Autoimmune Encephalomyelitis and Uveitis Induced by T Cell Immunity to Self β-Synuclein[1]

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β-synuclein is a neuronal protein that accumulates in the plaques that characterize neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. It has been proposed that immunization to peptides of plaque-forming proteins might be used therapeutically to help dissociate pathogenic plaques in the brain. We now report that immunization of Lewis rats with a peptide from β-synuclein resulted in acute paralytic encephalomyelitis and uveitis. T cell lines and clones reactive to the peptide adoptively transferred the disease to naive rats. Immunoblotting revealed the presence of β-synuclein in heavy myelin, indicating that the expression of β-synuclein is not confined to neurons. These results add β-synuclein to the roster of encephalitogenic self Ags, point out the potential danger of therapeutic autoimmunization to β-synuclein, and alert us to the unsuspected possibility that autoimmunity to β-synuclein might play an inflammatory role in the pathogenesis of neurodegeneration. *The Journal of Immunology,* **2003, 170: 628–634.**

Parkinson's and Alzheimer's diseases are common human neurodegenerative disorders in which abnormal aggregates of proteins accumulate in the brain, resulting in neurotoxicity and apoptosis (1, 2). The success of immune therapy in a mouse model of Alzheimer's disease (3–5) has prompted clinical studies of immunotherapy as a way to disrupt plaques in human disease (6). The aim is to induce Abs that might help resolubilize the denatured proteins that make up the plaques.

Experimental autoimmune encephalomyelitis (EAE),[3] one of the best-studied models of human autoimmune disease, was introduced over 50 years ago (7, 8). The autoantigens that are the focus of attack of the immune system in EAE have been extensively studied, and the vast majority of them, including myelin basic protein (MBP), proteolipid protein, myelin oligodendrocyte protein (MOG), myelin-associated glycoprotein (9, 10), myelin oligodendrocyte basic protein (11), and oligodendrocyte-specific protein (12), are related to myelin. αB-crystallin, which was isolated from myelin of multiple sclerosis patients (13), is expressed in glial cells and oligodendrocytes (14). To our knowledge, neuronal proteins have not been reported to induce EAE.

In this study, we set out to explore immunization to β-synuclein and to other self molecules associated with neurodegeneration, such as: prion protein, α-synuclein, γ-synuclein, and Presenilin 1 and 2. Our strategy was to immunize Lewis rats with a panel of peptides predicted to fit the peptide-binding motif for the MHC class II I-A molecule in the Lewis rat, previously characterized in our laboratory (15). We found that the peptides were immunogenic in Lewis rats; T cell lines could be generated to them; immunization to self β-synuclein induced acute paralytic encephalomyelitis; and uveitis could be adoptively transferred with T cell line and clones specific for β-synuclein that was detected by Western blot in myelin.

Materials and Methods

Animals

Inbred female Lewis rats were supplied by the animal breeding center of the Weizmann Institute of Science (under the supervision of Harlan Laboratories, Haslett, MI, and an animal welfare committee) and were used at 2–3 mo of age.

Ags and Abs

Mycobacterium tuberculosis H37Ra was purchased from Difco (Detroit, MI). Peptides were synthesized using the F-MOC technique with an automatic multiple peptide synthesizer (AMS 422; ABIMED, Langenfeld, Germany). The sequences of peptides are shown in Table I. MHC anchor positions 3, 4, 6, and 9 are indicated (15). The purity of the peptides was analyzed by HPLC and amino acid composition. Polyclonal rabbit anti-β-synuclein was purchased from Sigma-Aldrich (St. Louis, MO); rabbit anti-MBP (644) and rabbit anticyclic nucleotide phosphodiesterase were a gift from Dr. G. L. Boccaccio (Fundacion Campomar Institute, Buenos Aires, Argentina); and rabbit anti-MOG was a gift from Dr. N. Kerlero de-Rosbo (Department of Immunology, Weizmann Institute of Science, Rehovot, Israel). Secondary HRP anti-rabbit IgG was from Santa Cruz Biotechnology (Santa Cruz, CA).

Immunizations

The peptides were dissolved in DMSO (20 mg/ml) and then in PBS (1 mg/ml), and an oil emulsion was prepared (1:1 ratio) with IFA containing 4 mg/ml *M. tuberculosis* H37Ra (CFA). Naive female Lewis rats were immunized in both hind footpads with 50 μl of the emulsion; each rat was injected with 50 μg of peptide. Some groups of rats received i.p. injection of cyclophosphamide (25 mg/kg) 3 days before peptide/CFA injection. Draining popliteal lymph nodes were removed on day 12 after injection, a single cell suspension was prepared by pressing the organs through a fine wire mesh, and the cells were studied in vitro.

