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How special is a pathogenic CNS autoantigen? Immunization to many CNS self-antigens does not induce autoimmune disease

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Abstract

Recent work has shown neuro-protective effects of immunization with self-CNS antigens in animal models of Alzheimer's disease, prion diseases and CNS trauma. The major concern with such an approach is the inadvertent induction of autoimmune disease. The present work was initiated to study the incidence of autoimmune disease associated with the induction of T cell autoimmunity to a panel of 70 peptides derived from CNS proteins. Using a MHC class II motif developed in our laboratory to identify candidate peptides, we selected 70 peptides from 40 different CNS proteins. The proteins were selected randomly and represented various biological functions (surface receptors, structural proteins, synaptic proteins, neurodegeneration related proteins). Each peptide was emulsified in CFA and injected to autoimmune-prone Lewis rats. Immunogenicity was verified by peptide-specific LN cell proliferation. In addition, T cell lines were generated for many peptides and tested by adoptive transfer. Except for the previously reported pathogenicity of beta-synuclein, none of the 68 peptides from 39 proteins was found to induce CNS disease in recipient rats. These findings underscore the efficiency of immunological regulation in preventing CNS autoimmune disease, and confirm the uniqueness of the well-known pathogenic CNS auto-antigens.

Keywords: EAE; Alzheimer's disease; Autoimmunity; Peptides

1. Introduction

The study of auto-antigens in CNS autoimmune diseases and specifically in experimental autoimmune encephalomyelitis (EAE) spans over 70 years (Rivers et al., 1933). In early experiments, whole brain or spinal cord homogenate was injected to experimental animals to induce EAE (Kabat et al., 1947). In subsequent years, following a reductionistic approach based on chemical purification, encephalitogenic proteins were identified including myelin basic protein (MBP) (Eylar et al., 1969), proteolipid protein (PLP) (Yoshimura et al., 1985), myelin oligodendrocyte glycoprotein (MOG) (Wekerle et al., 1994), myelin associated glycoprotein (MAG) (Wekerle et al., 1994), and more recently myelin associated oligodendrocytic basic protein (MOBP) (Maatta et al., 1998). Extensive work has mapped the encephalitogenic peptides of these proteins in various strains of experimental animals. The common denominator of most encephalitogenic proteins is that they represent structural myelin antigens (Steinman, 1995). In other autoimmune diseases, the targets of autoimmune reaction are different and include surface receptors (Hoedemaekers et al., 1997), intracytoplasmic enzymes (Matsumoto et al., 2002), structural proteins (Hertl and Veldman, 2003), hormones (Eisenbarth, 2003), transcription factors (Lee and Craft, 1995), and ubiquitously expressed heat shock proteins (Cohen, 1991).

Recent work has documented the efficacy of immunization with CNS peptides in the treatment of experimental Alzheimer's disease (Schenk et al., 1999), experimental prion disease (Magri et al., 2005) and experimentally induced CNS trauma (Moalem et al., 1999). In view of the beneficial effects of amyloid-beta immunization in mice with experimental Alzheimer's disease, a human trial investigating the therapeutic effects of amyloid-beta vaccination in AD patients was launched (Helmuth, 2000). Unfortunately, the human trial had to be terminated prematurely as some patients developed encephalitis (Birmingham and Franz, 2002), possibly caused

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Table 1
Proteins and peptides used for immunization

