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Autoimmune spread to myelin is associated with experimental autoimmune encephalomyelitis induced by a neuronal protein, β-Synuclein

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ABSTRACT

Accumulating evidence suggests that autoimmunity against neuronal proteins is important for MS pathogenesis. We have characterized T- and B-cell responses associated with experimental autoimmune encephalomyelitis (EAE) induced in Lewis rats with recombinant β-Synuclein (βSync), a neuronal component. The encephalitogenic βSync-specific T cells recognize a single immunodominant region with an epitope delineated at amino acids 97–105; B-cell specificity is more widespread, albeit directed mostly to the C-terminus of βSync. Most interestingly, βSync-induced autoimmune T- and B-cell responses spread not only to other neuronal antigens but also to myelin encephalitogens, raising the possibility that anti-neuronal immune attacks could also result in demyelination.

1. Introduction

Multiple sclerosis is an autoimmune neurological disease characterized by demyelination believed to result from autoreactivity against CNS myelin. Extensive efforts by many laboratories worldwide to identify primary target Ags in MS, defined as CNS components that can induce the development of experimental autoimmune encephalomyelitis (EAE), the purported animal model for MS, and are recognized by peripheral blood lymphocytes of MS patients, have focused on myelin Ags. Thus, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin oligodendrocyte-associated basic protein (MOBP), oligodendrocyte-specific protein (OSP), and possibly also myelin-associated glycoprotein, could be demonstrated as bona fide primary target Ags in MS on the basis of these two criteria (Kerlero de Rosbo and Ben-Nun, 1998).

More recently, studies have confirmed that MS pathogenesis is also associated with primary axonal damage and neuronal loss prompting research into the role of neuronal proteins as potential target Ags in MS (Bjartmar and Trapp, 2001; Bjartmar and Trapp, 2003; Bjartmar et al., 2003; Ferguson et al., 1997). Thus, the encephalitogenic potential of Amyloid beta, which was suggested upon observation of clinical signs characteristic of CNS inflammation in Alzheimer patients that participated in clinical trials of treatment with the protein (Thatte, 2001), has been reported for mice, albeit of low extent (Furlan et al., 2003). Two recent reports indicate that immunization of mice with Tau, a mostly neuronal microtubule-associated protein that is incorporated in neurofibrillary tangles in Alzheimer's disease, and neurofilament light protein (NF-L), a major component of the axonal cytoskeleton, leads to neurological deficit (Huizinga et al., 2007). In MS, CSF concentrations of TAU proteins can be altered (Teunissen et al., 2005) and increased levels of Abs against NF-L and neurofilament medium protein have been reported (Silber et al., 2002), with Abs against NF-L correlating with cerebral atrophy (Eikelenboom et al., 2003). Abs against neurofascin, a cell-adhesion molecule present at nodes of Ranvier, are also elevated in secondary progressive MS; they have been shown in vitro to inhibit neural transmission in hypocampal slices, suggesting their potential pathogenic role in MS (Mathey et al., 2007). These possibilities, together with recent immunohistopathological and neuroimaging studies demonstrating that axonal damage is also associated with early MS (Bjartmar et al., 2000; Bjartmar and Trapp, 2001; Bjartmar and Trapp, 2003; Bjartmar et al., 2003; Ferguson et al., 1997), suggest that autoimmunity against neuronal components may also play a primary role in MS pathogenesis. In a previous study by one of the co-authors, where peptides representing epitopes of neurodegeneration-related proteins that were predicted to bind rat class II I-A molecule, RT1.B1, were tested for their encephalitogenic potential, a peptide encompassing amino acids

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; βSync, beta synuclein; IPTG, isopropyl-{beta}-D-thiogalactopyranoside; SI, stimulation index; NFL, neurofilament; LNC, lymph node cells; CP, cyclophosphamide; TMB, tetramethylbenzidine; DeltaCX, delta connexin; MOBP, myelin oligodendrocyte-associated basic protein; OSP, oligodendrocyte-specific protein; dhPLP, delta human proteolipid protein; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; Abeta1-42, amyloid beta 1–42; AlphaSync, alpha synuclein; GAD, glutamic acid decarboxylase.

