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Characterization of a new rat model for chronic inflammatory demyelinating polyneuropathies

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ABSTRACT

Our objective was to develop a chronic model of EAN which could be used as a tool to test treatment strategies for CIDP. Lewis rats injected with S-palmitoylated P0(180–199) peptide developed a chronic, sometimes relapsing–remitting type of disease. Our model fulfills electrophysiological criteria of demyelination with axonal degeneration, confirmed by immunohistopathology. The late phase of the chronic disease was characterized by accumulation of IL-17^{+} cells and macrophages in sciatic nerves and by high serum IL-17 levels. In conclusion, we have developed a reliable and reproducible animal model resembling CIDP that can now be used for translational drug studies.

1. Introduction

Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are autoimmune-mediated inflammatory diseases of the peripheral nervous system (PNS). They are thought to involve both cellular and humoral immunity that can be directed against specific components of the myelin sheath and/or the axon (Hughes et al., 2006; Vucic et al., 2009). GBS comprises several distinct subtypes with specific clinical, electrophysiological, and pathological features, namely: acute inflammatory demyelinating polyneuropathy (AIDP), the most frequent form in Europe and North America; acute motor axonal neuropathy (AMAN), the most prevalent form in China and Japan; acute sensory motor axonal neuropathy (ASMAN); and Miller Fisher syndrome (MFS). CIDP can present with a chronic progressive or relapsing–remitting course, and can be either a sensory or motor polyradiculoneuropathy causing weakness of proximal and distal muscles. It is the most common chronic autoimmune neuropathy, and is pathologically characterized by focal inflammatory demyelination followed by axonal degeneration (Vallat et al., 2010; Van den Bergh and Rajabally, 2013).

Most of the present knowledge about mechanisms involved in the pathogenesis of immune-mediated demyelination and putative autoantigens in GBS has been obtained through studies in the experimental autoimmune neuritis (EAN) animal model (Mäurer and Gold, 2002; Meyer zu Hörste et al., 2007), which involves active immunization of Lewis rats with peripheral myelin homogenates, myelin proteins, or peptides derived from those proteins; however this monophasic model really only mimics AIDP. Animal models of axonal subtypes have been generated by immunizing rabbits with gangliosides: they support the idea that antigangliosides antibodies cause axonal GBS (Yuki et al., 2001). This rabbit model has also been useful for the pathophysiological understanding of these subtypes of GBS, but is difficult to use to study treatment strategies. Up to now, very few mouse EAN models have been characterized (Xia et al., 2010), mainly because most mouse strains are resistant to EAN induction (Meyer zu Hörste et al., 2007).

CIDP is more difficult to mimic in animal models and in spite of active research in the field, no animal model for CIDP has reached common use (Salomon et al., 2001; Meyer zu Hörste et al., 2007; Soliven, 2012). A relevant chronic EAN model closely resembling CIDP (as shown by electrophysiologic and histologic data), has been described in rabbits following immunization with purified bovine PNS myelin

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(Harvey et al., 1987), but rabbits are less convenient to use in preclinical studies. More recently, spontaneous autoimmune polyneuropathy (SAP) in B7-2-deficient non-obese diabetic (NOD) mice, which has some similarities to the human disease and represents an alternative model of CIDP, has been characterized: in this model, the mice are protected from diabetes, and all the females exhibit limb paralysis with histologic and electrophysiologic evidence of severe demyelination in the peripheral nerves without spontaneous recovery (Salomon et al., 2001). Although the SAP model has provided some important information about potential immunopathogenic mechanisms of CIDP (Soliven, 2012; Ubogu et al., 2012), one drawback for its use in translational therapy strategies studies is its late onset (20 weeks) and slow progression. Thus, there is a need for new animal models of CIDP in order to obtain further insights into the immunopathogenic mechanisms and to allow testing of new immunotherapeutic strategies for human CIDP.

Previously we reported that thiopalmitoylation (S-palmitoylation i.e., the covalent attachment of palmitic acid via a thioester linkage to a cysteine residue in the polypeptide backbone) of peptides from the PNS myelin P0 protein enhanced their neuritogenic properties and active immunization of Lewis rats with thiopalmitoylated P0(180–199) peptide generated a relapsing–remitting type of disease in some animals (Beaino and Trifilieff, 2010).

In the present study we confirm that thiopalmitoylated P0(180–199) (S-palm P0) can induce a chronic and relapsing–remitting disease resembling CIDP, and we describe the clinical, histological, electrophysiological, and immunological features of this new chronic rat model in comparison to the classical monophasic EAN induced with P0(180–199), and discuss its possible relevance to human CIDP.

2. Materials and methods

2.1. Peptide synthesis

Peptide P0(180–199) (ACKRGRQTPVLYAMLDHSRS) was manually synthesized by solid phase-synthesis using the Fmoc/tBu strategy. To obtain S-palm P0 peptide [AC(palm) KRGRQTPVLYAMLDHSRS], thiopalmitoylation of residue Cys at position 181 was performed on the resin bound peptide after selective deprotection of the Cys(Mmt) side chain, as we have previously described (Beaino and Trifilieff, 2010). After cleavage from the resin, the crude peptides were lyophilized and purified by reverse phase HPLC. The purity of the peptides was assessed by analytical HPLC and their identities were confirmed by MALDI-TOF mass spectrometry.

