reverse-transcribed into first-strand cDNA by using NotI-d(T) 18 primers and a cDNA synthesis kit (Pharmacia). The cDNA was used at a dilution of 1:50 for enzymatic amplification with specific TCR V-element primers and a common constant-region Cα or Cβ primer. The PCR mixture consisted of 1 μl of diluted cDNA, 5 μl of 10× PCR buffer (Applied Biosystems), 1.25 units of *Taq* polymerase (Applied Biosystems), and 25 pmol of the respective TCR C-element and V-element primers. Amplifications were performed with a model 9600 thermocycler (Perkin–Elmer), using a cycle profile consisting of denaturation at 95°C for 60 s and extension at 72°C for 60 s for 30 cycles with a prolonged extension for 5 min during the last cycle. The primers detect the known rat Vα (27) and Vβ (28) families in lymph node cells and thymocytes of Lewis rats (data not shown). Reaction products purified by QIAquick spin PCR purification kits (Quiagen, Hilden, Germany) were cloned into PCRscript vectors (Stratagene) and sequenced (T7 sequencing kit; Pharmacia).

RESULTS

TCR Vβ Usage of MBP-Specific T-Cell Lines. We typed four encephalitogenic MBP-specific T-cell lines for TCR usage by FACS analysis. All lines were composed of α/β T cells. There was a strong usage of the Vβ8.2 TCR during early restimulation cycles of all cell lines, from 12% to 23%. However, up to 75% of the T cells could not be stained by the limited panel of mAbs specific for TCR Vβ regions. Longitudinal studies on the variability of TCR usage by line MBP13 were performed along several activation cycles with guinea pig MBP and propagation in interleukin 2-enriched medium. The percentage of Vβ8.2-positive T cells varied between 30% and 60%, and we observed no selection toward predominant Vβ8.2 TCR usage during subsequent activation cycles. Apart from Vβ8.2-positive T cells, only Vβ10-expressing cells made up a major subpopulation in T-cell line MBP13.

When we injected the cell lines characterized above into naive Lewis rats and studied inflammatory infiltrates in spinal cord 4 days later, we found that up to 30% of the α/β T cells in the lesion expressed the Vβ8.2 TCR. More than 60% of T lymphocytes were not stained by Vβ-specific antibodies. We observed 5% Vβ10-positive T cells only when EAE was induced by MBP13 cells (data not shown).

Sorting and Characterization of MBP-Specific T-Cell Lines. These findings prompted us to purify cell line MBP13 by repetitive sorting cycles with the MACS system to study TCR usage. The cytofluorometric T-cell typing is summarized in Fig. 1. We obtained sorted T-cell lines with homogeneous expression of Vβ8.2 TCR or Vβ10 TCR or a cell line with α/β T cells depleted of cells expressing these two Vβ gene segments.

We compared reactivity toward different MBP peptides that are recognized by encephalitogenic MBP-specific T-cell lines (3, 29) or dominate in the immune response to MBP in naive rats (30). As expected, reactivity of Vβ8.2 T cells was limited to the main encephalitogenic epitope of guinea pig MBP spanning aa 68–88 (Fig. 2 *Left*). However, the depleted T-cell line also displayed strong reactivity toward that peptide and