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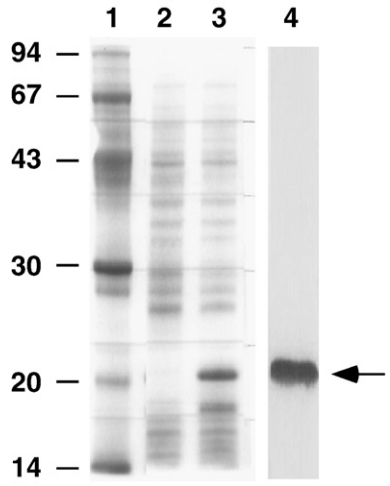

Fig. 1. Generation of recombinant βSync. Coomassie Blue-stained gel of recombinant βSync expression in bacterial host: lane 1, molecular weight markers (kDa); lanes 2 and 3, before and after induction with IPTG, respectively (bacterial proteins from pellets of 150 μl bacterial culture); lane 4, purified βSync.

92–110 of rat β-Synuclein (βSync) was shown to induce EAE in Lewis rats (Mor et al., 2003).

βSync, a neuronal protein demonstrated in human, rat, and bovine brain, is highly abundant in adult rat brain, accounting for as much as 0.1% of the total brain proteins; it has not been demonstrated in the peripheral nervous system. It is strongly expressed in the gray matter, where it is localized in the presynaptic nerve terminals (Quilty et al., 2003). The function of βSync is poorly understood; it is a soluble presynaptic protein that may play a role in synaptic transmission. In this study, we have used a recombinant preparation of βSync and overlapping synthetic peptides thereof to characterize the autoimmune T- and B-cell responses to the whole βSync molecule in Lewis rats. We confirm the encephalitogenicity of the region spanning amino acids 92–110 and show for the first time that a primary encephalitogenic response to a neuronal Ag can lead to T- and B-cell autoimmune spreading not only to epitopes on other neuronal Ags, but most importantly to epitopes of known myelin encephalitogens, raising the possibility that an autoimmune response directed against a neuronal component might also result in demyelination.

2. Materials and methods

2.1. Rats

Female Lewis rats were purchased from Jackson laboratories (Bar Harbor, ME) or obtained from the Weizmann Institute colony. All rats were 2–3 month-old when experiments were carried out. The IACUC of the Weizmann Institute has approved the experiments, which were performed in accordance to its relevant guidelines and regulations.

2.2. Generation of recombinant human βSync

A DNA fragment coding for human βSync was amplified by PCR using human brain cDNA as a template and relevant primers (5′ forward primer: 5′-ATGGCTAGCGACGTGTTCATGAAGGGCCTG-3′ and 3′ reverse primer: 5′-GATCTCGAGCTACGCCTCTGGCTCATACTCCTG-3′, containing NheI and XhoI restriction sites (underlined), respectively), gel-purified, and cloned into pRSET bacterial expression vector (Invitrogen Life Technologies, San Diego, CA) via NheI/XhoI. The nucleotide sequence of the PCR product within the construct, obtained by direct sequencing using pRSET-specific primers (forward primer, 5′-ATGCGGGGTTCTCATCAT-3′, and reverse primer, 5′-TAGCAGCCGGATCAAGCT-3′) and an Applied Biosystems 373A DNA sequencer (Foster City, CA), confirmed an open reading frame coding for human βSync (GenBank accession number NM003085), preceded by (Met)-Arg-Val-Ser-(His)6-Ala-Ser.

