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Immunology

Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to nested epitopes of myelin basic protein

(autoimmunity/immunotherapy/experimental allergic encephalomyelitis)

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ABSTRACT **The peptide p89-101 (Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro) of myelin basic protein is encephalitogenic in mice expressing H-2^q and H-2^s antigens. Six of 13 encephalitogen-specific T-cell clones were shown to express the variable β-chain (V_β) 17a gene product (KJ23a$^+$), whereas seven clones were KJ23a$^-$. Both KJ23a$^+$ and KJ23a$^-$ subpopulations were encephalitogenic in SJL/J mice when adoptively transferred. Depletion of KJ23a$^+$ cells *in vivo* with the administration of the antibody KJ23a suppresses experimental allergic encephalomyelitis induced with KJ23a$^+$ T-cell lines. However, experimental allergic encephalomyelitis induced with either (*i*) encephalitogenic peptide p89-101, (*ii*) intact myelin basic protein, or (*iii*) KJ23a$^-$ T cells reactive to p89-101 cannot be prevented with monoclonal antibody KJ23a. These data indicate that in spite of the V_β 17a gene expression in a relatively large proportion of p89-101-specific T cells, such V_β gene use is not essential for the induction of experimental allergic encephalomyelitis in SJL/J mice. These results contrast with the predominance of V_β gene use (V_β 8.2) in T cells reactive to the encephalitogenic fragment (pR1-11) in PL/J mice. One reason for this lack of dominant use of a particular T-cell receptor V_β gene family in the autoimmune response to myelin basic protein in SJL/J mice stems from the observation that two encephalitogenic epitopes exist in p89-101. KJ23a$^-$ T cells are stimulated by the deleted peptide p89-100, whereas KJ23a$^+$ T cells are not. Thus, in the response to an encephalitogenic fragment of myelin basic protein containing two nested epitopes, at least two distinct T-cell receptor V_β genes are expressed. These distinct T-cell subpopulations can each trigger experimental allergic encephalomyelitis. These findings have implications for therapy of autoimmune disease with antibodies to the T-cell receptor gene products.**

Experimental allergic encephalomyelitis (EAE) is a murine model of autoimmune inflammatory diseases in the central nervous system; it has been clearly demonstrated that this disorder is mediated by CD4$^+$ T cells (1). EAE can be induced by a monoclonal population of myelin basic protein (MBP)-specific T cells (2) and is prevented or ameliorated by *in vivo* injection with anti-Ia (3) or anti-L3T4 antibody (4, 5). Although T cells clearly play an essential role in the pathogenesis of this disease, the availability of encephalitogenic T-cell clones has permitted studies of the molecular genetic basis of EAE. Recently, various rearrangement patterns of the T-cell receptor (TCR) genes have been clarified, and differences in antigen or major histocompatibility complex specificity could be attributed to sequence changes in particular regions of the TCR genome (6). A previous report from our laboratory (7) showed a preferential expression of a single TCR variable β-chain (V_β) gene family in encephalitogenic T cells from PL/J mice, a strain highly susceptible to EAE. This indicated that such a preferential use of a TCR V_β gene in autoimmune effector T cells might correlate with susceptibility to EAE and raised the question whether such a predominant use of a single TCR V_β gene could be generalized to EAE in other genetic strains.

In this communication we examined another highly susceptible homozygous mouse strain, SJL/J, which lacks the V_β 8 gene family (8) that is predominantly expressed in encephalitogenic T-cell clones from PL/J mice. In SJL/J mice, an encephalitogenic epitope (p89-101) of MBP was characterized (9). Encephalitogenic T-cell clones specific for this determinant are available (10). Furthermore, a monoclonal antibody, KJ23a, against the TCR V_β 17a gene product has been produced by Kappler *et al.* (11). The initial studies revealed that the SJL/J-derived encephalitogenic T-cell clone 4b.14a (14a) was positively stained with KJ23a antibody. To determine whether preferential use of a single V_β gene exists in SJL/J mice, we examined the contribution of this V_β gene to the pathogenesis of EAE in this strain. It was found that about half of the independently derived p89-101-specific T-cell clones are KJ23a$^+$. Moreover, the KJ23a$^-$ T cells are also encephalitogenic. In addition, we show that there is more than one encephalitogenic epitope within p89-101; KJ23a$^-$ T cells recognize a nested epitope within p89-101—namely, p89-100.