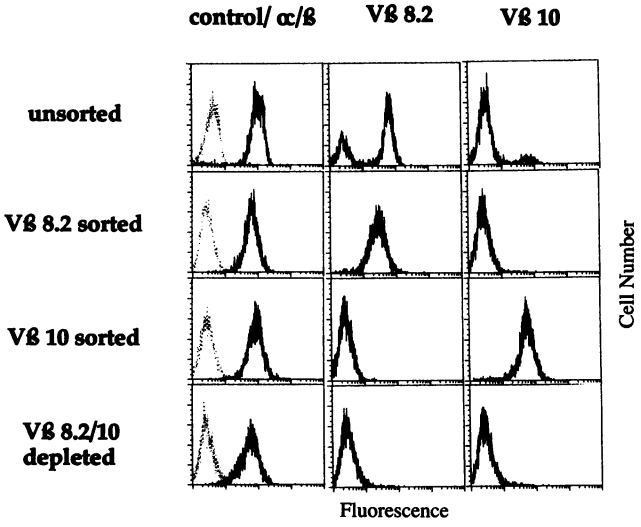

FIG. 1. FACS analysis of the unsorted T-cell line MBP13 and sorted sublines. (*Left*) α/β TCR expression vs. controls, where the primary antibody was omitted. Usage of Vβ8.2 TCR (*Middle*) and Vβ10 TCR (*Right*) is depicted.

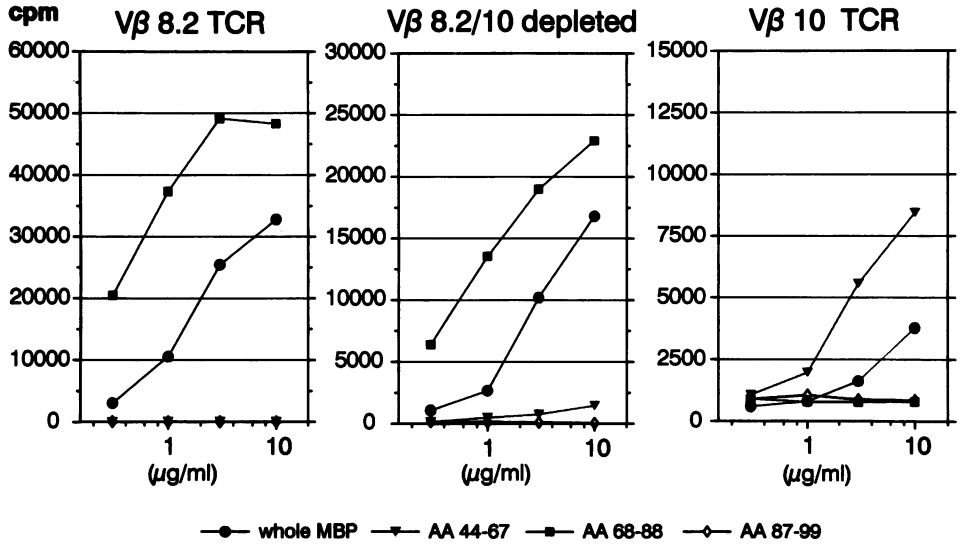

FIG. 2. Summary of T-cell proliferation data. Sorted T-cell lines were activated by syngeneic thymic antigen-presenting cells in the presence of increasing concentrations of antigen as indicated by the symbols. The *x* axis gives the concentration of antigen; the *y* axis gives the mean cpm of triplicate wells. Standard deviations were usually <10%. The experiment was repeated once with similar results.

had only a minor response to MBP-(44–67) (Fig. 2 *Middle*). Vβ10 T cells proliferated in response to MBP-(44–67) only (Fig. 2 *Right*). The peptide and MBP reactivity of all sorted T-cell lines was almost completely suppressed after addition of mAb OX-6 but not after addition of mAb OX-17, indicating major histocompatibility complex class II restriction by RT1.B[l] (data not shown).