Name	Accession	#	Origin	Sequence	Site	Function
				34 6 9[a]		
Acetylcholinesterase precursor	584716	1	Rat	GTPNGPWATVSAGEARRRAT	261–280	Enzyme
Amphiphysin II	2160719	2	Human	EEELIKAQKVFEEMNVDLQE	205–225	Synaptic protein
		3		PPKHTPSKEVKQEQILSLF	344–363	
		4		LLSATDTLDEAERQWKA	570–586	
Bassoon	3413504	5	Rat	KQASATAPGRESPRETRA	94–111	Presynaptic cytoskeleton
		6		SGTSPTSLSSLEEDSDSSPSR	987–1006	
Brain-1	2760445	7	Rat	DPSSVKMVQSDFMQGAMAA	65–83	Oligodendrocyte transcription factor
Brain specific protein	206781	8	Rat	TVSILCSTQSNPDPILTIF	34–52	Myelin associated glycoprotein
		9		GLNSHDPHSDEDTPTSDDLE	301–320	
ComplexinII	2137226	10	Mouse	QEEERKAKHARMEAEREKV	44–62	Presynaptic protein
Diacylglycerol kinase	1083642	11	Rat	LFWSFHSSQDLDEETESKAN	71–90	Oligodendrocyte specific enzyme
		12		IEISKVVYLDRWLLEVIPQQ	481–500	
Glutamate receptor GluR7	92440	13	Rat	SSSSRPLLKEMKRGREFR	211–228	Surface receptor
		14		TFFKKSKISTFEKMWAFMS	694–712	
Huntingtin associated prot.	2143798	15	Rat	ENNKLETMLGSAREEILHLR	181–200	Cytosolic protein
		16		YSVPLDALPSFPETLAEEL	416–434	
Huntingtin	1170192	17	Human	LTGGKNVLVPDRDVRVSVK	696–714	Unknown
		18		RLRDGDSTSTLEEHSEGKQ	1716–33	
		19		LTELRRVHPSEDEILAQYL	2725–44	
Myelin oligodendocytic basic protein	1408057	20	Rat	MSQKVAKEGPRLSKNQKF	1–18	Myelin protein
Myelin MVP17	914968	21	Rat	PSGFSVFVTFPDLLFIFE	13–30	Myelin protein
		22		SVLEALATITMFDGFTYR	103–120	
Neurokinin	135884	23	Bovine	AVIFFISTQLSAEEIGAND	8–26	neurotransmitter
Nerve growth factor receptor	128157	24	Rat	PCGANQTVCEPCLDNVTFS	57–75	Surface receptor
Tenascin-R	57962	25	Rat	LGCSSRGVCVDGQCICDSE	206–224	Brain extracellular matrix
		26		GISDRSIELEWDGPMAVTEY	336–345	
Neurodegeneration associated protein 1	971275	27	Rat	MFLTPSYSRVTPREAERHRA	371–390	Synaptic protein
		28		MSQYTEKEPSVMDQDSSKAA	1–20	
Oligodendrocyte specific protein	1083742	29	Rat	MSQKVAKEGPRLSKNQKFSE	1–20	Myelin protein
Phosphoprotein	1066848	30	Rat	LILSPRSKESVPEFPLSPP	32–50	Signal transduction?
		31		EEVRKNKESKDPADETEAD	131–149	
PSD93	2497503	32	Rat	PSVTLQRAISLEGEPRKVVLH	405–425	Postsynaptic density
		33		KPKSLEPLMEMNKRLTEEQA	779–798	
PSD95	400891	34	Rat	APGYELQVNGTEGEMEYEEI	47–66	Postsynaptic density
		35		VDVREVTHSAAVEALKEAG	123–141	
Synaptic vesicle prot-2	730883	36	Rat	SVFAIGALTTQPESPRFFLEN	344–364	Membrane glycoprotein transporter
		37		AVLPGNIVSALLMDKIGRLR	607–626	
Synapse associated protein(SAP) 102	1236953	38	Rat	SYGDILHVINASDDEWWQAR	546–565	Synaptogenesis and cell proliferation
		39		FHARTGMIESNRDFPGLSD	598–616	
SAP90	424013	40	Rat	VDVREVTHSAAVEALKEAGS	123–142	
		41		HVIDAGDEEWWQARRVHSDS	461–480	
Synaptotagmin4	1711652	42	Rat	ALPNLSLHLDLEKRDLNGN	86–104	Synaptic vesicle transport?
		43		PESLKSSTSLTSEEKQEKL	136–154	
Synaptotagmin5	1351177	44	Rat	AFSFEVPCDQVQKVQVELTV	309–328	Synaptic vesicle transport?
Synapsin-1	135153	45	Rat	GNWKTNTGSAMLEQIAMSDR	334–352	Synaptic protein
Synaptoporin	135158	46	Rat	GGYSQQASLGPTSDEFGQQPS	239–256	Synaptic vesicle membrane
Synuclein-beta	464424	47	Rat	LKPEEVAQEAAEEPLIEPL	93–111	Neuronal protein
Synaptotagmin2	135088	48	Rat	DDDDAETGLTEGEGEGEEEK	119–138	Synaptic vesicle transport?
		49		TKVHRKTLNPAFNETFTFKV	196–215	
Synaptophysin	135163	50	Rat	CVKGGTTKIFLVGDYSSSAE	81–100	Synaptic vesicle organization?
Synaptotagmin 3	730879	51	Rat	RPLTQQTLTTQADPSSEER	234–251	Synaptic vesicle transport?
		52		TLNPTYNEALVFDDVAPESVE	491–510	
Synaptotagmin 1	1351176	53	Rat	NESFSFEVPFEQIQKVQVVV	340–359	Synaptic vesicle transport?
		54		VVVTVLDYDKIGKNDAIDKV	357–376	
Tryptophan hydroxylase	136122	55	Rat	PLLKSHTTVLSVDSPDQLP	78–96	Enzyme
		56		KSITSAMNELRHDLDVVND	415–433	
Cannexin associated protein 1	4505463	57	Human	HEVNFVAQENHAVISIDDVE	445–465	Myelin protein