MATERIALS AND METHODS

Mice. All female mice were purchased from The Jackson Laboratory.

Antigens. Synthetic MBP peptides p89-101 and p89-100 were synthesized by solid-phase techniques. The purity was determined by high-pressure liquid chromatography and by amino acid analysis.

Monoclonal Antibodies. KJ23a (11) was a gift from P. Marrack (National Jewish Hospital, Denver), F23.1 (12) was a gift from M. Bevan (Scripps Clinic and Research Foundation, La Jolla, CA), and GK1.5 (13) was a gift from F. Fitch (University of Chicago). Hybridomas were grown as ascites in BALB/c mice. Ascites were purified over DEAE-Sephacel.

T-Cell Clones. MBP peptide-specific T-cell clones were isolated as described (10, 14), using intact MBP or synthetic MBP peptides. Briefly, SJL/J mice were injected on the flank with an emulsion containing 400 μg of intact rat MBP or 200

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Abbreviations: EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; TCR, T-cell receptor; FACS, fluorescence-activated cell sorter; V_β, variable β chain.

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nmol of synthetic peptide p89-101 and complete Freund's adjuvant. After 10–14 days a single-cell suspension was made from the draining inguinal and axillary lymph nodes and cultured for 5 days with the immunizing antigen (MBP at 100 μg/ml or 6.7 μM of p89-101). The cells were then cultured in RPMI 1640 medium containing 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, penicillin at 100 units per ml, streptomycin at 100 mg/ml, and 8–15% supernatant of rat spleen cells cultured with Con A every 3 or 4 days. Antigenic stimulation was done every 14 days in the presence of γ-irradiated syngeneic splenic antigen-presenting cells. T-cell clones were established from these T-cell lines by limiting dilution at 0.3 cell per well in 96-well microtiter plates in the presence of antigen-presenting cells, antigen, and Con A supernatant. The cloning efficiencies were <20%.

Proliferative Assay. The proliferative responses were determined as described previously (9). T cells (1×10^4) were cultured with 5×10^5 γ-irradiated (3300 rad; 1 rad = 0.01 Gy) SJL/J splenic antigen-presenting cells in 0.2 ml of culture medium in 96-well flat-bottomed microtiter plates. At 72-hr incubation, each well was treated with 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) and harvested 16 hr later. The mean cpm of [^{3}H]thymidine incorporation was calculated for triplicate cultures.

Fluorescence-Activated Cell Sorter (FACS) Analysis. Each clone or population of lymph node cells was stained with KJ23a (11) and GK1.5 (13) antibodies using standard techniques. T cells (5×10^5) were incubated with 1 μg of fluorescein-conjugated KJ23a and 1 μg of allophycocyanin-conjugated GK1.5. GK1.5 allophycocyanin fluorescence is shown on the vertical axis and KJ23a fluorescence on the horizontal axis (Fig. 1). Immunofluorescence analysis was performed on a dual-laser modified FACS II (Becton Dickinson Immunocytometry). Two-color staining data are presented as contour plots, in which the levels of green and allophycocyanin fluorescence per cell define the location on a two-dimensional surface. The elevation at each location represents a 5% frequency of cells with a given fluorescence intensity (5).