Histological Studies on TCR Vβ Gene Usage. EAE was induced by sorted T-cell lines to investigate the composition of the inflammatory infiltrate and the variability of TCR Vβ usage in the lesion during the evolution of the disease. Only occasional Vβ8.5- and Vβ16-positive cells were seen, excluding a significant contribution of these Vβ gene segments. In the brain, composition of the inflammatory infiltrate was similar to that in spinal cord. Inflammation was maximal in the lumbar spinal cord. Serial sections from different regions of lumbar spinal cord were stained for α/β-TCR, TCR Vβ8.2, and TCR Vβ10. In Lewis rats injected with Vβ8.2 cells, EAE started at day 3, and animals had lost 15% of their weight and displayed severe paraparesis on day 5. The peak of infiltration by α/β T lymphocytes was observed on days 3 and 4. At this time the percentage of Vβ8.2-positive cells was 48% of all α/β T lymphocytes and decreased to 18% on day 6. Only occasionally did we observe Vβ10 TCR expression. Vβ10 T-cell blasts were encephalitogenic when activated and injected immediately after positive sorting. However, inflammation and disease started 2 days later than in EAE induced by Vβ8.2-positive cells and resulted in weight loss of only 10% and milder paraparesis. The number of T cells expressing Vβ10 TCR was 38% of T cells stained for α/β TCR by mAb R73 on day 5. Other TCR Vβ regions studied represented negligible subpopulations. Of special note were the findings obtained with the T-cell line depleted for Vβ8.2 and Vβ10. Lewis rats injected with these cells exhibited a mean weight loss of 15% and moderate paraparesis at the peak of the disease on day 5. The composition of the inflammatory T-cell infiltrate in the

a

	Vβ	N - Dβ - N	Jβ	
	C A S S	D S S	Y E Q Y	
Vβ8.2	TGT GCC AGC AGT.....	GAC AGC TCT.....	TAT GAG CAG TAT	Jβ2.6
	C A S S	S L R G	L F F G	
Vβ10	TGT GCC AGC AGC.....	TCT CTC AGG GGG.....	TTG TTT TTC GGC	Jβ1.4
	C A S S	L S Q G G	T E V F	
Vβ3.3	TGT GCC AGC AGT.....	CTG TCC CAG GGT GG.....	C ACA GAA GTT TTC	Jβ1.1
	C A S S	I Q R T G A	Q A Q Y	
Vβ6	TGT GCC AGC AGC...	ATA CAG AGG ACA GGG GC...	C CAG GCC CAG TAT	Jβ1.5
	C A S S	P G T P I	Y E Q Y	
Vβ12	TGT GCC AGC AGT.....	CCC GGG ACT CCG AT.....	C TAT GAG CAG TAT	Jβ2.6
	C A W	T R P E	Q E T Q	
Vβ14	TGT GCC TGG A.....	CC CGG CCG GAG.....	CAA GAG ACC CAG	Jβ2.5

b

	Vα	N - Jα	
	C A A	R G Y G N E K M T F	
Vα2 (Vβ8.2)	TGT GCA GCA.....	AGG GGA TAT GGA AAT GAG AAA ATG ACT TTT	Jα rVA19
	C A A	Y N S N A G K L T F	
Vα2 (Vβ3.3)	TGT GCA GCC.....	TAT AAC AGC AAT GCA GGC AAA TTA ACC TTT	Jα TRA29
	C A M	S S G P G A G T G K	
rVα19 (Vβ10)	TGC GCT ATG.....	AGT AGT GGG CCT GGA GCT GGC ACT GGA AAG	Jα G101

FIG. 3. Nucleic acid and predicted amino acid sequences of rat TCR β (*A*) and α (*B*) chains in the naive T-cell line MBP13 or in cell lines obtained after sorting MBP13. Assignments to V, D, and J segments are based on refs. 28 and 31–33. Dβ and Jα elements are underlined.

spinal cord was screened in serial sections stained for α/β TCR and Vβ usage. Despite marked infiltration by α/β T cells, <5% Vβ8.2 T cells were detected, reflecting the normal proportion of Vβ8.2 TCR usage in peripheral lymphoid tissue. Only single Vβ10-positive T cells were found in the meninges. Usage of Vβ8.5 or Vβ16 TCR was not observed in inflammatory lesions (data not shown).

