Immunology Letters 185 (2017) 93–97

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

sNCAM as a specific marker of peripheral demyelination☆

Adam Niezgoda[a,*], Sławomir Michalak[b,c], Jacek Losy[c,d], Alicja Kalinowska-Łyszczarz[a], Wojciech Kozubski[a]

[a] Chair and Department of Neurology, Poznan University of Medical Sciences, Poznań, ul. Przybyszewskiego 49 Poznań, 60-355, Poland
[b] Department of Neurochemistry and Neuropathology, Poznan University of Medical Sciences, Poznań, ul. Przybyszewskiego 49, Poznań, 60-355, Poland
[c] Neuroimmunological Unit, Miroslaw Mossakowski Medical Research Center of the Polish Academy of Sciences, Poznań, ul. Przybyszewskiego 49, Poznań, 60-355, Poland
[d] Department of Neuroimmunology, Chair of Neurology Poznan University of Medical Sciences, Poznań, ul. Przybyszewskiego 49, Poznań, 60-355, Poland

ARTICLE INFO

Article history:
Received 20 October 2016
Accepted 15 March 2017
Available online 21 March 2017

Keywords:
Adhesion molecules
NCAM
Demyelinating polyneuropathy
Axonal polyneuropathy
Peripheral nerve inflammation
Electroneurography
ELISA immune assay
Overall Neuropathy Limitations Scale

ABSTRACT

Adhesion molecules are involved in nerve growth, synaptic plasticity and myelin formation and maintenance process. Neural cell adhesion molecule (CD56 or NCAM) seems to play a crucial role in all the above-mentioned events. Having found poly-sialylated NCAM increased re-expression on demyelinated axons within multiple sclerosis plaques we assessed soluble NCAM (sNCAM) in sera of patients with various types of peripheral nerve affections – demyelinating, axonal "inflammatory", axonal metabolic polyneuropathies and healthy controls. These data were compared with the clinical state using Overall Neuropathy Limitations Scale (ONLS) and nerve conduction studies. We found significantly increased sNCAM concentration in demyelinating polyneuropathies in comparison to axonal group and healthy controls as well as significantly increased sNCAM level in axonal group in comparison to healthy subjects. We also found high positive correlation between sNCAM and ONLS and strong negative correlation between sNCAM level and the lowest conduction velocity (V_{min}) found in a patient. We conclude that sNCAM might be thought as a specific marker of peripheral nerve demyelination and as a sensitive marker of peripheral nerve injuries.

1. Introduction

Adhesion molecules are involved in nerve growth, re-growth, synaptic plasticity, cognitive functions as well as in the process of myelin formation and its maintenance. Neural cell adhesion molecule (CD56 or NCAM) seems to play a crucial role in all the above-mentioned events.

NCAM is a member of the immunoglobulin (Ig) superfamily expressed on the surface of several types of neural cells and its ubiquitous presence in the nervous system has been documented across species [1]. It is involved in cell-to-cell interactions during brain development, regeneration and synaptic modeling [2–5]. NCAM can act homophilically with another NCAM and was first identified by this function [6]. It may also interact by heterophilical adhesion with other molecules like L1 [7], signal transduction molecules [8], cytoskeletal components [9], laminin [10].

Neural cell adhesion molecule is a glycoprotein coded by a 70 kb single gene localized by Cunningham et al. [11] to human chromosome 11; the molecule has an intracellular, transmembrane and extracellular portion. The final product of the NCAM gene becomes polysialylated in its extracellular part and may appear in more than 20 different isoforms (e.g. 120, 140 i 180 kDa). Like other adhesion molecules NCAM may appear as a soluble form (sNCAM) – which is cleaved of the cell surface from its cell-bound form.

It is generally believed that posttranslational polysialylation gives NCAM (PSA-NCAM) high flexibility in its extracellular portion made of five immunoglobulin and two fibronectin domains which is necessary for space orientation changes and close contact with other cells [12]. Polysialylation of NCAM is assured by two different enzymes. It is assumed that PSA chain increases elasticity, cell mobility or diffusion by decreasing NCAM–NCAM or NCAM-nonNCAM (e.g.NCAM-cytoskeleton) adhesion [13]; [14]. PSA-NCAM is present on immature non-myelinated axons and negatively correlates with the progress of myelination. As described Nait-Oumesmar et al. [15] it may reappear on denuded demyelinated axons, that is why it has been called the "young" or "embryonic" form of NCAM.

☆ This work was supported by Chair of Neurology, University of Medical Sciences, Poznań, Poland.
* Corresponding author.
E-mail address: adamniezgoda@wp.pl (A. Niezgoda).

http://dx.doi.org/10.1016/j.imlet.2017.03.011
0165-2478/

We showed using *in vitro* myelinating cultures that PSA-NCAM is involved in the process of (re)myelination and may be thought to slow down the repair of the myelin sheaths [16].

Soluble form of NCAM is present normally in blood and CSF in man, its concentration is physiologically more elevated in blood compared to CSF. Serum levels in the postnatal period and childhood are much higher than in the adults, with a progressive reduction starting from young adult age to elderly age [17]. It is generally admitted that concentration of the soluble form reflects the intensity of the cell-bound form production and expression on the cell surface.