T cell proliferation assay

T cell proliferation was performed by seeding 5×10^4 line cells (at day 4–5 in propagation phase) with 5×10^5 irradiated thymocytes (2500 rad) as APCs, in stimulation medium for 3 days, in 96 microtiter round-bottom wells (Nunc, Rosklide, Denmark), as described (16). The cultures were incubated in triplicate for 72 h at 37°C in humidified air containing 7% CO_2. Each well was pulsed with 1 μCi of [^{3}H]thymidine (10 Ci/mmol sp. act.; Amersham, Buckinghamshire, U.K.) for the final 4 h. The cultures

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; RT, reverse transcriptase.

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were then harvested using a MicroMate 196 Cell Harvester, and cpm were determined using a Matrix 96 Direct beta counter using avalanche gas (98.7% helium; 1.3% C_4H_{10}) ionization detectors (Packard Instrument, Meriden, CT). The results of proliferation are expressed as cpm.

T cell lines and clones

Ag-specific T cell lines were established from lymph node cells that had been stimulated with peptide (10 μg/ml) for 3 days in stimulation medium, as described above. Following stimulation, the T cell blasts were isolated on Lympho-prep (Nycomed Pharma, Oslo, Norway) and seeded in propagation medium. Propagation medium was identical with stimulation medium without autologous serum, but supplemented with 10% FCS and 10% T cell growth factors from the supernatant of Con A-stimulated spleen cells (17). Four to seven days seeding, the cells (5×10^5/ml) were restimulated with peptide, and irradiated thymocytes as APCs (10^7/ml) for 3 days in stimulation medium. T cell lines were expanded by repeated stimulation with Ag and irradiated thymocytes as APCs every 10–12 days (18). After each stimulation cycle, the lines were tested in a proliferation assay at the end of the propagation phase, and supernatants were collected for cytokine ELISA. T cell clones to $SynB_{93-111}$ were generated by limiting dilution, as described (18), and tested for encephalitogenic potential by i.p. injection (2×10^7 T cell blasts).

Induction of EAE

Active EAE was induced by injection of 50 μg of peptide in CFA. Adoptive EAE was transferred by i.p. injection of 2×10^7 peptide-activated cells of the lines, as described (16). Clinical EAE was observed 4–5 days following administration of T cell lines. Clinical scoring was: +1, paralysis of tail; +1.5, paresis of posterior paws and ataxia; +2, paraplegia; +3, paralysis extending to thoracic spine; +4, a moribund state (16).

Cytokine ELISA

Supernatants were collected after 3 days of stimulation of the T cell lines with $SynB_{93-111}$. IL-10, TNF-α, and IFN-γ in the culture supernatants were measured by ELISA using BD PharMingen's OPTEIA kit (BD PharMingen, San Diego, CA). BD PharMingen recombinant rat cytokines were used as standards for calibration curves. A standard ELISA was performed, as described (19).

Cytokine PCR assay

Total cellular RNA was isolated by the single-step method using the TRI REAGENT (Molecular Research Center, Cincinnati, OH). A total of 5 μg total RNA was used for the reverse transcriptase (RT) reaction. RNA was incubated with oligo(dT) (12–18) (200 ng) for 5 min at 65°C and left to cool to 42°C. The RT reaction contained: dNTPs (0.25 mM each), RNAsin (3 U), DTT (10 mM), sodium pyrophosphate (4 mM), avian myeloblastosis virus RT (12 U; Promega, Madison, WI), and RT buffer. The mixture was incubated for 120 min at 42°C and then heat inactivated for 5 min at 95°C. The primer sequences and PCR protocol were as described (20).

Myelin preparation

Myelin was isolated from rat brain extract using a 0.8 M sucrose gradient. Brain extracts were loaded in ultracentrifuge tubes and covered with buffer containing 0.25 M sucrose. Centrifugation was performed at 35,000 rpm using a Beckman SW 41 rotor (Beckman Coulter, Fullerton, CA), for 30 min at 10°C. The myelin fraction from the interface 0.25–0.8 M was recovered and kept at −80°C. Myelin was fractionated on discontinuous sucrose gradients. After washing the crude myelin by spinning at 14,000 × *g* for 30 min at 4°C, the myelin was suspended in water and loaded on 0.32 M/0.6 M/0.7 M sucrose. The bands at the interface 0.32–0.6, 0.6–0.7, and the pellet (heavy myelin) were collected separately and analyzed in Western blots.