Table 1 (*continued*)

Name	Accession	#	Origin	Sequence	Site	Function
				34 6 9[a]		
		58		VDGQLVNLTLVEGRRLG	512–528	
Synuclein alpha	420292	59	Rat	AAATGFVKKDQMGKGE	89–104	Neuronal protein
Synuclein beta	2501106	60	Rat	AAATGLVKKEEFPTDL	78–93	Neuronal protein
Synuclein gamma	2501107	61	Rat	RGTSVTSVAEKTKEQA	48–63	Neuronal prot.
		62		VSSVNTVATETVEEAE	71–86	
		63		VVTTGVVRKEDLEPPA	89–104	
Presenilin 1	6174931	64	Rat	KSVSFYTRKDGQLIYT	101–116	Transmembrane protein
		65		VWLVNMAEGDPEAQRR	293–308	
		66		KYSTQGTERETQDTG	315–329	
		67		STPESRAAVQELSGSIL	354–370	
		68		LSGSILTSEDPEERGV	365–380	
Presenilin 2	6174932	69	Rat	SQGALQLPYDPEMEED	311–326	Transmembrane protein
		70		ESPTSRSCQDSRPGPE	24–39	

[a] Number of MHC ancor position in the peptide groove Reizis, B., Mor, F., Eisenstein, M., Schild, H., Stefanovic, S., Rammensee, H.G., Cohen, I.R., 1996. The peptide binding specificity of the MHC class II I-A molecule of the Lewis rat, RT1.BI. Int Immunol 8, 1825–1832.

by induction of pathogenic CNS autoimmunity (Furlan et al., 2003). This adverse effect raised the question of how we might predict the pathogenicity of a self-protein. Will immunization with any self-protein induce disease? Or is disease limited to particular self-antigen?

The CNS is known to express a large and diverse proteome consisting of thousands of different proteins (Gauss et al., 1999; Yu et al., 2004). Immunity to some of these proteins may have beneficial effects in ameliorating devastating human diseases. The present study was designed to test the pathogenic effects of immunization with a large panel of peptides derived from various CNS-specific proteins including surface receptors, enzymes, and structural proteins as well as disease related proteins. The peptides were chosen to fit a MHC class II IA motif in the Lewis rat identified in our lab (Reizis et al., 1996). While the vast majority of peptides were immunogenic and induced T cell responses as strong as previously identified encephalitogens, none were found to be pathogenic. These results highlight the unique specificity of the pathogenic autoimmune reaction: not any immunogenic self-antigen can induce a disease.

2. Materials and methods

2.1. Animals

Inbred female Lewis rats and NOD mice were supplied by the animal breeding center of the Weizmann Institute of Science under the supervision of Harlan Laboratories and an animal welfare committee and were used at 2–3 months of age. Experiments were approved by the Institutional Animal Care and Use Committee.

2.2. Antigens and antibodies

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 1. MHC anchor positions 3,4, 6 and 9 are indicated (Mor et al., 1996). The purity of the peptides was analyzed by HPLC and amino acid composition.

2.3. Immunizations

The peptides were first 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 *Mycobacterium tuberculosis* H37Ra (complete Freund's adjuvant-CFA). Naive female Lewis rats or NOD mice, were immunized in both hind footpads with 50 μl of the emulsion; each animal was injected with a total 50 μg of peptide. As pertussis toxin is known to enhance the pathogenic potential of immunogens (Hofstetter et al., 2002), some of the immunized rats were injected intraperitoneally with 200 ng of pertussis toxin (Sigma) on day of peptide/CFA inoculation and 2 days later. Rats and mice were inspected for clinical signs from day 10 after injection for 3 weeks. Draining popliteal lymph nodes were removed on day 12 after injection and a single cell suspension was prepared by pressing the organs through a fine wire mesh, and the cells were studied in vitro.

