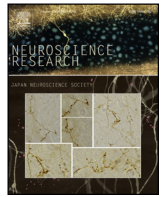
Neuroscience Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

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



A possible serologic biomarker for maternal immune activation-associated neurodevelopmental disorders found in the rat models

Arata Oh-Nishi [a,*,1], Kaori Koga [b], Tadakazu Maeda [c], Tetsuya Suhara [a]

[a] Department of Functional Brain Imaging, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Sciences and Technology, Chiba 263-8555, Japan
[b] Anatech Corporation, Tokyo 113-0034, Japan
[c] Professor Emeritus, Kitasato University, Kanagawa 252-0373, Japan

ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form 21 June 2016
Accepted 7 July 2016
Available online xxx

Keywords:
Maternal immune activation
Serologic biomarker
Ig light chain
Schizophrenia
Autism
Neurodevelopmental disorders

ABSTRACT

Epidemiological studies have shown that maternal infection during early pregnancy increases the risk of neurodevelopmental disorders (i.e., schizophrenia or autism) in offspring. Recently, diagnostic/stratification biomarkers for the maternal immune activation background in patients with neurodevelopmental disorders have been energetically searched for in the patient blood. Here, we report a novel serologic marker candidate for the disorders found in the maternal immune activation (MIA) rat model. Serum proteome analysis of the MIA rat showed that the immunoglobulin (Ig) light chain is reproducibly augmented. The Ig light chain in sera takes two forms — free form or bound to the Ig heavy chain. Only the former is an inflammatory disease marker, but pro-inflammatory cytokine levels in the sera of the MIA rats were below detectable limits of the ELISA protocol we used. We thereby carried out serum assays of Ig light chains and pro-inflammatory cytokines of commercially available schizophrenia patient sera for research. Although the number of samples was limited, we found augmentation of free Ig light chains but not pro-inflammatory cytokines in sporadic schizophrenia patient sera. Our findings suggest that Ig light chain assay of the schizophrenia/autism patient sera would be worthy to be validated in larger scale.

© 2016 Elsevier Ireland Ltd and Japan Neuroscience Society. All rights reserved.

1. Introduction

Emerging literature has provided epidemiological evidence that maternal immune activation (MIA) during the first half of gestation increases the risks of neurodevelopmental disorders including schizophrenia and autism in offspring, based on the fact that these increased risks are correlated not only with influenza infection at pregnancy (Barr et al., 1990; Brown et al., 2004; Kendell and Kemp, 1989) but also with other maternal infectious diseases (Blomstrom et al., 2012; Brown and Susser, 2002; Lee et al., 2015; Suvisaari et al., 1999) such as *poliovirus*, *rubella* and *T. gondii*. From this, we can infer that not a specific viral infection but MIA itself would increase the risks in offspring. It is estimated that 30% of schizophrenia cases could be prevented if certain prenatal infections were entirely eliminated from the pregnant population (Brown and Derkits, 2010; Cannon et al., 2014). MIA would be a common immune-related background for neurodevelopmental disorders in offspring. In fact, a number of studies have been published that searched for clues to diagnostic/stratification biomarkers of such disorders (Blomstrom et al., 2015; Tomasik et al., 2012; Vargas, 2014), but candidates for related diseases with immune-related background have not yet been fully found.

MIA of pregnant rodents can be induced by injection of synthetic polyriboinosinic-polyribocytidilic acid (Poly I:C), which mimics viral infections through Toll-like receptor 3 stimulation and causes their offspring to have permanent immune system disturbances and behavioral abnormalities (Hsiao et al., 2012, 2013; Meyer,

Abbreviations: MIA, maternal immune activation; Ig, immunoglobulin; Poly I:C, synthetic polyriboinosinic-polyribocytidilic acid; GD, gestation day; I.P., intraperitoneally; 2-DE, two-dimensional electrophoresis; CHAPS, 3-((3-cholamidopropyl)dimethylammonium)-1-propanesulfonate; DTT, dithiothreitol; IPG, immobilized pH gradient; BPB, bromophenol blue; KLH, keyhole-limpet hemocyanin; HRP, horseradish-peroxidase; IL-6, interleukin 6; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; κFLC, free immunoglobulin κ light chain; λFLC, free immunoglobulin λ light chain; FLCs, free Ig light chains; rFLC, κ-to-λ FLC ratio; MHC, major histocompatibility complex.