Western blotting

Rat brain was ground with a tissue grinder in lysis buffer. The homogenate was centrifuged 14,000 × *g* for 15 min in 4°C, and the supernatant was used for Western blotting. The protein concentration was determined using the Bio-Rad Dc protein assay (Bio-Rad Laboratories, Hercules, CA). From brain and myelin lysates, 15 μg of protein was loaded in each well. Following electrophoresis in 15% SDS gel in a minigel apparatus (Bio-Rad), the gels were electrotransferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). The nitrocellulose membranes were washed with distilled water for 5 min, and then blocked for 60 min using a blocking solution composed of 2% BSA (Fraction V; Sigma-Aldrich), 2.5% milk powder (Bio-Rad), 10 mM Tris (Sigma-Aldrich), pH 7.5, 150 mM NaCl, and 0.02% thimerosal (Sigma-Aldrich). After three 10-min washes in PBS/Tween 20 (PBS/T, 0.02%; Sigma-Aldrich), primary Abs (1/1000) were incubated with the membranes in blocking solution for 60 min. Following another series of washes in PBS/T (3 × 10 min), the membranes were incubated with a secondary Ab (peroxidase-conjugated anti-rabbit IgG; Santa Cruz Biotechnology) at a 1/2500 dilution in blocking solution for 60 min. After another three 10-min washes, the membranes were incubated with the ECL reagent (for 2 min) and exposed to x-ray film.

Results

Immunogenicity of peptides selected to fit the MHC class II motif

The MHC class II motif for the Lewis rat class II I-A molecule RT1.Bl was characterized in our laboratory (15). Based on the motif, we selected peptides from several neurodegeneration-related proteins: α-, β-, and γ-synuclein; Presenilin 1 and 2; and prion protein (Table I). The peptides were injected in CFA. Groups of five rats were immunized with individual peptides and observed for the development of an immune response. On day 12 postimmunization, popliteal and inguinal lymph nodes were removed and

Table I. *Peptide sequences*

Source	Peptide Sequence	Ac. EAE	Line[a]	EAE[b]
	34 6 9[c]			
Prion[d] $_{182-202}$	ITIKQHTVTTTTKG**E**[e]NFTETD	Negative	++	−
$Prion_{211-230}$	QMCVTQYQK**E**SQAYYDGRRS	Negative	++	−
β-$Synuclein_{93-111}$	LKPEEVAQEAAE**E**PLIEPL	Positive	++	++
β-$Synuclein_{78-93}$	AAATGLVKK**E**EFPTDL	Negative	++	++
α-$Synuclein_{89-104}$	AAATGFVKK**D**QMGKGE	Negative	++	−
γ-$Synuclein_{48-63}$	RGTSVTSVA**E**KTKEQA	Negative	NA[f]	
γ-$Synuclein_{71-86}$	VSSVNTVAT**E**TVEEAE	Negative	NA	
γ-$Synuclein_{89-101}$	VVTTGVVRK**E**DLEPPA	Negative	NA	
$Presenilin1_{101-116}$	KSVSFYTRK**D**GQLIYT	Negative	NA	
$Presenilin1_{293-308}$	VWLVNMAEG**D**PEAQRR	Negative	NA	
$Presenilin1_{315-329}$	KYSTQGTER**E**ETQDTG	Negative	NA	
$Presenilin1_{354-370}$	STPESRAAVQ**E**LSGSIL	Negative	++	−
$Presenilin2_{311-326}$	SQGALQLPY**D**PEMEED	Negative	NA	
$Presenilin2_{24-39}$	ESPTSRSCQ**D**SRPGPE	Negative	++	−

[a] T cell line generated by repeated in vitro stimulation with peptide.
[b] Passive EAE mediated by inoculation of 2×10^7 T cell line.
[c] Nos. indicate the position of MHC anchor positions (15).
[d] The effects of prion peptide immunization were previously described (38).
[e] Anchor position no. 9 is boldface.
[f] NA, Not attempted.

T cell lines were derived, as described (16). The results of proliferation assays of the lines to different concentrations of stimulating peptide are shown in Fig. 1. All peptides tested were immunogenic, and T cell lines could be readily propagated in culture.

Immunization with $SynB_{93-111}$ causes EAE and uveitis

From day 10 postimmunization, the rats were scored for clinical signs. On day 13–14 after immunization, the rats inoculated with β-synuclein peptide ($SynB_{93-111}$) developed typical signs of EAE with a clinical score between 2 and 3.5, marked by paralysis of the posterior paws extending caudally (Fig. 2). The rats were scored daily, and were found to recover from paralytic EAE on day 19.

Cyclophosphamide can be used to amplify autoimmune responses, probably by suppressing immune down-regulation (21). We found that rats that had received a low dose cyclophosphamide (25 mg/kg) 3 days before immunization (22) developed more severe disease that lasted longer (Fig. 2).