Induction of EAE. Mice were immunized in the base of the tail with a total of 0.1 ml of an emulsion containing 400 μg of MBP or 200 nmol of synthetic peptide with 50 μg of *Mycobacterium tuberculosis* (H37Ra) in incomplete Freund's adjuvant. Four hundred nanograms of pertussis toxin (List Biological Laboratories, Cupertino, CA) was injected immediately and 48 hr after immunization. Mice were examined daily for clinical signs of EAE. For passive transfer, T cells (2×10^5 cells per ml) were stimulated with 6.7 μM of synthetic peptide p89-101 in the presence of antigen-presenting cells (1×10^7 cells per ml). After 96-hr cultivation, 1×10^7 viable cells were injected i.v. into the tail vein. Severity of EAE was graded as follows: 0, no abnormality; 1, decreased tail and body tone; 2, a clumsy but otherwise normal gait; 3, definite weakness of one or more limbs; 4, a paraplegic or monoplegic state; and 5, a premorbid state.

Depletion of KJ23a$^+$ Cells *in Vivo*. For active EAE, 0.1 mg of KJ23a antibody was injected i.p. on the day before and the day after immunization. For passive EAE, 0.1 mg of this antibody was injected i.p. immediately after passive transfer. F23.1 monoclonal antibody specific for the V_β 8 TCR gene products (12) was used as a control antibody in some experiments.

RESULTS

Staining of Clone 4b.14a (14a) with KJ23a Antibody. The MBP synthetic peptide p89-101 (Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro) is common to the amino acid sequence of rat, mouse, guinea pig, bovine, rabbit, and human MBPs. It is highly encephalitogenic in SJL/J mice (9). An encephalitogenic clone 4b.14a (14a) was derived from rat MBP-immunized SJL/J mice, which specifically responded to p89-101. FACS analysis using monoclonal antibodies revealed that this clone was L3T4$^+$ and KJ23a$^+$ (data not shown), indicating that KJ23a$^+$ T cells are involved in autoimmune encephalomyelitis in SJL/J mice.

***In Vitro* Responses of KJ23a$^+$ and KJ23a$^-$ T Cells from Immunized Mice.** Because the involvement of KJ23a$^+$ T cells in the development of EAE in SJL/J mice was indicated from the above data, we examined whether or not all the encephalitogen-primed T cells might belong to a single subpopulation. Lymph node cells from SJL/J mice immunized with p89-101 were isolated and sorted by FACS into CD4$^+$, KJ23a$^+$ and CD4$^+$, KJ23a$^-$ subpopulations using KJ23a and GK1.5 monoclonal antibodies. Sorted subpopulations were assessed for their proliferative response to the encephalitogen, p89-101 (Table 1). Both CD4$^+$, KJ23a$^+$ and CD4$^+$, KJ23a$^-$ subpopulations were stimulated by the encephalitogen equally well. Thus, encephalitogen-responsive T cells belong not only to the KJ23a$^+$ subpopulation, but also to the KJ23a$^-$ subpopulation.

The Effect of Depletion of KJ23a$^+$ T-Cell Subpopulation on the Development of EAE. To determine the participation of KJ23a$^+$ T cells in EAE, we depleted KJ23a$^+$ cells *in vivo* and tested whether or not such mice could develop EAE with the encephalitogen. Naive mice bear KJ23a$^+$ T cells in 4–15% of peripheral T cells (ref. 11 and Fig. 1*A*). By a single i.p. injection of 0.1 mg of KJ23a monoclonal antibody, KJ23a$^+$ cells were depleted *in vivo* even 22 days postinjection (Fig. 1*B*). When challenged with p89-101 and complete Freund's adjuvant, these KJ23a$^+$ T cell-depleted SJL/J mice did develop EAE. The clinical symptoms in these KJ23a$^-$ mice were as severe as those in naive or control antibody F23.1-injected mice (Table 2). KJ23a$^+$ T cells could not be detected (<5%) in the lymph nodes from paralyzed mice after KJ23a antibody treatment in the midst of the acute stage of disease (Fig. 1*C*). In contrast, in paralyzed mice given F23.1 antibody treatment, a similar percentage of KJ23a$^+$ cells could be detected as seen in naive mice (Fig. 1*D*). The disease induced with native MBP and complete Freund's adjuvant also was not influenced by depletion of KJ23a$^+$ T cells. These results indicate that KJ23a$^-$ T cells are definitely involved in the induction of EAE in SJL/J mice.