2.4. T cell proliferation assay

T cell proliferation was performed by seeding 10^5 LN cells or 5×10^4 line cells at 4–5 days of the propagation phase with 5×10^5 irradiated thymocytes (2500R) as antigen presenting cells, in stimulation medium for 3 days, in 96 micro-titter round bottom wells (Nunc, Rosklide, Denmark), as described (Mor and Cohen, 1993). 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, England) for the final 4 h. The cultures 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 Company, Meriden, CT, USA). The results of proliferation are expressed as cpm.

2.5. T cell lines

Antigen-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 to stimulation medium without autologous serum, but supplemented with fetal calf serum 10% and T cell growth factors from the supernatant of Con A stimulated

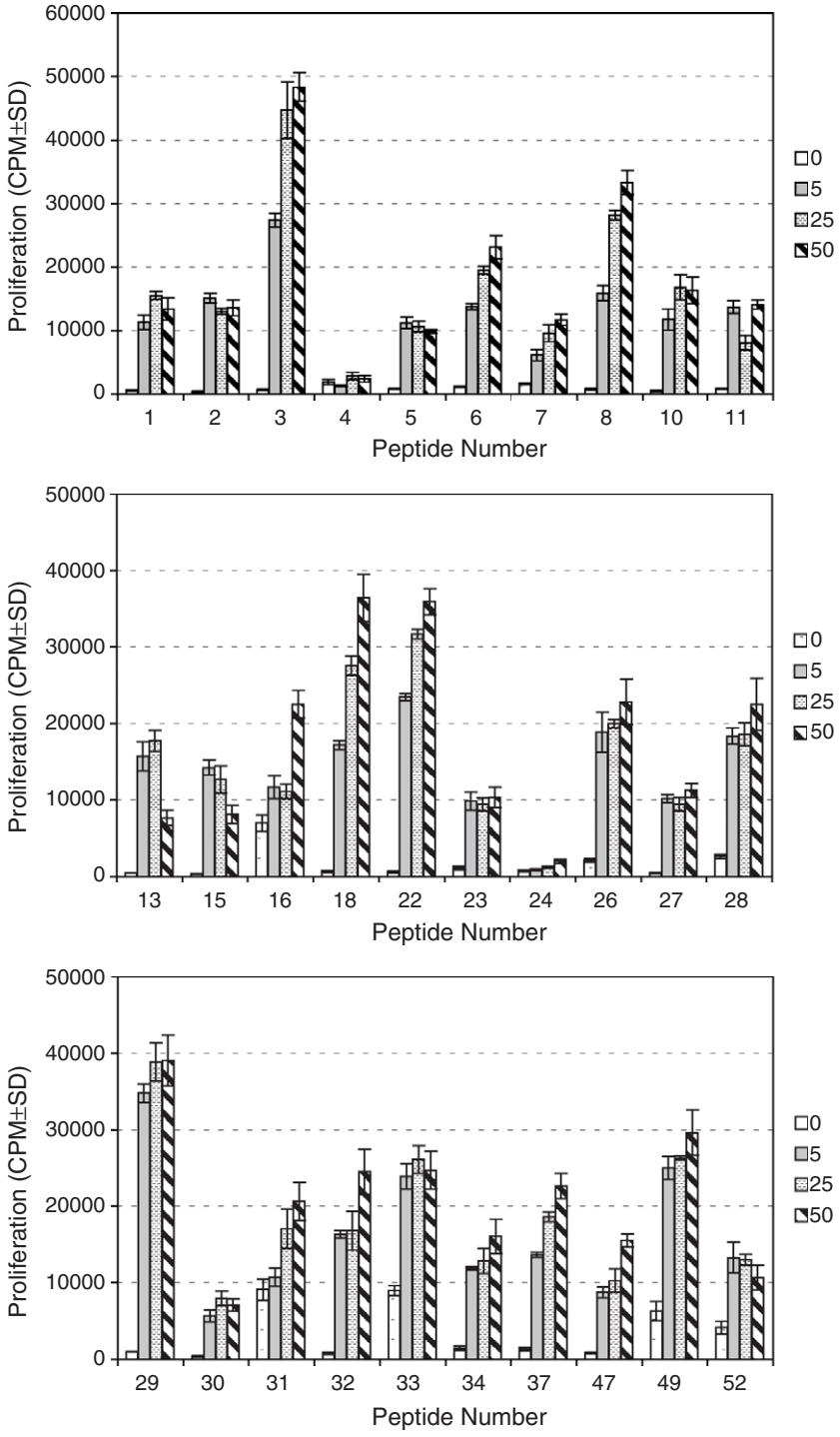

Fig. 1. Proliferation profiles of CNS derived peptide lines. Lines were tested at the second cycle of in vitro stimulation. Each line was tested with 0, 5, 25 and 50 μg/ml of the specific peptide. 5×10^4 line cells were incubated with 5×10^5 irradiated thymocytes as APC's. Cultures were pulsed with 3[H] thymidine and proliferation was tested as cpm. Peptide numbers correspond to the numbers on Table 1.

Table 2
Testing pathogenic potential of peptides

Pep	Active immunization	Pertussis[a]	T cell line[b]	Irradiation[c]	NOD mice
1	No disease	Yes	Yes		No dis.
2	No disease	Yes	Yes	Yes	No dis.
3	No disease	Yes	Yes		
4	No disease				
5	No disease	Yes	Yes	Yes	No dis.
6	No disease				
7	No disease	Yes	Yes		No dis.
8	No disease	Yes	Yes	Yes	No dis.
9	No disease	Yes	Yes		
10	No disease	Yes	Yes	Yes	No dis.
11	No disease	Yes	Yes	Yes	No dis.
12	No disease				
13	No disease	Yes	Yes	Yes	No dis.
14	No disease				
15	No disease	Yes	Yes	Yes	No dis.
16	No disease				
17	No disease	Yes			No dis.
18	No disease	Yes	Yes		
19	No disease	Yes	Yes		
20	No disease	Yes			No dis.
21	No disease	Yes			No dis.
22	No disease	Yes	Yes		
23	No disease	Yes	Yes	Yes	No dis.
24	No disease	Yes	Yes		