At the peak of disease, rats were sacrificed and tissues were tested by histology. As seen in Fig. 3, the lumbar and thoracic spinal cord showed many perivascular and parenchymal mononuclear cell

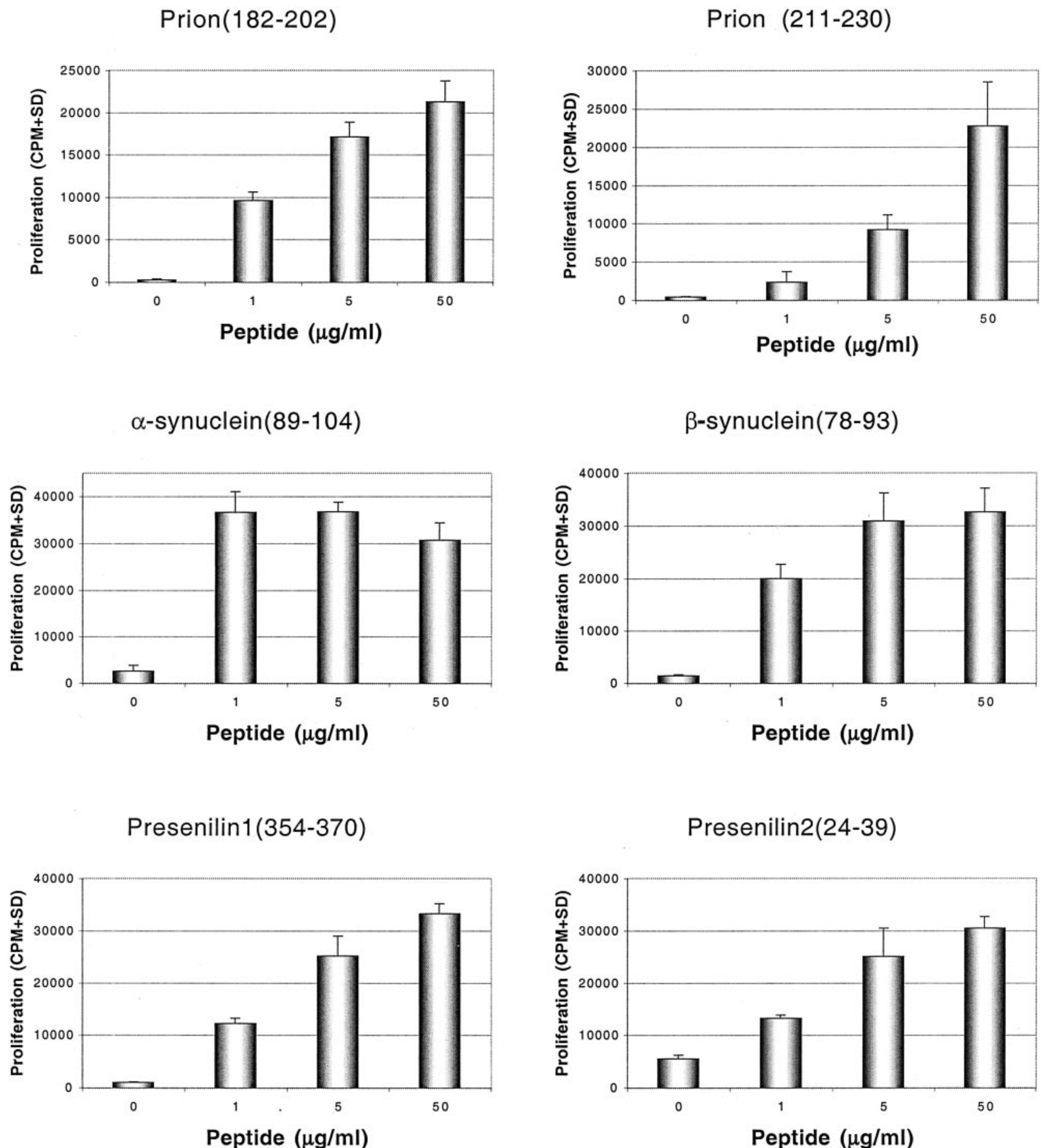

FIGURE 1. Immunogenicity of peptides selected on MHC class II motif. T cell proliferation assays of T cell lines at the second in vitro stimulation to neurodegeneration protein peptides. A total of 5×10^4 line cells at the end of propagation phase were plated in microtiter round-bottom 96-well plates, with 5×10^5 irradiated thymocytes as APCs, with various concentrations of peptide. In the last 4 h, the wells were pulsed with [^{3}H]thymidine and harvested.

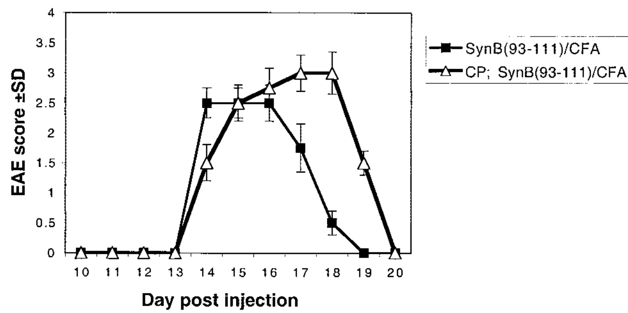

FIGURE 2. Immunization with $synB_{93-111}$ induces EAE. Groups of five Lewis rats were immunized with $SynB_{93-111}$ in CFA. Each rat was inoculated intrafootpad with 50 μg of peptide. One group (designated CP; $SynB_{93-111}$/CFA) received 25 mg/kg cyclophosphamide 3 days before peptide/CFA immunization. Clinical scoring was performed as described.