Thomaidou [18] showed in 2001 that soluble forms of NCAM stimulate Schwann cell migration indicating that NCAM is also involved in the development, maturation and repair processes as well in the peripheral nervous system.

Carenini et al. [19] demonstrated that, in P0-deficient mice displaying a severe dysmyelinating phenotype in peripheral nerves, during formation of myelin sheaths, absence of NCAM caused transient delay of Schwann cell spiralling, whereas the absence of myelin associated glycoprotein (MAG) prevented Schwann cell from spiralling for a longer period. These data show that MAG and NCAM play important roles during myelin formation in the peripheral nervous system and that the functions of the molecules involved in the control of myelin maturation in PNS are partially overlapping.

2. Objective

The aim of the study was to assess possible involvement of the neural cell adhesion molecule (soluble NCAM, sNCAM) in the pathomechanism of polyneuropathy (PN).

3. Methods

We examined patients with various types of peripheral nerve involvement:

80 patients with demyelinating PN, 40 subjects with axonal non-diabetic PN, 20 patients with diabetic PN and 20 healthy controls.

The study was approved by the local ethics committee.

Group characteristics

We enrolled in the study:

A.) 80 patients with peripheral demyelinating neuropathy (Group A):
- 40 had acute demyelination fulfilling clinical, laboratory and electrophysiological criteria for Guillain-Barré syndrome of AIDP type [20], 23 men/17 women
- 40 patients were diagnosed as having CIDP (29) or MMN (11) (according to EFNS/PNS 2010 criteria), 27 men/13 women

B.) 40 patients with axonal sensori-motor "inflammatory" PN with increased cerebrospinal fluid (CSF) protein level (normal range 15–40 mg%) and without electrophysiological signs of demyelination; they will be called further "axonal PN group" (Group B): 21 men/19 women

C.) 20 patients with diabetes and sensori-motor "non-inflammatory" PN (CSF protein level within normal range) (Group C): 10 men/10 women

D.) 20 healthy controls; (Group D): 10men/10 women

3.1. Clinical assessment

We examined all the subjects clinically using overall neuropathy limitations scale (ONLS) [21]

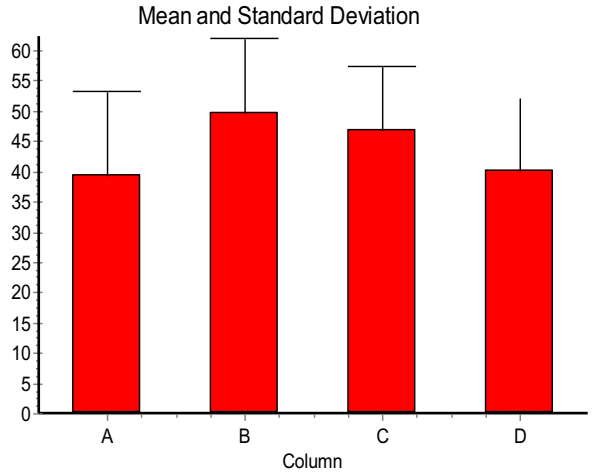

Fig. 1. demographic data – **age (years)** mean ± standard deviation (SD), range. A – demyelination (39,3 ± 13,9; 18–68); B – axonal polyneuropathy (49,5 ± 12,4; 20–69); C – diabetic polyneuropathy (46,8 ± 10,7; 28–65); D – healthy controls (40,3 ± 11,9; 22–61) Tukey-Kramer multiple comparisons test (A ↔ B $p < 0{,}01$; no statistically significant difference between B ↔ C, B ↔ D or C ↔ D $p > 0.05$).

3.2. Electrophysiological analysis

Electroneurographic assessment (ENG) was carried out by the same experienced neurologist using SYNERGY-VIASYS machine within 10 days of clinical onset for the acute demyelination group. Motor studies were performed at conventional sites on tibial, peroneal, median and ulnar nerve. Sensory studies included sural, superficial peroneal, median, ulnar and radial nerve. The amplitude of the negative phase was measured for compound muscle action potentials (CMAPs) and sensory nerve action potentials (SNAPs). In the group of demyelination the lowest value of motor conduction velocity (V_{min} m/s) in the most affected nerve was taken into consideration for further analysis. F waves were obtained using 15 stimulations and the shortest latency F response velocity was taken for the analysis.

3.3. sNCAM assessment

Concentration of sNCAM in sera was measured using ELISA assay (WuHan EIAAB Science Co Ltd). Sera were stored at −80 °C until the day of laboratory analysis.

Statistical analysis (distribution evaluation, comparison of means and correlation tests) was performed using GraphPad InStat software version 3.00; p value <0.05 was considered significant;

4. Results

4.1. Demographic data

The groups B, C and D did not differ significantly in age, significant difference was found between the group with demyelination (A) in comparison to axonal polyneuropathies (B) (Fig. 1).

The demographic structure of all the studied groups of patients was comparable in terms of age and gender, however demyelination had a tendency to occur in slightly younger patients (Fig. 1) (Fig. 2).

5. Clinical status

5.1. Serum sNCAM

We found significantly increased sNCAM concentration in the demyelination group in comparison to all the other groups