25	No disease	Yes			No dis.
26	No disease				
27	No disease	Yes	Yes	Yes	No dis.
28	No disease				
29	No disease	Yes	Yes		
30	No disease	Yes	Yes	Yes	No dis.
31	No disease				
32	No disease	Yes	Yes	Yes	No dis.
33	No disease				
34	No disease	Yes	Yes	Yes	No dis.
35	No disease				
36	No disease	Yes			No dis.
37	No disease				
38	No disease	Yes			No dis.
39	No disease				
40	No disease	Yes			No dis.
41	No disease				
42	No disease	Yes			No dis.
43	No disease				
44	No disease				
45	No disease	Yes			No dis.
46	No disease	Yes			
47	EAE	Yes	Yes EAE		No dis.
48	No disease	Yes			No dis.
49	No disease				
50	No disease	Yes			No dis.
51	No disease	Yes			No dis.
52	No disease	Yes			
53	No disease	Yes	Yes		No dis.
54	No disease				
55	No disease	Yes	Yes		No dis.
56	No disease	Yes			
57	No disease	Yes	Yes		
58	No disease				
59	No disease	Yes	Yes	Yes	
60	No disease	Yes	Yes EAE	Yes	
61	No disease	Yes			
62	No disease	Yes			
63	No disease	Yes			
64	No disease	Yes			
65	No disease	Yes			
66	No disease	Yes			
67	No disease	Yes	Yes	Yes	
68	No disease	Yes			
69	No disease	Yes			
70	No disease	Yes	Yes	Yes	

[a] Intraperitoneal injection of 200 ng of pertussis toxin on day of peptide/CFA immunization and 48 h later.
[b] Intraperitoneal injection of 2×10^7 peptide activated T cell line.
[c] Irradiation with 600R prior to T cell line inoculation.

spleen cells 10% (Mor et al., 1990). Animals were injected intraperitoneally with 2×10^7 peptide stimulated T cells, following 3–5 cycles of in vitro stimulations. In some experiments rats were irradiated (600R) prior to line inoculation, as irradiation is known to enhance susceptibility to passive transfer of autoimmune disease (Williams et al., 1987).

2.6. Induction of disease

Active EAE was induced by injection 50 μg peptide in CFA. Adoptive EAE was transferred by intra-peritoneal injection of 2×10^7 peptide-activated cells of the lines as described (Mor and Cohen, 1995). Clinical EAE was observed 12 days after peptide/CFA or 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 (Mor and Cohen, 1995).