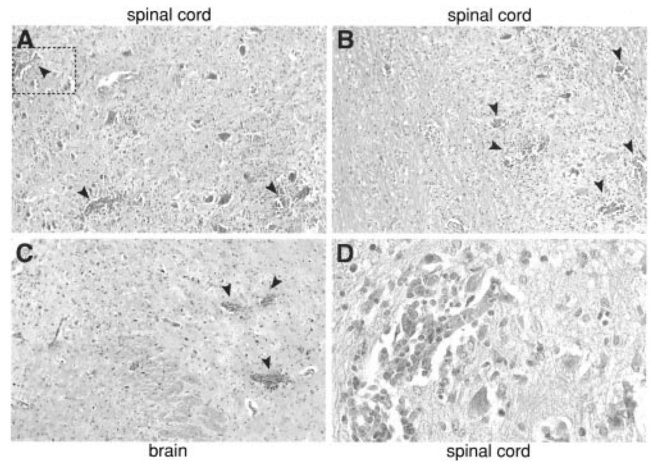

FIGURE 3. Inflammatory infiltrate in the spinal cord of $SynB_{93-111}$/CFA-injected rats. Histological photomicrographs of sections of lumbar (*A*, *D*, magnification ×100 (*A*), ×400 (*D*)), thoracic (*B*) spinal cord, and cerebral cortex (*C*) of rats injected with $SynB_{93-111}$ in CFA, 18 days postinjection. Typical mononuclear infiltrates are seen (arrowheads) in lumbar (*A*, *D*) and thoracic spinal cord (*B*) and cerebral cortex (*C*), more abundant at the interface of white and gray matter. *D*, Magnification of the marked area in *A*.

infiltrates that are typical of EAE. The infiltrates were more abundant at the interface between white and gray matter. The number of infiltrates was higher in the lumbar spinal cord, and it decreased in thoracic and cervical regions.

We have previously detected anterior uveitis in rat EAE induced by MBP (23, 24), so we also searched for uveitis in the rats autoimmunized to β-synuclein. As shown, the iris (Fig. 4, *A*, *B*, and *D*) and posterior uvea (Fig. 4*C*) were thickened and infiltrated with inflammatory cells, which were also seen in the anterior chamber. The iris showed signs of anterior and posterior synechiae (adhesion of the iris to the posterior layer of the cornea and the anterior surface of the lens; Fig. 4, *A* and *B*). Immunization with CFA alone or with CFA and other peptides did not induce EAE or uveitis, excluding the possibility that other components of the immunogen (mycobacterial Ags, oil) were responsible for the observed pathology.

T cell line and clones to $SynB_{93-111}$ are Th0-Th1 and transfer EAE and uveitis

EAE is thought to be mediated by Th1 T cells (8, 25, 26). We tested whether T cell lines or clones reactive to the $SynB_{93-111}$ could mediate paralysis. We generated a T cell line specific for the $SynB_{93-111}$ peptide and cloned it. The proliferation profile of the line and several clones is shown in Fig. 5. The line was tested against a panel of overlapping MBP peptides and to crude rat