Expression of the recombinant protein was induced in BL21-DE3 bacterial host using isopropyl-{beta}-D-thiogalactopyranoside (IPTG; cat. no. R0392; MBI Fermentas, Vilnius, Lithuania). The bacterial pellets from two 12-L fermentor cultures were pooled, solubilized in lysis buffer (6 M guanidine–HCl, 0.5 M NaCl, 100 mM $Na_2HPO_4{\cdot}H_2O$, 10 mM Tris, pH 8.0), sonicated (UltraSonic Processor; Misonix, Farmingdale, NY) extensively, and centrifuged (12,000 rpm, 15 min). The supernatant was adsorbed (60 min at room temperature) to Ni^{2+}-NTA agarose (cat. no. 30230; Qiagen, Chatsworth, CA) pre-equilibrated with lysis buffer containing 20% glycerol and washed twice in wash buffer (8 M urea, 0.5 M NaCl, 100 mM $Na_2HPO_4{\cdot}H_2O$, 10 mM Tris, 20% glycerol, pH 6.3). Elution of the recombinant protein with elution buffer (8 M urea, 0.5 M NaCl, 100 mM $Na_2HPO_4{\cdot}H_2O$, 10 mM Tris, 20% glycerol, pH 3.5) was monitored by SDS-PAGE and fractions containing βSync were combined and diluted 1:10 in refolding buffer (20 mM Na_2HPO_4, 50 μM β-mercaptoethanol, pH 8.0). This mixture (final pH 6.5–7.0) was left to stand overnight at room temperature. Aggregates were removed by centrifugation and the soluble βSync was dialyzed for 6 h against dialysis buffer (0.4 M urea, 1 mM Tris, 10 mM NaH_2PO_4, 50 mM NaCl, pH 7.0), followed by extensive dialysis against several changes of double-distilled H2O (made to pH 3.0 with acetic acid) for at least 48 h. Fig. 1 shows the bacterial expression of βSync before (lane 3) and after (lane 2) IPTG induction and βSync after purification on Ni^{2+}-NTA agarose (lane 4). All other recombinant proteins used in this study, including Delta CX, AlphaSync, Abeta1-42, mMOBP, SmOSP, dhPLP, mMOG, and YDMP, were purified in the same way.

2.3. βSync peptides

The amino acid sequences of the synthetic overlapping peptides (pβSync) spanning mouse βSync that were used in this study are given in Table 1. All the peptides were synthesized in the laboratory of Prof. M. Fridkin, Department of Organic Chemistry, the Weizmann Institute of Science, using the Fmoc technique with an automated peptide synthesizer (AMS422; Abimed, Langenfeld, Germany).

2.4. T-cell lines and T-cell proliferative responses

Ag-specific T-cell lines were selected in vitro as described previously (Ben-Nun and Lando, 1983) from lymph node cells (LNC) of rats that had been primed 9 days before with Ag (150 μg βSync or 100 μg pβSync) emulsified in CFA containing 150 μg *Mycobacterium tuberculosis* H37Ra (Cat. No: 3114-25, Difco Laboratories, Detroit, MI). All T-cell lines were maintained in vitro in medium containing IL-2 with alternate stimulation with the Ag, every 10–14 days as described (Ben-Nun and Lando, 1983). Proliferation assays of selected T-cell lines were performed exactly as previously described (Ben-Nun and Lando, 1983).

Table 1
Sequences of pβSync used in this study

Peptide	Sequence
pβSync1–24	MDVFMKGLSMAKEGVVAAAEKTKQ
pβSync12–36	KEGVVAAAEKTKQGVTEAAEKTKEG
pβSync24–48	QGVTEAAEKTKEGVLYVGSKTSGVV
pβSync36–60	GVLYVGSKTSGVVQGVASVAEKTKE
pβSync48–72	VQGVASVAEKTKEQASHLGGAVFSG
pβSync60–84	EQASHLGGAVFSGAGNIAAATGLVK
pβSync72–96	GAGNIAAATGLVKKEEFPTDLKPEE
pβSync84–108	KKEEFPTDLKPEEVAQEAAEEPLIE
pβSync92–110	LKPEEVAQEAAEEPLIEPL
pβSync96–120	EVAQEAAEEPLIEPLMEPEGESYED
pβSync96–108	EVAQEAAEEPLIE
pβSync108–133	EPLMEPEGESYEDSPQEEYQEYEPEA