TCR Sequence Analysis. To characterize the TCR Vβ and Vα chains expressed by the sorted T-cell lines, we used a panel of primers amplifying all known rat Vβ and Vα chains. Cloning and sequencing of PCR products of the Vβ8.2- and Vβ10-positive T-cell lines confirmed the results obtained by immunostaining. Sequences of the TCR Vβ junctional regions are given in Fig. 3. The Vβ8.2 sequence showed the typical Asp-Ser sequence of encephalitogenic T cells recognizing the main MBP epitope (5). The junctional sequence of the Vβ10-positive cells did not show similarity to published sequences of MBP-specific T cells (3–5, 13, 16, 17). To characterize the TCR elements in non-Vβ8.2 non-Vβ10 T cells obtained by depletion of cell-line MBP13, we used cDNA prepared from these sorted T cells for a Vβ-specific RT–PCR. Positive amplification products were detected for Vβ3, Vβ6, Vβ12, and Vβ14 (Fig. 3*A*). Cloning and sequencing of the PCR products revealed that none of these Vβ chains had high similarity in the V(D)J-junctional regions (where D is diversity and J is joining) to any published TCRβ sequence used by MBP-specific T cells or inflammatory infiltrates in the central nervous system. To identify the Vα gene elements associated with the TCR in the non-Vβ8.2 non-Vβ10 T cells, we generated T-cell hybridomas. cDNA prepared from cloned hybridomas was screened by PCR as described above. In 10 of 12 clones, positive signals were seen for Vβ3 primers, but none of the hybridomas used Vβ6 or Vβ14 TCR. By cloning and sequencing, the PCR products were identified as Vβ3.3 chains, displaying V(D)J junctional regions identical to those obtained from the depleted T-cell line used for fusion (Fig. 3*A*). By using a panel of oligonucleotide primers specific for rat TCR Vα elements, the TCR α chains expressed by Vβ8.2, Vβ10-sorted T cells and by Vβ3.3-positive hybridomas were amplified by RT–PCR, cloned, and sequenced. As reported (3, 4), we detected TCR Vα2 usage in the Vβ8.2-sorted T cells (Fig. 3*B*). Surprisingly, the TCR Vβ3.3 chain was also associated with Vα2 in all hybridomas analyzed (Fig. 3*B*). The TCR α chains expressed by the Vβ8.2-positive and Vβ3.3-positive cells used distinct Vα2 family members and differed in their junctional regions from the Vα–Jα sequences described for Tcc510 (4) and Z1 (34). The Vβ10-positive T cells used the Vα element rVA19 described in ref. 31 (Fig. 3*B*).

Encephalitogenicity of T-Cell Lines Depleted of TCR Vβ8.2 and Vβ10 Cells in Vβ8.2-Depleted Rats. Further analyses were performed to study trafficking of injected T cells from the depleted cell line and to exclude a contribution of Vβ8.2-positive cells recruited from the recipient rat. By using fluorescent membrane labeling, PKH-stained cells were found in spleen but not in spinal cord on days 1 and 2 after injection. On day 3, PKH-labeled T cells made up only 5% of the α/β T cells in parenchyma and meninges of lumbar spinal cord (data not shown). That Vβ3 was indeed expressed by T cells in the lesion, along with several other Vβ chains, was confirmed by RT–PCR on RNA prepared from spinal cord (Fig. 4).

