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DNA vaccination efficiently induces antibodies to Nogo-A and does not exacerbate experimental autoimmune encephalomyelitis

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ABSTRACT

Antibodies against the neurite outgrowth inhibitor Nogo-A enhance axonal regeneration following spinal cord injury. However, antibodies directed against myelin components can also enhance CNS inflammation. The present study was designed to assess the efficacy of DNA vaccination for generating antibodies against Nogo-A and to study their pathogenic potential in a mouse model for multiple sclerosis. Mice were immunized by a single i.m. injection of a plasmid expression vector encoding either full length membrane-integral Nogo-A equipped with a signal peptide or two versions of its large N-terminal extramembrane region. The presence of serum antibodies to Nogo-A was measured 4 weeks after injection by ELISA, Western blotting and immunohistochemistry. DNA vaccination efficiently induced production of Nogo-A-specific antibodies that recognized recombinant, intracellular Nogo-A in cell culture but also stained native Nogo-A on the oligodendrocyte surface. Experimental autoimmune encephalomyelitis was induced in DNA-vaccinated mice by immunization with proteolipid peptide (a.a. 139–154). In contrast to vaccination with DNA encoding myelin oligodendrocyte glycoprotein that exacerbates this disease, Nogo-A DNA vaccination did not enhance clinical severity of disease. In summary, DNA vaccination is a simple and efficient method for generating an antibody response to Nogo-A. No pathogenicity was observed even during a full-blown inflammatory response of the central nervous system.

1. Introduction

The extremely limited ability of central nervous system (CNS) axons to regenerate following injury is in part attributed to factors present in CNS myelin that inhibit neurite outgrowth (Schwab, 2002; Schwab and Bartholdi, 1996). One such inhibitor is the myelin-associated protein Nogo-A, which was described simultaneously by three independent groups (Chen et al., 2000; GrandPre et al., 2000; Prinjha et al., 2000). The role of antibodies in neutralizing the inhibitory activity of Nogo-A is well documented, as the inhibition of neurite outgrowth by CNS myelin can be neutralized by both monoclonal and polyclonal Nogo-A-specific antibodies in vitro (Chen et al., 2000). In vivo, Nogo-A-specific antibodies show a strong therapeutic potential after spinal cord injury or stroke where their intrathecal application enhances axonal sprouting and functional recovery (Bregman et al., 1995; Merkler et al., 2001; Wiessner et al., 2003). Recently, these findings were extended to primates in which intrathecal infusion of Nogo-A-specific antibody was shown to enhance motor recovery after spinal lesion (Freund et al., 2006).

Alternatively to the infusion of recombinant antibodies, anti-Nogo-A antibodies may be generated by immunization (Merkler et al., 2003). An effective technique for inducing highly specific antibodies to myelin antigens is DNA vaccination. DNA vaccine delivery systems in many ways mimic the antigenicity of infectious organisms and have become one of the fastest growing fields in vaccine technology. Furthermore, vaccination with DNA encoding the antigen of interest is a technically simple and effective method of generating neutralizing antibodies (Boyer et al., 1997; Lodmell et al., 1998). Because DNA vaccination induces expression of target antigens by host cells, it can result in production of protein with native conformation and post-translational modifications that elicit antibodies of optimal specificity (Attanasio et al., 1997). The efficacy of DNA vaccination was demonstrated in a spinal cord injury model in which vaccination with DNA encoding myelin inhibitors enhanced axonal regeneration (Xu et al., 2004).

Abbreviations: CHO, Chinese hamster ovary; CNS, central nervous system; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein.

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Although the possibility of using Nogo-A-specific antibodies to promote axonal growth following CNS injury is tantalizing, one major concern is that anti-Nogo-A antibodies may disturb the structural integrity of CNS myelin and even initiate demyelination by antibody-dependent immune effector mechanisms. Indeed, antibodies to myelin components such as the myelin oligodendrocyte glycoprotein (MOG) can be highly pathogenic, causing severe clinical disease and extensive demyelination in animal models, and are implicated in the immunopathogenesis of demyelination in a subset of multiple sclerosis patients (Bourquin et al., 2003; Genain et al., 1999; Kerlero de Rosbo et al., 1990; Linington et al., 1988; Piddlesden et al., 1993). Importantly, the presence of MOG-specific B cells and antibodies in the periphery is benign, and mice fail to develop either spontaneous neurological disease or pathological evidence of demyelination. However, MOG-specific B cells both accelerate and exacerbate experimental autoimmune encephalitis, an autoimmune disease that reproduces many of the clinical and pathological features of multiple sclerosis (Bourquin et al., 2000; Litzenburger et al., 2000).

In this study, we examined the pathogenic potential of Nogo-A-specific antibodies during the clinical course of experimental autoimmune encephalomyelitis induced by an unrelated antigen. To induce a humoral immune response against Nogo-A, mice were vaccinated with plasmid DNA encoding either the N-terminal extramembrane fragment of Nogo-A or the complete amino acid sequence. We show that a single injection of Nogo-A cDNA efficiently induces antibodies to the gene product. Following an encephalitogenic challenge by the proteolipid protein peptide $PLP^{139-154}$, Nogo-A DNA vaccination does not enhance the clinical severity of experimental autoimmune encephalomyelitis. This is in strong contrast to the response induced by MOG-DNA vaccination, which exacerbates the clinical course of disease. These findings provide important information as to the use and safety of DNA vaccination for inducing Nogo-A-specific antibodies.