The Encephalitogenicity of KJ23a$^+$ and KJ23a$^-$ T-Cell Subpopulations. To clarify the encephalitogenicity of KJ23a$^+$ and KJ23a$^-$ T-cell subpopulations, we expanded FACS-sorted cells by several cycles of stimulation with antigen and with interleukin 2, and established KJ23a$^+$ and KJ23a$^-$ T-cell lines specific for the encephalitogen p89-101. These

Table 1. The proliferative response of KJ23a$^+$ and KJ23a$^-$ populations of encephalitogen-sensitized lymph node cells

Cell population	Antigen	[^{3}H]Thymidine uptake, cpm × 10^{-3} ± SD
Unfractionated	None	4.0 ± 0.7
	p89-101	26.2 ± 10.6
CD4$^+$/KJ23a$^+$	None	2.6 ± 0.1
	p89-101	26.2 ± 10.6
CD4$^+$/KJ23a$^-$	None	2.5 ± 0.5
	p89-101	31.2 ± 5.4

Proliferative responses of lymph node cells that were removed from SJL/J mice immunized with MBP synthetic peptide p89-101 (200 nmol) in complete Freund's adjuvant 11 days before, sorted by FACS into the populations indicated above, and cultured with or without antigen p89-101 (6.7 μM). For sorted cells (4×10^5 cells per ml) γ-irradiated (3300 rad) splenic antigen-presenting cells (2×10^7 cells per ml) were added. Unfractionated cells (5×10^6 cells per ml) were cultured in the absence of antigen-presenting cells. Response was assessed by [^{3}H]thymidine incorporation in the last 16 hr after 72-hr culture.

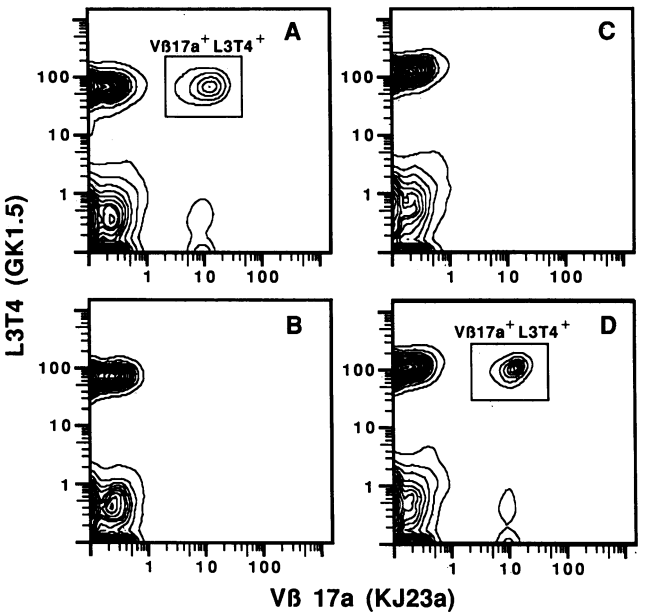

FIG. 1. Fluorescence-activated cell sorter profile for lymph node cells stained with GK1.5 and KJ23a antibodies from naive SJL/J mice (*A*), from mice at 22 days after injection of 0.1 mg of KJ23a antibody (*B*), from paralyzed mice (grade 4) with the injection of KJ23a antibody at 14 days postinoculation (*C*), and from paralyzed mice (grade 4) with the injection of F23.1 antibody at 14 days postinoculation (*D*). For paralyzed mice, 0.1 mg of KJ23a or F23.1 monoclonal antibody was injected i.p. at the day before and the day after immunization.

T-cell lines were tested for encephalitogenicity in adoptive transfer experiments. When KJ23a$^+$ T cells were transferred to γ-irradiated (350 rad) recipients that were injected i.p. with the irrelevant monoclonal antibody F23.1, immediately after transfer four of six mice developed EAE (Table 3). On the other hand, none of four recipients given KJ23a$^+$ T cells and injected with the antibody KJ23a developed EAE. Mice transferred with KJ23a$^-$ T cells developed EAE after injection with either KJ23a or F23.1 antibodies. These results indicate that both p89-101-specific KJ23a$^+$ and KJ23a$^-$ T cells are encephalitogenic in SJL/J mice and that *in vivo* administration of anti-TCR antibody is effective in the suppression of the disease induced with a specific population of these T cells.