2.7. Cytokine ELISA assays

Supernatants were collected after three days of stimulation of the T cell lines with peptides. IL-10, TNFα and IFNγ in the culture supernatants were measured by ELISA using Pharmingen's OPTEIA kit (Pharmingen, San Diego, CA). Pharmingen recombinant rat cytokines were used as standards for calibration curves. A standard ELISA assay was performed as described (Mor and Cohen, 2002).

3. Results

3.1. Peptides identified by their MHC class II motif are immunogenic

Extensive experimental work has characterized the MHC class I and II motifs in various strains of laboratory animals (Rammensee, 1995). Our group previously reported the MHC class II motif of the IA molecule in the Lewis rat (Reizis et al., 1996) and NOD mouse (Reizis et al., 1997).

Selection of peptides based on this algorithm was found to predict T cell immunogenicity (Mor et al., 2003, 1996). To select potentially pathogenic CNS proteins, we searched the protein database for brain-specific and oligodendrocyte-specific proteins. We selected proteins with various cellular functions. The proteins were scanned, and 70 peptides that conformed to the IA motif of the Lewis rat were selected. Table 1 shows the proteins and peptide sequences. The bold and underlined amino acids represent positions 3,4,6 and 9 in the MHC motif. To test for immunogenicity of the selected peptides, we performed T cell proliferation assays on the LN cells after one cycle of in vitro stimulation. Fig. 1 shows the proliferation profile of 30 of the peptides. As can be seen, the vast majority of peptides were highly immunogenic, only 2 of the peptides (peptides 4 and 24) did not induce antigen-specific T cell proliferation. Thus, the motif correctly identified immunogenic peptides from the proteins selected.

3.2. Most immunogenic self-CNS peptides do not induce active EAE

To test for their pathogenic potential, the peptides were injected in CFA (Billiau and Matthys, 2001) to groups of 4 Lewis rats. As seen in Table 2, the vast majority of peptides (69 of 70) did not induce EAE upon active immunization. Only peptide 47 from the synuclein beta molecule induced EAE in Lewis rats (Mor et al., 2003). To extend the experiment to other species, NOD mice (which are known to be susceptible to experimental autoimmune encephalomyelitis (Ichikawa et al., 2000) and bear a similarity in the MHC class II motif of IAG7 to the class II IA of the Lewis rat (Reizis et al., 1998)), were injected with the peptides indicated and with pertussis toxin on day 0 and 2; none of the mice developed EAE.

In previous work, we found that a T cell line may cause disease even though active immunization to the target peptide was not pathogenic (Mor and Cohen, 1995). Hence, we generated T cell lines (Table 2) to a sample of 29 different peptides and tested the lines for pathogenicity. The lines were expanded for 3–4 cycles in vitro and were injected to naïve Lewis rats. Some of the lines were also injected to rats that had been irradiated with 600R, since irradiation of recipients is known to enhance the pathogenicity of adoptively transferred cells (Williams et al., 1987). Except for the T cell line reactive to peptides 47 and 60 (Mor et al., 2003), no other line mediated EAE in recipient rats.

3.3. Cytokine profile of the T cell lines

Pathogenic T cells in EAE are of the Th1 phenotype (Lafaille, 1998). To test whether the lack of pathogenicity of injected peptides was due to induction of Th2 type of responding cells, we tested in parallel several CNS peptides: the encephalitogenic peptide GBP71-90 was used as a