2. Materials and methods

2.1. Construction of expression plasmids

2.1.1. pSecNogo

An 850 bp 3' fragment of rat *nogo-A* cDNA was amplified by PCR from previously cloned *nogo-A* cDNA (Chen et al., 2000) with primers 5'-gcttagaattgccctgtgac-3' and 5'-gttcgcctcgagaatctgctttgcgcttcaatcc-3' in order to remove the stop codon and insert an XhoI site. The 5' 2.9 kb EcoRI fragment of *nogo-A* and the modified 3' fragment cut with EcoRI and XhoI were inserted into the expression vector pSecTag2C (Invitrogen AG, Basel, Switzerland) digested with EcoRI and XhoI. The construct encodes Nogo-A with an N-terminal Igκ-chain leader sequence and C-terminal myc and His tags. The integrity of the plasmid was verified by restriction analysis and sequencing.

2.1.2. $pNogo_{1-979}$

nogo-A cDNA cloned in the EcoRI site of pBluescript was partially digested with HindII and religated, resulting in the deletion of the last 558 base pairs of *nogo-A* that encode the transmembrane segments. This plasmid containing the truncated *nogo-A* ($nogo_{1-979}$) was digested with BamHI, filled in with Klenow, and digested with ApaI. The resulting fragment was cloned into pSecTag2A (Invitrogen) digested with EcoRV and ApaI. The Igκ leader sequence was deleted by digestion with NheI and SfiI and blunt ends were created with Klenow and T4 DNA polymerase. The plasmid was self-ligated and encodes the extramembrane N-terminal part of Nogo-A, with 3' myc/His tags and without the additional leader sequence.

2.1.3. $pSecNogo_{1-979}$-GPI

The GPI anchor recognition site of Thy1.1 was cut with HindIII and PmeI from pcDNA3.1BDNFmyc-Thy1 (gift of Dr. M. Hoener, Max-Planck Institute, Martinsried), blunt ends were created with Klenow. pSecTag-Nogo-Aext was cut with ApaI and PmeI and blunt ends were created with T4 DNA polymerase. These fragments were ligated and the orientation of the GPI anchor recognition site was checked by restriction analysis. The plasmid encodes the extramembrane N-terminal part of Nogo-A with the 5' Igκ leader sequence and is anchored to the membrane by a GPI anchor. All enzymes were purchased from Roche Diagnostics (Rotkreuz, Switzerland), except PmeI (New England Biolabs GmbH, Frankfurt am Main, Germany). DNA for vaccination was isolated from the transformed *E. coli* strain XL1-Blue with Wizard midiprep kits (Promega, Madison, WI).

2.1.4. pMOG

The full-length MOG coding sequence together with its signal sequence was cloned into the expression vector pcDNA3.1 (Invitrogen) as described previously (Bourquin et al., 2000). The plasmid pcDNA 3.1 was used as control DNA. DNA quality was checked by DNA electrophoresis and concentration was measured via absorption at 260 nm.

2.2. ELISA

Peripheral blood was collected by tail bleeding and, after coagulation, serum was obtained by centrifugation and stored at −20 °C. 96-well vinyl assay plates (Costar, Cambridge, MA) were coated either with 10 μg/ml of a recombinant N-terminal Nogo-A fragment comprising residues 233–940 (Fiedler et al., 2002) or of recombinant myelin oligodendrocyte glycoprotein (Bourquin et al., 2000) or myelin basic protein (both a gift from Dr. F. Kurschus, Max-Planck Institute for Neurobiology). After blocking with 1% (w/v) bovine serum albumin, the assay plates were incubated with the test serum. Specific binding was detected with alkaline phosphatase-conjugated goat anti-mouse IgG, IgG1 or IgG2a (Southern Biotechnology Associates, Birmingham, AL). Signal was developed by the addition of p-nitrophenyl phosphate and the absorption at 405 nm was measured on a MR-4000-ELISA-Reader (Dynatech, Embrach, Germany).

2.3. Cell culture and immunocytochemistry

Stable CHO-Nogo-A cells described previously (Chen et al., 2000) were cultured in MEM-alpha (Gibco, Invitrogen), supplemented with 5% fetal calf serum, 10 mM HEPES and 400 μg/ml G418. For immunocytochemistry, cells cultured on glass coverslips were fixed with 4% PFA, 5% sucrose in PBS for 20 min at room temperature. The cultures were permeabilized with 0.1% Triton X-100 and unspecific binding sites were blocked with 10% fetal calf serum. Mouse antisera were added as primary antibodies to the cells for 1 h, diluted 1:100 in PBS/ 0.1% Triton X-100. The cells were then washed, incubated with goat anti-mouse conjugated with TRITC (Jackson Immunoresearch Laboratories, PA) 1:200, washed and mounted on slides for fluorescence microscopy.

Optic nerve oligodendrocytes isolated from postnatal day 7 rats were cultured as described (Schwab and Caroni, 1988). For cell surface staining, four day-old cultures were incubated with the mouse antisera diluted 1:50 in culture medium for 30 min at room temperature, washed, and fixed. Cells were blocked with 0.1 M maleic acid with 2% (w/v) blocking reagent (Roche, Basel, Switzerland) for 1 h. Secondary goat anti-mouse antibodies conjugated with alkaline phosphatase (Milan Analytica, Lausanne, Switzerland) were used at 1:5000 in 0.1 M maleic acid with 1% (w/v) blocking reagent for 1 h at room temperature. The cultures were washed twice with maleic acid buffer, once with alkaline phosphatase buffer (0.1 M Tris–HCl pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$) and the staining was developed for 2–3 h at room temperature with 0.175 mg/ml BCIP (Sigma-Aldrich, Germany) and 0.338 mg/ml NBT (Sigma-Aldrich) in alkaline phosphatase buffer.