2.2. Animals

Male Lewis rats (Charles River, L'Arbresele, France), 7–8 weeks old and weighing 230–250 g were used in the present study. All experiments were approved by the animal experimentation ethical committee of Université de Strasbourg, France.

2.3. Induction of EAN and assessment of clinical signs

Rats were anesthetized i.p. with ketamine chlorhydrate (37 μg/g)/xylazine (Rompun) (5.5 μg/g). Rats were then immunized with peptide P0(180–199) (n = 16) and S-palm P0(180–199) (n = 24), by s.c. injection at the base of the tail of 200 μL of an emulsion containing 200 μg of peptide and 0.5 mg of *Mycobacterium tuberculosis* (strain H37 RA, Difco, Detroit, Michigan, USA) emulsified in 100 μL saline and 100 μL incomplete Freund adjuvant (IFA) (Sigma-Aldrich, St. Quentin Fallavier, France). Two non-injected rats were used as negative controls and complete Freund adjuvant (CFA) injected rats (n = 8) were used as sham control. Body weights and clinical scores were assessed daily from day 0 until 74 days post-immunization (dpi). Severity of paresis was graded as follows: 0 = no illness; 1 = flaccid tail; 2 = moderate paraparesis; 3 = severe paraparesis; 4 = tetraparesis; 5 = death; intermediate scores of 0.5 increments were given to rats with intermediate signs.

2.4. Electrophysiological studies

Rats were anesthetized as described above, and the sensory nerve action potential (SNAP) was recorded on the caudal nerve using two needle electrodes (ALPINE-bioMed, NATUS France) inserted at the tail base, with the stimulating electrodes placed about 50 mm distally. Sciatic nerve motor conduction was assessed by examining the amplitude and the latency of the evoked compound muscle action potentials (CMAP). A stimulating needle electrode was inserted at the hip of the animal (proximal) or at the knee (distal) and two recording needle electrodes (Alpine-bioMed, Natus France) were inserted in the gastrocnemius muscle. Recordings were obtained on a differential amplifier DAM8 (WPI, London, UK) with a filter setting of 10 Hz to 10 kHz. The stimulus waveform (square shape stimuli, 0.1 ms duration) was generated with Clampex acquisition software (P-clamp8 software package, Axon Instruments, LA, US) and the signal was acquired through a Digidata 1224 (Axon Instruments, LA, US).

2.5. Immunohistochemical studies

Rats were deeply anesthetized with Ketamine/Rompun and perfused intracardially with 4 °C, 4% paraformaldehyde (PFA) in PBS. Left and right sciatic nerves, spinal nerve roots, brains and spinal cord were quickly removed and post-fixed overnight at 4 °C in Bouin (left sciatic nerve), periodate lysine-paraformaldehyde (PLP) (right sciatic nerve) or in 4% PFA (for the other organs) and then preserved in 4% PFA. Sciatic nerves and spinal nerve roots were cut in three segments and embedded in paraffin, serially sectioned (5 μm) and mounted on gelatin-covered slides.

After dewaxing, cross sections were heated at 80 °C for 10 min in citrate buffer. Endogenous peroxidase was inhibited with 0.02% H_2O_2 in water for 10 min. Non-specific binding sites were blocked with 5% fetal calf serum (Gibco Invitrogen, Camarillo, CA, USA) in PBS for 30 min and then with the following monoclonal antibodies: anti-MBP (1:50; produced in house) for CNS and PNS myelin; anti-P0 (1:4000; from Dr. Archelos, University of Würzburg, Germany) for PNS myelin; SMI-311 (1:1000; Abcam, Paris, France) for neurofilaments; ED1 (1:400; Serotec, Oxford, UK) for activated macrophages; CD3-clone G4.18 (1:100 on PLP-fixed sciatic nerve; BD Biosciences, Le pont de Claix, France) for T-cells; and anti-interleukin-17 (IL-17; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antibody binding to tissue sections was visualized with biotinylated anti-mouse IgG (1:200; Vectastain®, Vector Laboratories, Burlingame, CA, USA) and Avidin–Biotin-complex (ABC-peroxidase kit; Vectastain®, Vector Laboratories), followed by development with DAB substrate (Vector® DAB SK-4100, Vector Laboratories) for IL-17, and VIP substrate (Vector® VIP SK-4600, Vector Laboratories) for other antibodies.

Slides were viewed using a Nikon Eclipse E600® optical microscope connected to a Nikon® Digital Sight DS-Fi1 digital camera. Images were processed using Nikon NIS Elements® software supplied with the camera. Macrophage counting was performed on 3 rats per group and 3 cross-sections per rat in a region of interest of 330 × 430 μm per slide labeled with ED1 antibody. Results are given as mean values per mm^2. Myelinated fibers were divided in two groups according to their normal size distribution in control sciatic nerve (i.e. small fibers with a diameter < 6.5 μm and large fibers with a diameter > 6.5 μm) and counted as described above on MBP-labeled slides.

Fluorescent immunohistochemistry was performed on 5 μm cross-sections of sciatic nerve embedded in paraffin. After dewaxing, non-specific binding sites were blocked with 5% horse serum (Vector Laboratories) in PBS for 30 min at RT. Sections were then incubated for 1 h at RT with monoclonal anti-P0 (1:4000; from Dr Archelos) and rabbit anti-neurofilament 200 antibody (1:200; from Sigma-Aldrich). Antibody