* Corresponding author. Fax: +81 043 253 0396.
E-mail addresses: arata.ohnishi@gmail.com, arata@resvo-inc.com (A. Oh-Nishi).
[1] Present address: RESVO Inc. 3-15-5-201, Omorikita, Ota-ku, Tokyo, 143-0016, Japan.

http://dx.doi.org/10.1016/j.neures.2016.07.003
0168-0102/© 2016 Elsevier Ireland Ltd and Japan Neuroscience Society. All rights reserved.

2013a,b; Meyer et al., 2005; Patterson, 2009). A non-human primate Poly I:C complex-induced MIA model has also presented alteration of social attention, abnormal behavioral development and neuropathology (Bauman et al., 2014; Machado et al., 2015; Weir et al., 2015). Although the total picture of the MIA model animal phenotype has still to be fully clarified, we herein suggest its usefulness in our quest for diagnostic/stratification markers of MIA-associated neurodevelopmental disorders.

In the present study, we explored a novel serologic MIA-associated marker candidates in the serum proteome of the rat model. To see whether we can find corresponding markers in human samples, we carried out a pilot study using sporadic schizophrenia patient sera for research.

2. Materials and methods

2.1. Experimental animal care

All experiments were performed in accordance with international guidelines on the ethical use of animals (NIH Guide for the Care and Use of Laboratory Animals) and the guidelines of the National Institute of Radiological Sciences in Chiba, Japan, and all efforts were made to minimize suffering and the number of animals used. The present study was approved by the Animal Ethics Committee of the National Institute of Radiological Sciences, Chiba, Japan. The rats were housed under controlled temperature and at a 12-h light/dark cycle with food and water ad libitum until the experiments.

2.2. Preparation of MIA rat

Female Wistar rats (Japan SLC, Shizuoka, Japan) were mated at 12–15 weeks of age. The first day after copulation was defined as gestation day 1 (GD1). Poly I:C potassium salt (Product No. P9582, Lot No. 012M4032V, Sigma–Aldrich, Tokyo, Japan) (4 mg/kg) dissolved in saline was injected into the pregnant Wistar rats intraperitoneally (I.P.) daily for 4 consecutive days from GD15 to GD18. Their neonatal- or mature-male offspring (postnatal days 3 or postnatal weeks 9–16) were used as MIA rats. We also injected saline (vehicle) into pregnant Wistar rats by the same procedures and their male offspring were used as controls. In the present study, all pregnant Wistar rats having been injected with Poly I:C potassium salt or with only saline could give birth to living pups (Poly IC treated dams: $N = 9$ and saline treated dams: $N = 7$). The number of offspring per dam were not significantly different between the two groups of dams (Poly I:C treated dam: 9 ± 1.11 and saline treated dam 9.5 ± 0.75 (mean ± SEM), $t(14) = 0.39$, $P = 0.69$ (Student's t-test)). All offspring were culled to 6–8 pups on postnatal day 3. Offspring were separated from their mothers 21 days after birth. We prepared 1–3 male offspring from each dam for each serum analyses.

2.3. Antipsychotic drug injection

The MIA rats (9 weeks postnatal) were injected intramuscularly (I.M.) with haldol-decanoate (Sumitomo Dainippon Pharma, Tokyo, Japan) (0.5 mg/kg) or propylene glycol (Vehicle) (Vincent et al., 1994). Sera were collected 3 weeks later.

2.4. Rat serum sampling

Blood was collected from deeply anesthetized rats with an overdose of pentobarbital (Kyoritsu Seiyaku Co., Tokyo, Japan) by cardiac puncture with 23-gauge needles attached to 2.5 ml syringes. Blood was incubated for 3 h at 4 °C and centrifuged for 40 min at 1000 × g at 4 °C. Supernatant sera were collected and stored at −80 °C until use.