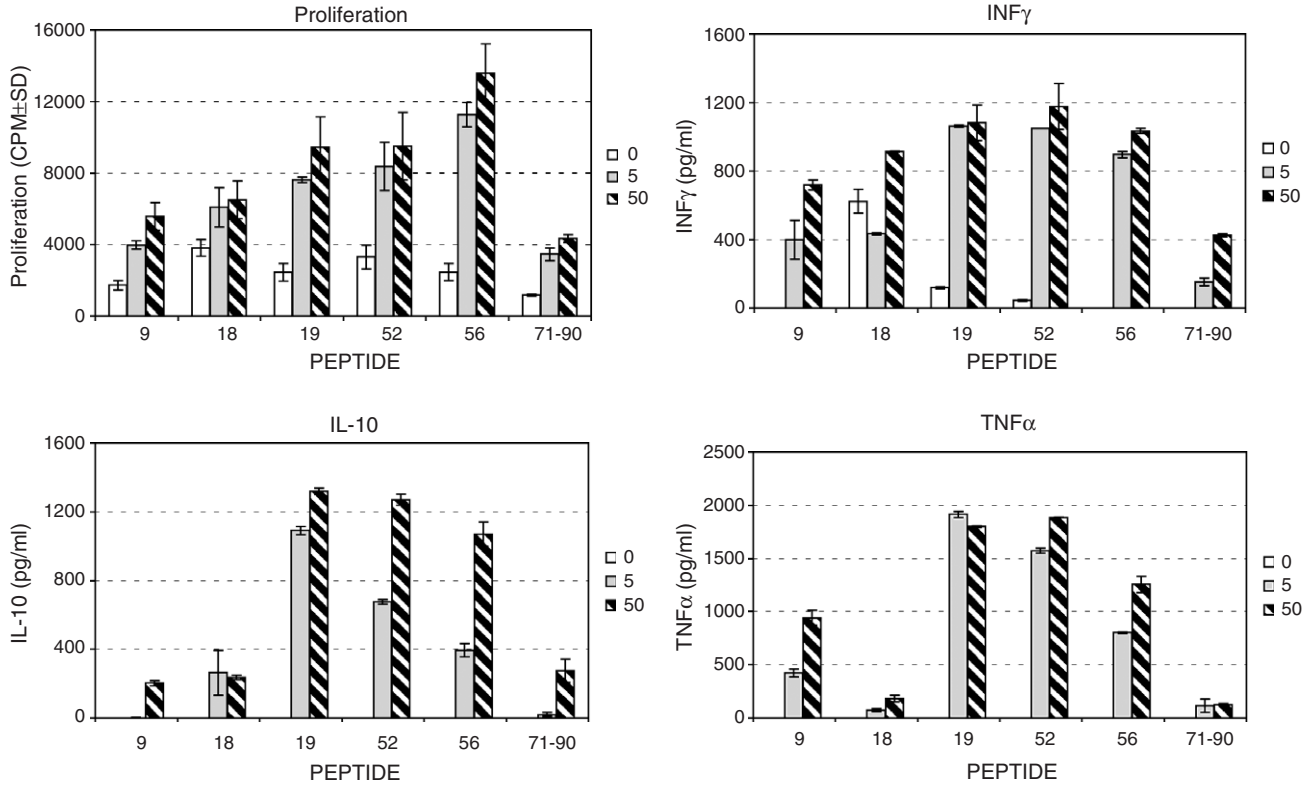

Fig. 2. Proliferation and cytokine secretion by peptide-specific T cell lines. The proliferation and cytokine secretion profile of a panel of 5 CNS peptides and guinea-pig MBP peptide 71–90 (as a positive control) were tested on the second cycle of in vitro stimulation.

positive control. The cytokine profile of these T cell lines, similar to our previous experience (Mor et al., 1996), was compatible with a Th0/Th1 phenotype, secreting γ-INF TNFα and IL-10 (Fig. 2). Thus, the lack of pathogenic activity was not due to a skewed differentiation to a Th2 phenotype.

4. Discussion

The central nervous system is known to express the largest variety of proteins in the body (Yu et al., 2004). With current technology, over 8500 proteins can be detected in the brain (Klose et al., 2002), and the nervous system expresses 30–50% of mammalian genes (Fountoulakis, 2004). Approximately 10% of brain proteins are specific to the brain: structural or regulatory proteins, involved in neurogenesis, neuronal modeling, synaptic transmission and neurotransmitter function (Fountoulakis, 2004). Recently, immunization with CNS derived peptides and proteins was found to ameliorate devastating diseases such as Alzheimer's disease (Schenk et al., 1999) prion disease (Magri et al., 2005) and nerve degeneration following CNS trauma (Moalem et al., 1999). Ideally, one would like to use immunogens that are effective in treating CNS disease and devoid of the potential to cause CNS autoimmune disease. The importance of this issue is highlighted by the recent cessation of an amyloid beta immunization trial in patients with Alzheimer's disease following the induction of encephalitis in some patients (Birmingham and Franz, 2002). On a more basic level, can we predict whether a given protein will cause autoimmune disease upon immunization?