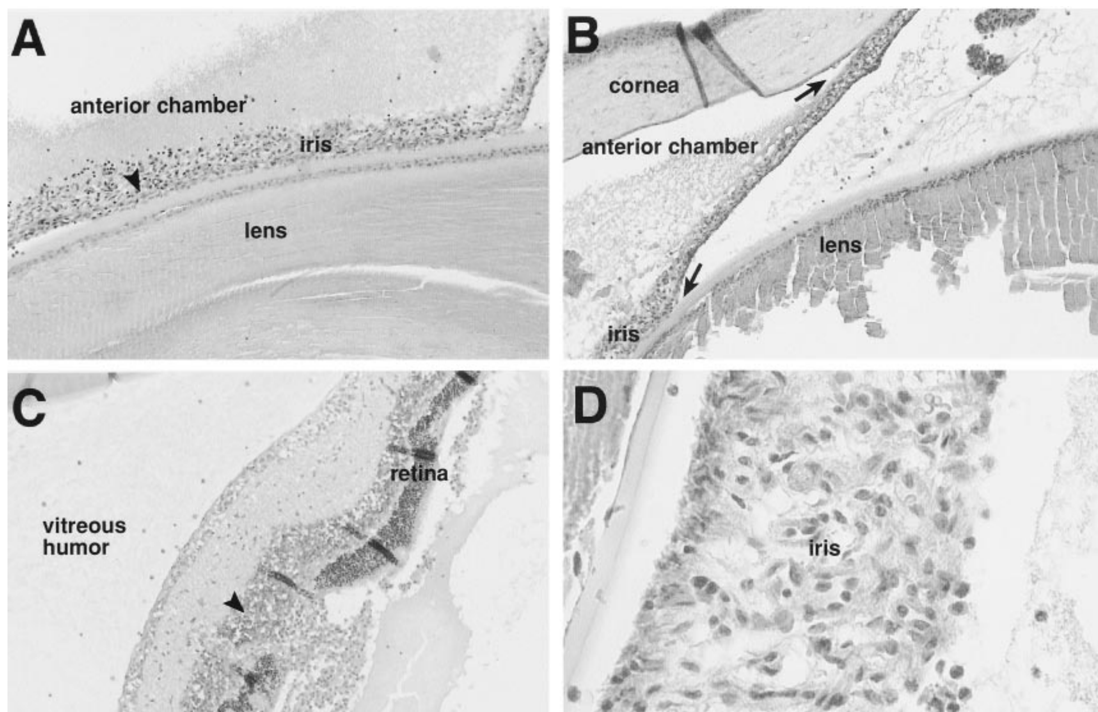

FIGURE 4. Uveitis in $SynB_{93-111}$/CFA-immunized rats. The iris and posterior uveal tract were thickened and infiltrated with inflammatory cells that were also detected in the anterior chamber. The iris is adherent to the lens (*A*, arrowhead; *B*, lower arrow) and posterior surface of the cornea (*B*, upper arrow). Inflammatory infiltrate in the posterior uvea (*C*, arrowhead). Higher magnification (×400) of the thickened and inflamed iris (*D*).

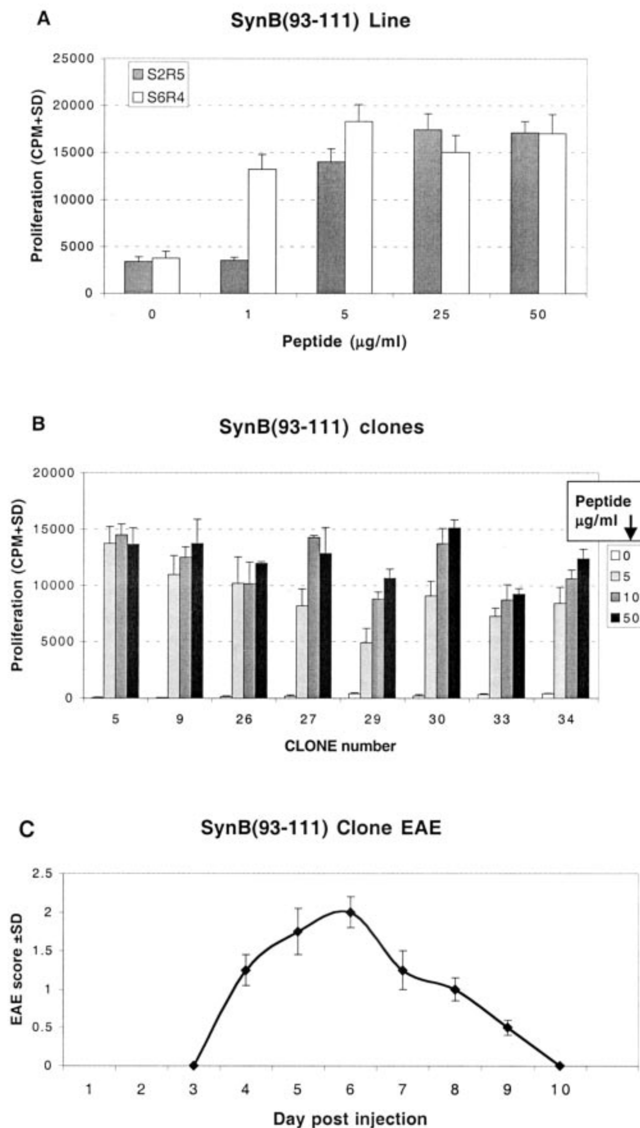

FIGURE 5. T cell proliferation of line and clones to SynB_{93-111}. Proliferation profile of T cell line (*A*) and clones (*B*) specific for SynB_{93-111} to various concentrations of peptide. The line was tested after two cycles (designated S2R5) and after six in vitro stimulations (designated S6R4). In the *lower part* of the figure (*C*), EAE clinical score of a group of five rats injected with 2×10^7 activated clone cells is shown.

MBP, and was found to be immunologically specific for β-synuclein and negative to MBP. The line and clones were pathogenic and caused paralysis in naive recipient rats. The EAE clinical scores of a group of five rats injected with 2×10^7 T cells from clone number 9 are shown in Fig. 5*C*. The histological pathology in the spinal cord and uveal tract of rats injected with a T cell clone reactive to the SynB_{93-111} peptide was similar to the peptide/CFA-injected rats (data not shown).