Table 2. Active EAE in SJL/J mice with or without injection of anti-T-cell receptor antibodies

Antigen	mAb*	Incidence of EAE†	Severity‡	Day of onset‡
p89-101	None	12/18	2.2 ± 1.1	12.8 ± 2.3
	F23.1	11/18	2.3 ± 0.6	12.6 ± 1.6
	KJ23a	10/18	2.3 ± 1.0	12.9 ± 2.6
MBP	None	6/6	2.5 ± 1.0	11.6 ± 0.8
	KJ23a	5/6	2.0 ± 1.0	11.8 ± 1.3

Active EAE was induced by immunization on the tail base with 0.1 ml of an emulsion of p89-101 (200 nmol) or guinea pig MBP (400 μg) and complete Freund's adjuvant. Pertussis toxin (400 ng) was injected i.v. immediately and 48 hr after immunization.
*Monoclonal anti-TCR antibodies (0.1 mg) were injected i.p. on the day before and the day after immunization.
†See Table 1.
‡See Table 3.

Nested Antigenic Epitopes Recognized by KJ23a$^+$ and KJ23a$^-$ T-Cell Subpopulations. We established 13 independent T-cell clones derived from 13 different SJL/J-specific T-cell lines specific for p89-101 and examined their KJ23a positivity and antigen fine specificity. Staining analysis revealed that six of these clones are CD4$^+$, KJ23a$^+$ and seven clones are CD4$^+$, KJ23a$^-$. To determine antigen fine