2.5. Two-dimensional electrophoresis (2-DE) and image analysis

We carried out standard 2-DE as described previously (Koga and Minohata, 2011). An aliquot of a serum sample was mixed at a volume ratio of 1:1 with an extraction medium (7 M urea, 2 M thiourea, 2% 3-((3-cholamidopropyl)dimethylammonium)-1-propanesulfonate (CHAPS), 0.1 M dithiothreitol (DTT), and 2.5% Pharmalyte (pH 3–10)), and was homogenized using an ultrasonic disruptor (UR-20P; Tomy Seiko, Tokyo, Japan), followed by 20-min centrifugation at 15,000 rpm, 4 °C, using Centrifuge 3500 (Kubota, Gunma, Japan). The supernatant was collected and stored at −80 °C until use. An 18-cm-long dry immobilized pH gradient (IPG) gel strip (GE Healthcare, Tokyo, Japan) was rehydrated for 8–24 h at 20 °C in rehydration buffer (7 M urea, 2 M thiourea, 2% triton X-100, 13 mM DTT, 1% Pharmalyte (pH 3–10), 25 mM acetic acid, and 0.025% bromophenol blue (BPB)). The sample solution (0.054 ml) was applied to filter paper, which was placed on the cathodic edge of an immobilized pH gradient (IPG) gel strip. IPG gel strips were subjected to 1st dimension isoelectric focusing (IEF) electrophoresis in CoolPhoreStar IPG IEF Type PX (Anatech, Tokyo, Japan). After the 1st dimension IEF, the IPG gel strips were rinsed briefly in distilled water to remove silicon oil and were then equilibrated for 40 min at room temperature under gentle shaking in SDS treatment buffer (6 M urea, 32.5 mM DTT, 25% glycerol, 25 mM Tris–HCl (pH 6.8), 0.0025% BPB, and 2% SDS). The IPG gel strips were then placed on top of 12% SDS-polyacrylamide slab gels for 2nd dimension SDS-PAGE.

The 2nd dimension SDS-PAGE was carried out with CoolPhoreStar SDS-PAGE Dual 200 K (Anatech), after which the slab gels were stained with Flamingo fluorescent gel stain (Bio-Rad). FluoroPhoreStar 3000 (Anatech) was used for capturing fluorescent images of the Flamingo-stained slab gels. These fluorescent images were captured three times per gel, and were analyzed by Progenesis PG220 (Nonlinear Dynamics, Newcastle-upon-Tyne, UK). The integrated fluorescent intensity of a spot in a gel image was calculated after subtracting the lowest-on-boundary pixel intensity around the spot from the pixel intensity within the spot.

2.6. Identification of proteins by LC–MS/MS

We identified proteins in 2-DE gels by following a standard protocol with several modifications (Shevchenko et al., 2006). Flamingo-stained spots were excised from a 2-DE slab gel using FluoroPhoreStar3000 (Anatech). Excised gel pieces were submitted for protein identification by mass spectrometry, which were processed using a robot (ProGest, DigiLab, MA, USA) by the following protocol: the gel pieces were washed with 25 mM ammonium bicarbonate followed by acetonitrile, reduced with 10 mM DTT at 60 °C followed by alkylation with 50 mM 2-iodoacetamide at room temperature, and trypsin digestion (Promega) at 37 °C for 4 h. Finally, the samples were quenched with formic acid and the supernatant containing digested peptides was used for protein identification by mass-spectrometry without further processing. The experiments were performed by RelyOn Ltd. (Tokyo, Japan).

Our nano LC/MS/MS system was equipped with a Waters NanoAcquity HPLC system (Waters Corp., MA, USA) interfaced to a ThermoFisher Orbitrap Velos Pro (Thermo Fisher Scientific K.K., Kanagawa, Japan). Peptides were loaded on a trapping column of the nano LC and eluted over a 75-μm-diameter nano LC column at a flow rate of 350 nL/min; both columns were packed with Jupiter Proteo resin (Phenomenex, CA, USA). The mass spectrometer was operated in data-dependent mode, with MS performed in the Orbitrap at 60,000 FWHM resolution and MS/MS performed in LTQ™

Download English Version:

https://daneshyari.com/en/article/5739027

Download Persian Version:

https://daneshyari.com/article/5739027

Daneshyari.com