To test the Th phenotype of the T cell line and clones to SynB_{93-111}, PCR was performed on the cDNA of the line at the third cycle of peptide stimulation. Typically, Th1 cells produce IL-2, IFN-γ, IL-12, and TNF-β, and Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13, and very little or no IL-2 and IFN-γ, while both types secrete IL-3, GM-CSF, and TNF-α (27). The cytokine profile by PCR was of the Th0-Th1 phenotype, with high levels of IFN-γ (typical for Th1) and detectable IL-6 and IL-10 (secreted by Th2). TGF-β that was reported to be secreted by regulatory Th3 cells (28) was also detected (Fig. 6). The secretion of IFN-γ, IL-10, IL-4, and TNF-α was tested in culture supernatants of successive stimulations. The amounts of IFN-γ remained high, while the levels of TNF-α and IL-10 increased in successive stimulations (Fig. 6). IL-4 was not detected. The cytokine profile of the T cell clones was similar to that of the line (Fig. 6). Several synuclein peptides (Table I) did not induce EAE when injected in CFA. However, the T cell line to peptide SynB_{78-93} also induced EAE in naive Lewis rats. The fact that the SynB_{78-93} T cell line was encephalitogenic while the peptide in CFA did not induce EAE may indicate that upon active immunization the precursor frequency may not reach a threshold level to cause clinical paralysis. In our experience with cryptic MBP peptides, we found that T cells specific for MBP 11–30 were pathogenic, while the peptide in CFA did cause EAE (16).

β-synuclein is present in myelin

β-synuclein is known to be a presynaptic neuronal protein (29–32), but most proteins that can induce EAE are expressed in myelin (7). Therefore, we wondered whether β-synuclein might be present in myelin and not only in neurons. We isolated whole myelin from rat brain and fractionated it on sucrose gradients. As seen in Fig. 7, whole brain lysate, myelin preparation, and specifically the heavy myelin fraction contained β-synuclein detected by Western blot. Thymus, skin, and heart extracts were negative for β-synuclein (not shown). Thus, the in vivo encephalitogenic potential of the β-synuclein peptides alerted us to the unsuspected expression of β-synuclein in myelin. Interestingly, α-synuclein was reported to be expressed in oligodendrocytes (33). However, because axonal proteins may contaminate the myelin isolated on sucrose gradients, additional experiments that directly show the expression of β-synuclein in myelin are needed. In a preliminary immunohistochemical experiment, we detected β-synuclein staining in the paranode region of myelin (34).

Discussion

The present study has several important implications. First, similar to our past experience (20), we confirm that selecting peptides based on the MHC class II I-A-binding motif is effective in predicting immunogenicity; we were able to demonstrate both primary lymph node proliferations (data not shown) and we generated T cell lines to the peptides selected in this manner. Second, we found that immunization with β-synuclein peptide induces EAE and uveitis in immunized rats. The disease could be transferred with T cell line and clones specific for the peptide. Encephalitogenicity has been shown for myelin-specific proteins only. Because β-synuclein was not considered to be related to myelin, it was surprising to find that β-synuclein was encephalitogenic. The present study indicates that experimental immunization is necessary to test for the autoimmune disease-inducing potential of a protein. In a recent study of mRNA transcripts in the brains of patients with multiple sclerosis, β-synuclein was found to be upregulated 2.5-fold similar to the transcripts of MBP and αB-crystallin (35), suggesting it could be an autoantigen in human multiple sclerosis. Third, the in vivo effects of the immunization led us to discover the expression of β-synuclein in myelin.

The concomitant occurrence of EAE and uveitis is of interest. In a previous study, we found uveitis to accompany EAE induced by MBP immunization and by transfer of an encephalitogenic T cell clone (23). We suggested that MBP must be expressed in the uvea and is attacked by MBP-specific T cells. In confirmation and extension of our study, Buenafe et al. (24) found the Vβ and Vα restriction of T cells isolated from the uveitis lesion to be similar in composition to the EAE infiltrate, and the cells isolated from the