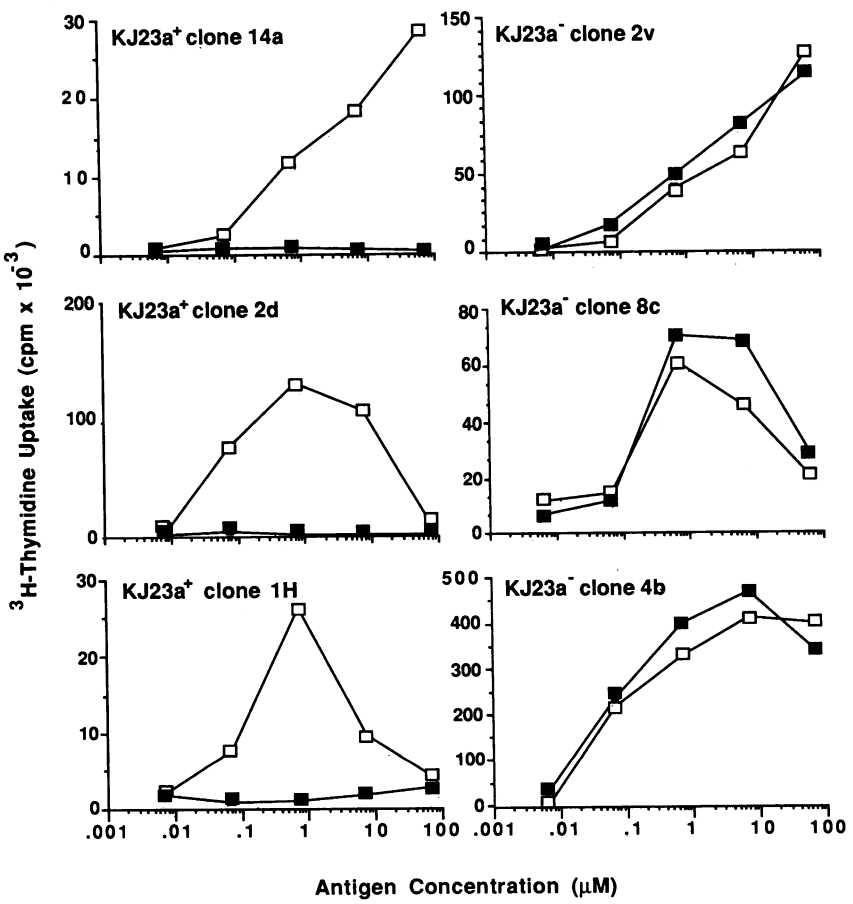

FIG. 2. The proliferative responses of representative KJ23a$^+$ and KJ23a$^-$ T-cell clones against the MBP synthetic peptide p89-101 (□) and p89-100 (■) at the various concentrations. Proliferation assays were done as described.

Table 3. Passive transfer of KJ23a$^+$ and KJ23a$^-$ T cells in SJL/J mice with treatment of anti-T-cell receptor antibodies

Transferred T cells*	mAb†	Clinical incidence of EAE‡	Severity§	Day of onset¶
CD4$^+$/KJ23a$^+$	F23.1	4/6	2.8 ± 1.3	8.0 ± 1.0
	KJ23a	0/4	—	—
CD4$^+$/KJ23a$^-$	F23.1	6/6	3.4 ± 1.3	8.6 ± 0.5
	KJ23a	6/6	3.0 ± 1.2	9.2 ± 1.2

*1 × 10^7 T cells, which were derived from lymph node cells of SJL/J mice immunized with p89-101, sorted into the populations indicated above by FACS, and expanded by several cycles of stimulation and rest, were transferred into γ-irradiated (350 rad) SJL/J mice via tail vein after 96-hr culture with antigen and antigen-presenting cells.
†Monoclonal antibodies (0.1 mg) were injected i.p. immediately after inoculation of T cells.
‡Number of mice with clinical EAE per number of mice passively transferred.
§Severity is determined using a scale of 1 to 5 as described in text and presented as mean severity of sick mice ± SD.
¶Mean day of onset ± SD.

specificity, we assayed the proliferative response of these clones by using a MBP synthetic peptide p89-100 (residues 89–100 of MBP, Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Phe-Arg-Thr), in which the proline residue at position 101 was deleted. The proliferative assay of these clones was performed in the range of 0.0067 to 67 μM of antigen. Six of six KJ23a$^+$ T-cell clones respond to p89-101, but not to p89-100,

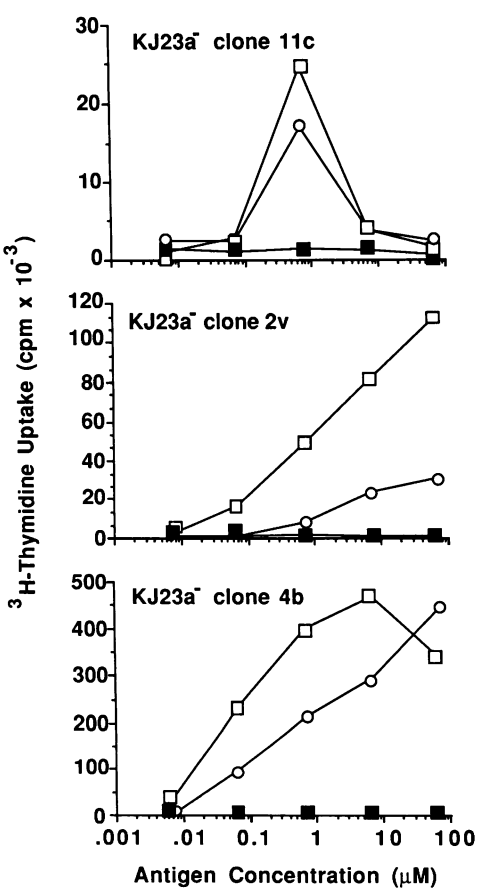

FIG. 3. The proliferative responses of representative KJ23a$^-$ T-cell clones against the MBP synthetic peptide p89-100 (Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr) (□), p89-100 substituted peptide 90K (Val-Lys-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr) (○), and p89-100 substituted peptide 97K (Val-His-Phe-Phe-Lys-Asn-Ile-Val-Lys-Pro-Arg-Thr) (■) at the various concentrations. Proliferative assays were done as described.