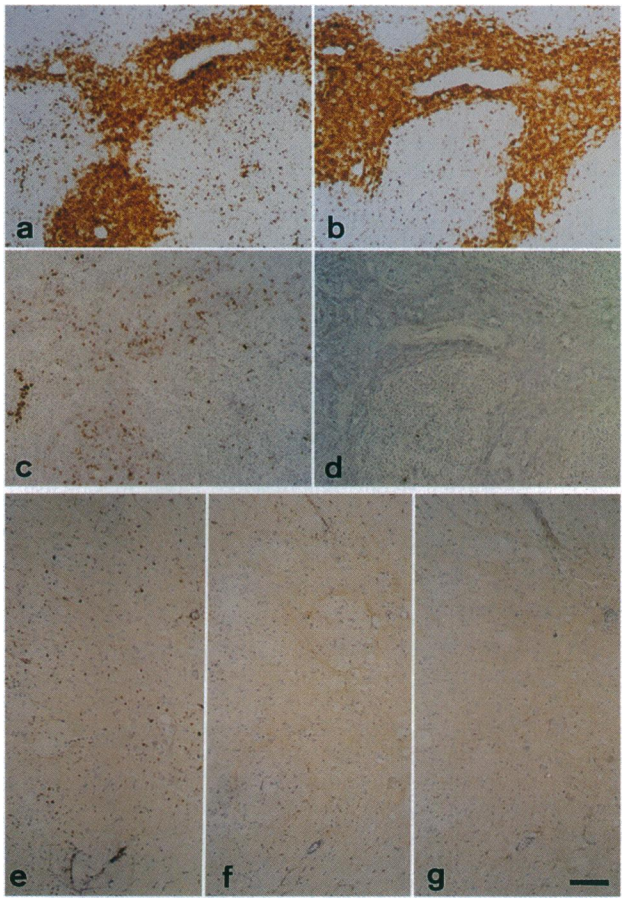

FIG. 5. Representative serial sections of splenic tissue from a rat treated with Vβ8.2 mAb (*b* and *d*) and a naive littermate (*a* and *c*) and serial sections of EAE spinal cord from the Vβ8.2-mAb-treated rat (*e–f*). The Vβ8.2-mAb-treated rat shows as many α/β T cells (*b*) as its naive littermate (*a*). However, Vβ8.2 T cells have almost disappeared in the spleen of the treated animal (*d*) but are abundant in the control (*c*). Spinal cord of the same Vβ8.2-mAb-treated rat on day 4 after induction of EAE by injecting the Vβ8.2/Vβ10-depleted T-cell line. Cryosections were stained for α/β TCR (*e*), Vβ8.2 TCR (*f*), or Vβ10 TCR (*g*) and counterstained with hematoxylin. There is a strong infiltrate of α/β T cells without any Vβ8.2 or Vβ10 T cells. (Bar in *g* = 100 μm.)

Fluorescent membrane labeling with Vβ8.2-positive T cells indicated that ≈70% of Vβ8.2-positive T cells in the lesion were recruited from the recipient rat in Vβ8.2 adoptive-transfer EAE (data not shown), thus suggesting a major contribution of host-derived Vβ8.2 T cells. In view of these findings, we decided to substantiate the pathogenicity of Vβ8.2/Vβ10-negative T cells by transferring them into rats that were neonatally injected with Vβ8.2 mAb. This treatment almost completely eliminated Vβ8.2 T cells in splenic tissue but did not affect overall number of α/β T cells in spleen as shown in Fig. 5 *a–d*. Disease course and histological findings (Fig. 5 *e–g*) after injection of the depleted T-cell line into neonatally treated rats were comparable to EAE in native animals. At the peak of the disease, rats in both groups exhibited a mean weight loss of 15% and a moderate paraparesis. These experiments provided strong evidence that non-Vβ8.2

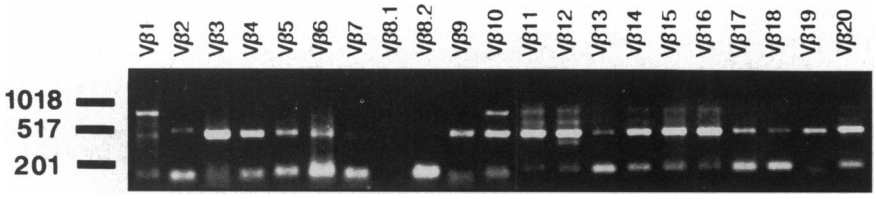

FIG. 4. DNA gel electrophoresis of RT–PCR fragments. RNA was prepared from spinal cord of a rat injected with the depleted T-cell line. Vβ-specific primers are indicated on the top of each lane. Positions of DNA size markers (in bp) are shown to the left.

non-Vβ10 T cells are capable of mediating EAE, even in the complete absence of host-recruited TCR-Vβ8.2-positive T cells.