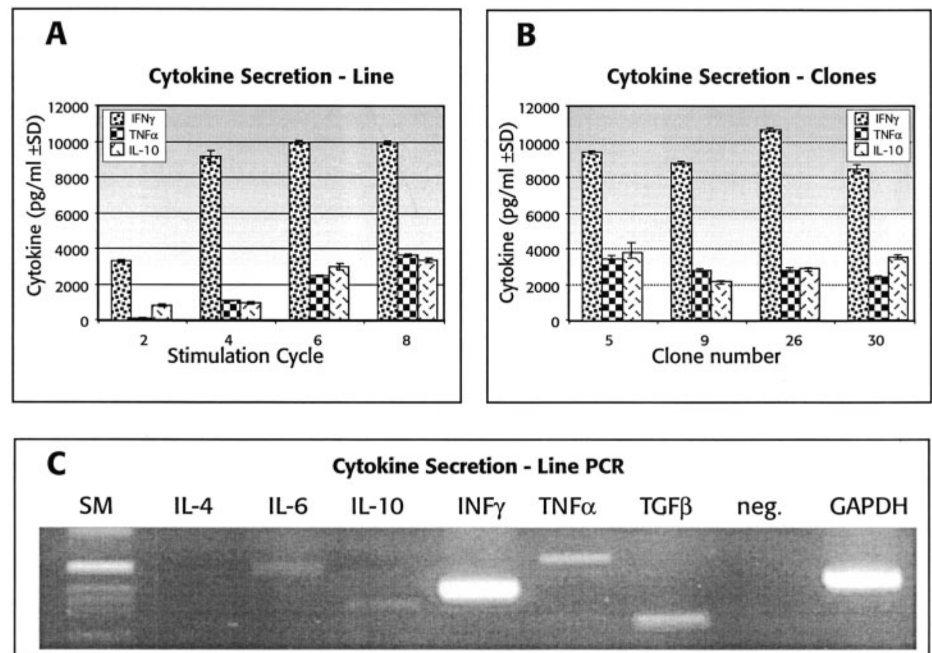

FIGURE 6. Cytokine secretion by line (*A*) and clones (*B*) to $SynB_{93-111}$. Cytokine secretion profile of the line after successive stimulations in vitro as determined by ELISA and cytokine secretion profiles of several clones to $SynB_{93-111}$. PCR of cytokines (*C*) of the T cell line to $SynB_{93-111}$ at the third cycle of stimulation. SM indicates molecular size marker; neg. indicates negative control.

affected iris proliferated specifically to MBP. In patients with multiple sclerosis, the frequent occurrence of uveitis has also been described (36). The fact that β-synuclein immunization also caused uveitis indicates that the eye inflammation is not selective for MBP, but accompanies EAE induced by different autoantigens.

The current idea is that neurodegenerative diseases are caused by abnormal protein folding, leading to neuronal death and clinical neurological deficits (37). The immune system is not considered to participate in these processes. In previous work, we found that in analogy to the therapeutic effect of amyloid-β immunization in a mouse model of Alzheimer's disease (3), immunization with prion protein peptide reduced the PrP^{Sc} content of a transplanted neuroblastoma (38). Moreover, recent publications have described inflammatory cells and molecules in several neurodegenerative diseases, including Parkinson's (39), Alzheimer's (40, 41), and experimental prion disease (42). These findings raise the possibility that Ag-specific T or B cells might influence the natural history of these neurodegenerative diseases. Our present study demonstrates the immunogenicity of β-synuclein peptides and raises the possibility that specific immune reactions to neurodegeneration-related proteins might arise in the course of such diseases with potential beneficial (43) or, as we show in this study, detrimental effects.

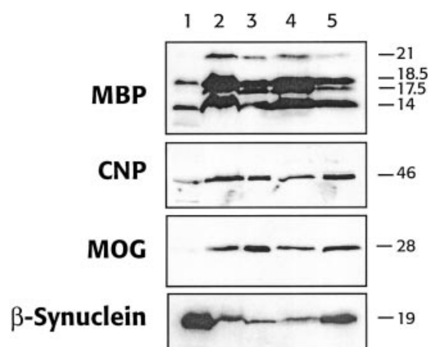

FIGURE 7. Detection of β-synuclein in myelin. Western blotting of brain and fractionated myelin. Samples of brain and myelin were run in 15% SDS-PAGE. The gels were transferred to nitrocellulose membranes and incubated with rabbit polyclonal Abs to MBP, cyclic nucleotide phosphodiesterase (CNP), MOG, and β-synuclein. MBP and MOG were more abundant in the lighter myelin, while β-synuclein band was stronger in heavy myelin. *Lanes*: *1* is whole brain homogenate, *2* is unfractionated myelin, *3* is light myelin (above 0.6 M sucrose), *4* is medium myelin (above 0.7 M sucrose), and *5* is heavy myelin (pellet at bottom of 0.7 M sucrose).

The present study, in addition to its novel findings, points out an actual danger in autoimmunization with β-synuclein peptides. Recently, a human trial of amyloid-β peptide immunization in patients with Alzheimer's disease had to be prematurely terminated due to the development of encephalitis in some of the injected patients. It was suggested that the cause of encephalitis in these patients might include activation of microglial cells and a reaction to the adjuvant used in immunization (44). Our study raises the possibility that amyloid-β immunization might also result in an autoimmune reaction in some of the treated patients. Finally, the finding that epitopes of β-synuclein can induce autoimmune inflammation raises the question as to whether such reaction might arise in the course of neurodegenerative disease and contribute to the pathology.

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