Table 4. The reactivity of T-cell clones to synthetic peptides

Clone	KJ23a	p89-101	p89-100	p89-100 (90K)*	p89-100 (97K)*
14a	+	+	−	−	−
3c	+	+	−	ND	ND
4c	+	+	−	ND	ND
5e	+	+	−	ND	ND
2d	+	+	−	−	−
1H	+	+	−	−	−
3f†	+	+	−	ND	ND
8c	−	+	+	+	−
1q	−	+	+	+	+
9b	−	+	+	+	−
4b	−	+	+	+	−
8b	−	+	+	+	−
2v	−	+	+	+	−
3r	−	+	+	+	−
F1.6g‡	−	+	+	+	−
F1.3c	−	+	+	+	+
F1.4c	−	+	+	+	−

Proliferative T-cell lines specific for p89-101 or rat MBP were generated from SJL/J, DDD/1, or (PL/J × SJL/J)F_1 mice immunized with 200 nmol of p89-101 or 400 μg of rat MBP in complete Freund's adjuvant following the described protocols. These T-cell lines were cloned after several cycles of antigenic stimulation and rest. Cloning procedure was done by limiting dilution at 0.3 cell per well. Only one clone was randomly picked up from each T-cell line. Proliferative responses of KJ23a$^+$ (+) and KJ23a$^-$ (−) T cell clones against MBP synthetic peptides are recorded as + or −. The concentration of antigen was examined at 0.0067–67 μM. ND, not determined.
*In p89-100-substituted peptides 90K and 97K lysine is substituted for histidine at position 90 and for threonine at position 97, respectively.
†Clone 3f is a T-cell clone from DDD/1 mice (14).
‡Clone F1.6g, F1.3c, and F1.4b are derived from (PL/J × SJL/J)F_1 mice.

whereas seven of seven KJ23a$^-$ T-cell clones respond to both peptides (Fig. 2). This indicates that KJ23a$^+$ and KJ23a$^-$ T-cell subpopulations might recognize different immunodominant epitopes within the encephalitogen p89-101. These results also indicate that the proline residue at position 101 might be necessary for the recognition of encephalitogen by KJ23a$^+$ clones. Furthermore, the analysis using substituted peptides 90K (K = Lys) (Val-Lys-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr) and 97K (Val-His-Phe-Phe-Lys-Asn-Ile-Val-Lys-Pro-Arg-Thr) revealed that the threonine residue at position 97 is essential for the recognition by six of seven KJ23a$^-$ clones (Fig. 3 and Table 4).

Encephalitogenicity of p89-100 in SJL/J Mice. Finally, to confirm that the peptide with proline deleted at position 101 of p89-101 is encephalitogenic in SJL/J mice, we immunized SJL/J mice with p89-100 in complete Freund's adjuvant. Six of ten mice immunized with proline-deleted peptides developed disease. This clinical incidence was equal to that seen in mice immunized with the p89-101.

DISCUSSION

The KJ23a monoclonal antibody derived by Kappler *et al.* (11) reacts with the TCR V_β 17a gene product on T cells. Expression of the TCR V_β 17a gene occurs in strains such as SWR/SuJ, SJL/J, or C57L mice, which do not express any I-E molecules. It has been shown that KJ23a$^+$ T cells are eliminated from the peripheral T cells and mature thymocyte pool of mice expressing the I-E molecule (15). In this report, we show that about half of independently derived encephalitogen-specific T-cell clones are KJ23a$^+$. Although it is clear from our results that KJ23a$^+$ T cells are involved in EAE, we

also show that encephalitogen-specific KJ23a$^-$ T cells are disease-inducing. Consistently, we find that SWR/SuJ (H-2^q) mice, which are I-E$^-$ and KJ23a$^+$ like SJL/J mice, are susceptible to EAE induced by p89-101 (data not shown). This peptide is also encephalitogenic in the ASW/SuJ (H-2^s) and B10.T(6R)/SgDvEg (H-2^{v2}, I-A^q) mouse strains, which are I-E$^-$ and KJ23a$^-$ (data not shown). Taken together, these data indicate that there is not an exclusive use of KJ23a$^+$ T cells in the development of EAE in the SJL/J mouse.