DISCUSSION

We have shown that a T-cell line depleted of TCR Vβ8.2 and Vβ10 T cells with preferential recognition of residues 68–88 of guinea pig MBP is encephalitogenic in Lewis rats depleted of Vβ8.2 T cells. We have traced this cell line in the lesion by fluorescent labeling techniques. Unfortunately, the lack of a complete set of mAbs specific for rat TCR elements hinders studies on the protein level. Predominant usage of Vβ3.3 TCR β chain associated with Vα2 was found in hybridomas generated from that T-cell line. Although the proportion of generated T-cell hybridomas may not reflect the actual frequency of T-cell subsets in the original population, Vβ3.3 was also detected in early spinal cord lesions by RT–PCR, in accord with findings by Sun *et al.* (18) who also showed Vβ3 TCR usage in Vβ8.2-negative encephalitogenic T-cell lines by using PCR techniques.

Our results corroborate previous reports (17, 18) on diverse TCR β-chain usage by rat encephalitogenic T cells reactive to guinea pig MBP-(68–88). TCR Vβ3.3 elements are used by >2% of the T lymphocytes in the Lewis rat, while the number of Vβ8.2-positive T cells in peripheral blood and lymph nodes is ≈5% (12, 35). This may explain why the pathogenic importance of Vβ8.2-positive T cells has been recognized much earlier. While the majority of rats neonatally treated with Vβ8.2 mAb are resistant to disease induction with MBP, some are still susceptible, indicating a recruitment of non-Vβ8.2 encephalitogenic T cells (H. Immrich, C. Kugler, N. Torres-Nagel, R. Dörries, and T. Hünig, personal communication).

As expected, Vβ8.2-positive T cells were encephalitogenic. Analysis of their TCR usage was in accord with previous studies (3, 4). Furthermore, Vβ10 T cells recognizing MBP-(44–67) were encephalitogenic only after engagement and crosslinking of their TCR by mAb and subsequent activation with guinea pig MBP but not after simple activation by MBP or MBP-(44–67). By FACS analysis, we ruled out contamination by TCR Vβ8.2 cells immediately after sorting, and even addition of a subencephalitogenic number of cells (1×10^5 Vβ8.2 T cells to 5×10^6 Vβ10 cells) during later stages of activation did not produce clinical disease. Thus it appears that TCR engagement may critically alter the ability of Vβ10 cells to enter the central nervous system through the blood–brain barrier. Similar effects of TCR engagement by mAb have been reported by Zhao *et al.* (36). Alternatively, one may hypothesize that the density of $RT1.B^1$ molecules presenting MBP-(44–67) on the surface of antigen-presenting cells in the brain is not high enough to activate the Vβ10-positive T cells, thus requiring costimulation by the TCR-specific antibody. Indeed, a low binding affinity of MBP-(53–67) for $RT1.B^1$ molecules has been shown by Joosten *et al.* (37).

Interestingly, Vα2 not only was associated with TCR Vβ8.2 chains, as described (3, 4), but also was coexpressed with Vβ3.3 in the T-cell hybridomas characterized in our studies. Thus one may hypothesize that recognition of MBP by encephalitogenic T cells in the Lewis rat is associated with a dominant usage of specialized Vα chains, irrespective of the MBP epitope recognized, rather than of Vβ chains. Only rare exceptions of coexpression of Vβ8.2 chains with non-Vα2 chains in encephalitogenic T cells have been reported (38). This issue needs to be explored with regard to TCR Vα gene usage by T cells reactive to other encephalitogenic epitopes of MBP, especially by MBP-(87–99)-specific encephalitogenic T cells (17, 39).

Our experiments with neonatally treated rats demonstrate that during the autoimmune process directed against the main encephalitogenic epitope MBP-(68–88) in Lewis rats, expression of TCR Vβ8.2 is necessary neither at the level of the encephalitogenic T cell transferring the disease nor at the level of host-recruited T cells. This argues against a unique importance of TCR Vβ8.2 in EAE induction.

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