We chose the model of EAE in the Lewis rat as a probe for pathogenic autoimmunity for several reasons: 1. Lewis rats are very sensitive to the induction of EAE. 2. A variety of self-antigens have been shown to cause the disease. 3. EAE is readily inducible by both active immunization and adoptive transfer of T cell lines. 4. The pathogenic T cells capable of mediating EAE have been well characterized with regard to proliferation and cytokine production. 5. We have extensive experience with the model. Previous work with EAE has demonstrated that most encephalitogenic proteins are expressed in myelin (Schmidt, 1999); these include MBP, PLP, MOG MAG and MOBP. In addition non-myelin self-antigens too can induce EAE (Furlan et al., 2003; Wekerle et al., 1994). Many of the proteins whose peptides we chose to test were derived from oligodendrocytes (such as 7, 11, 12) and some were proper myelin antigens (such as: 8, 9, 20–22, 57, 58), yet none of them induced EAE. Thus, among myelin proteins, some are clearly pathogenic and others seem to be devoid of pathogenic potential. However, the pathogenic and non-pathogenic T cells could not be distinguished by the magnitude of their proliferation or cytokine secretion (Fig. 2). Previous work has emphasized the importance of local reactivation of pathogenic T cells in the target organ by locally expressed antigen (Kawakami et al., 2005). One may speculate that the difference in pathogenicity may lie in differences in the MHC class II epitope presentation in the CNS.

Some years ago, our group introduced the term the Immunological Homunculus to describe the hierarchy among self-proteins in terms of their representation in the immune system including their pathogenic potential (Cohen, 1992). Thus, upon immunization with spinal cord homogenate containing several thousands of different proteins, the immune system reacts to a small number of "dominant" proteins such as PLP (Whitham et al., 1991), MBP and MOG. The mechanism underlying the observed hierarchy between proteins is not known, but it may be related to competition for presentation and the efficiency of thymic deletion. Thus, self proteins that are not adequately expressed in the thymus (Pitkanen and Peterson, 2003) may not delete or induce the regulation of the corresponding T cells that will mature, populate the peripheral repertoire and upon immunization will expand and cause disease.

Why might some immunogenic peptides not be pathogenic?

First, we screened peptides and not whole proteins. It is possible that the peptides chosen were not immunodominant in terms of their recognition, but cryptic (Sercarz et al., 1993); the T cell reactive against the immunizing peptide will react poorly to the whole protein. However, from our analysis of the cryptic T cell repertoire to MBP in the Lewis rat, we have learned that cryptic epitopes can be pathogenic (Drakesmith et al., 1998; Mor and Cohen, 1995). Thus, a peptide's crypticity does not preclude its pathogenic potential.

Secondly, most peptides were chosen from the self-(rat) sequence database (Table 1). From the experience with MBP we know that other species molecules can be much more pathogenic: guinea-pig MBP and its immunodominant peptide MBP-Gp-71–90 are more pathogenic than rat MBP and MBP-rat-71–90 in the Lewis rat (Mor et al., 2000). Yet, this heteroclitic behavior of a foreign peptide is empirically derived and cannot be predicted. Thus, the best way to ensure self-reactivity is to immunize with a self-peptide. Based on our experience with rat MBP sequences we know that many self peptide sequences are pathogenic including the immunodominant peptide 71–90, and the cryptic peptides 51–70, 11–30 and 91–110 (Mor and Cohen, 1995). To increase the potential pathogenicity of selected peptides, we injected rats with activated peptide-specific T cell lines (Table 2), and several lines were transferred to irradiated recipients. Except for the 2 known synuclein-beta peptides, none of the lines caused EAE.

Third, our method of scoring was based on clinical criteria (scoring for neurological signs of weakness and paralysis). It is possible that the disease induced by some of the peptides did not lead to clinically detectable disease but caused histological brain inflammation as was described for immunization with the non-myelin astroglial protein S100β (Kojima et al., 1994).

What can we learn from our work? First, we show that the T cell repertoire of the Lewis rat is populated with T cells reactive to the majority of the peptides chosen by the MHC class II motif. Thus, deletion of self-CNS proteome reactive T cells did not appear to be operative. Second, most anti-self reactivity was found not to be pathogenic. Even the T cell lines reactive to myelin proteins not already known to cause EAE, were not pathogenic. Third, the known biological function of a protein cannot be used to predict its pathogenic potential in vivo: bona fide myelin peptides (peptide number: 8, 9, 20–22, 29, 57, 58) were devoid of pathogenicity, yet beta-synuclein which is considered a neuronal protein, induced EAE (Mor et al., 2003). Thus, the only way to discover the pathogenic potential of a candidate protein is to actively immunize with the antigen in a suitable adjuvant or raise a specific T cell line and test its pathogenicity empirically.

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