In murine (16, 17) and human (18) autoimmune disorders, there are descriptions of a predominance in the use of the TCR V_β gene. These reports imply that variation in the TCR variable gene repertoire might play a significant role in the pathogenesis of autoimmune diseases. Recently, a report from this laboratory (7) demonstrated that there is a predominant use of the V_β 8 gene family in the encephalitogen-specific T cells from PL/J mice. About 80% of T-cell clones that are specific for the N-terminal peptide of MBP (2) and restricted to I-A^u, an encephalitogenic determinant in PL/J mice, use the TCR V_β 8 gene family. In addition, *in vivo* administration of monoclonal antibody F23.1, which reacts with this V_β gene family product, was shown to suppress T-cell-induced disease. The therapeutic use of the TCR-specific monoclonal antibody, F23.1, has been extended to EAE induced by native MBP (19).

In the SJL/J mouse, T cells using the V_β 17a gene comprise 10–15% of peripheral T cells. Our result that 50% of the clones responding to p89-101 are in the KJ23a$^+$ T-cell subpopulation initially suggested that there might be a dominant use of this V_β gene in encephalitogen-specific T cells for SJL/J mice. Nonetheless, depletion of this T-cell population *in vivo* was proven to be ineffective in suppressing disease induced by the encephalitogen, again emphasizing the involvement of KJ23a$^-$ T cells in EAE. These results contrast with our previous findings in the same disease, EAE, in different strains, PL/J and (PL/J × SJL/J)F_1. Thus, a simple correlation between autoimmune disorders and restricted TCR V_β gene use is not always found.

However, the results demonstrating that *in vivo* administration of KJ23a antibody suppressed the development of EAE induced with KJ23a$^+$ T cells still indicate the potential efficacy of anti-TCR antibody as a treatment. At present we have no information about the TCR gene use in the KJ23a$^-$ T-cell population. If there is a limited use of the TCR genes in this population, it might be possible to combine several anti-TCR antibodies to deplete an oligoclonal T-cell population involved in autoimmune disease. There is at least one description of oligoclonal T cells in the cerebrospinal fluid compartment of multiple sclerosis patients (20).

We demonstrate that there exists more than one encephalitogenic epitope for SJL/J mice within the residues 89–101 of MBP (p89-101). KJ23a$^+$ T cells are stimulated by p89-101, whereas KJ23a$^-$ T cells are stimulated by both p89-100 and p89-101. The data suggest that the epitope recognized by KJ23a$^+$ T cells requires the proline residue at position 101, and we further show that the peptide recognized by KJ23a$^-$ T cells requires threonine at position 97. Thus, a nested determinant within p89-101 is recognized by a distinct T-cell subpopulation. Although the existence of multiple encephalitogenic determinants on MBP for SJL/J mice has been suggested, we demonstrate the presence of more than one epitope even within a 13-amino acid stretch. The fact that there are some T-cell clones (data not shown) which respond to the substituted peptide at position 97 (lysine for threonine, Val-His-Phe-Phe-Lys-Asn-Ile-Val-Lys-Pro-Arg-Thr) suggests that there might exist other epitopes besides these two.

The role of multiple and nested epitopes within antigens responsible for an autoimmune disease is unclear, but perhaps significant. Proteolysis occurs as a consequence of antigen processing or *in situ* exposure of self-antigen. Proteolysis of epitopes could increase the number of distinct clones participating in an autoimmune response. This may influence the chronicity of autoimmune conditions, as well as their tendency to exacerbate. Moreover, it underscores the difficulty inherent in anticlonotypic approaches to therapy of autoimmune disease.

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