Rectracted: Anti-ribosomal-phosphoprotein autoantibodies penetrate to neuronal cells via neuronal growth associated protein, affecting neuronal cells in vitro

Shaye Kivity1, Yehuda Shoenfeld1, Maria-Teresa Arango1,2, Dolores J. Cahill3, Sara Louise O’Kane3, Margalit Zusev1, Inna Slutsky1, Michal Harel-Meir1, Joab Chapman5, Torsten Matthias6 and Miri Blank1

Abstract

Objective. Anti-ribosomal-phosphoprotein antibodies (anti-Ribos.P Abs) are detected in 10–45% of NPSLE patients. Intracerebroventricular administration of anti-ribosomal-P Abs induces depression-like behaviour in mice. We aimed to discern the mechanism by which anti-Ribos.P Abs induce behavioural changes in mice.

Methods. Anti-Ribos.P Abs were exposed to human and rat neuronal cell cultures, as well as to human umbilical vein endothelial cell cultures for a control. The cellular localization of anti-Ribo.P Abs was found by an immunofluorescent technique using a confocal microscope. Identification of the target molecules was undertaken using a cDNA library. Immunohistochemistry and an inhibition assay were carried out to confirm the identity of the target molecules. Neuronal cell proliferation was measured by bromodeoxyuridine, and Akt and Erk expression by immunoblot.

Results. Human anti-Ribos.P Abs penetrated into human neuronal cells and rat hippocampal cell cultures in vitro, but not to endothelial cells as examined. Screening a high-content human cDNA-library with anti-Ribos.P Abs identified neuronal growth-associated protein (GAP43) as a target for anti-Ribos.P Abs. Ex vivo anti-Ribos.P Abs bind to mouse brain sections of hippocampus, dentate and amygdala. Anti-Ribos.P Abs brain-binding was prevented by GAP43 protein. Interestingly, GAP43 inhibited in a dose-dependent manner the anti-Ribos.P Abs binding to recombinant-ribosomal-P0, indicating mimicry between the ribosomal-P0 protein and GAP43. Furthermore, anti-Ribos.P Abs reduced neuronal cell proliferation activity in vitro (P < 0.001), whereas GAP43 decreased this inhibitory activity by a factor of 7.6. The last was related to Akt and Erk dephosphorylation.

Conclusion. Anti-Ribos.P Abs penetrate neuronal cells in vitro by targeting GAP43. Anti-Ribos.P Abs inhibit neuronal-cell proliferation via inhibition of Akt and Erk. Our data contribute to deciphering the mechanism for anti-Ribos.P Abs’ pathogenic activity in NPSLE.

Key words: anti-ribosomal antibodies, autoantibodies, penetration, brain cDNA library, neuropsychiatric-systemic lupus erythematosus.

1 The Zabludowicz Center for Autoimmune Diseases, affiliated to Sackler Faculty of Medicine, Tel-Aviv University, Israel, *Doctoral Program in Biomedical Sciences, Universidad del Rosario, Bogota, Colombia, School of Medicine and Medical Sciences, Conway Institute of Biomedical and Biomolecular Research, University College Dublin, Belfield, Ireland, †Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, ‡Department of Neurology, Sagol Neuroscience Center, Sheba Medical Center, Tel-HaShomer, Israel and 5AESKU, KIPP Institute, Mikroforum Ring, Wendelsheim, Germany

Submitted 8 July 2015; revised version accepted 1 February 2016

Correspondence to: Yehuda Shoenfeld, Head of Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer 52621, Israel. E-mail: shoenfeld@post.tau.ac.il
Rheumatology key messages

- Anti-ribosomal-phosphoprotein antibodies penetrate into neuronal cells in vitro.
- Anti-Ribosomal-phosphoprotein penetration inhibits neuronal cells proliferation associated with inhibition of phosphorylation of Akt and Erk.
- Neuron-growth-associated-protein is a target for anti-Ribosomal-phosphoprotein antibodies binding to neuronal cells.

Introduction

In 1999 the ACR consensus document proposed that 19 different neurological and psychiatric cases of SLE patients be defined as NPSLE [1, 2]. Among several autoantibodies related to NPSLE [3], those directed to ribosomal-phosphoproteins (anti-Ribos.P Abs) were found to be specific to disease activity, especially psychosis and depression [4-7]. However, other reports failed to demonstrate this relationship [8], and other clinical associations with anti-Ribos.P Abs were found, such as in LN and hepatitis [9-11]. Anti-Ribos.P Abs target three highly conserved ribosomal phosphoproteins of molecular weights 38 kDa (P0), 19 kDa (P1) and 17 kDa (P2) on acidic 60S ribosomal protein P0 (accession number P05386) [12]. Epitope mapping shows that the major epitope is located mainly within the last 11 C-terminal amino acids [13, 14]. Previous studies demonstrated that intracerebro-ventricular passive transfer of affinity purified human anti-Ribos.P Abs into naïve mice induced autoimmune depression and smell deficits, and bound to the limbic system [15-17]. It was also shown that the anti-Ribos.P-mediated depression-like behaviour of the mice was significantly alleviated by specific anti-anti-Ribos.P (anti-idiotypic) Abs, as well as by long-term antidepressant therapy (fluoxetine) [15]. As the ribosomal phosphoproteins are located in the cytoplasm, the pathogenic effects of their cognate autoantibodies are still not deciphered. To assess the biological function of anti-Ribos.P Abs, one may assume that the intracellular ribosome is exposed to the circulation only if the cell is in an apoptotic/necrotic state, in which the intracellular clusters are modified and presented to the immune system. Koren et al. demonstrated the affinity of anti-Ribos.P Abs to bind proteins of the plasma membrane of different cell types, including neuroblastoma cells [18]. Later, the same group showed the antibody's ability to bind and penetrate human hepatoma cell lines, as well as inhibit their apolipoprotein B synthesis, causing cellular dysfunction [19]. In addition, Sun KH demonstrated the ability of anti-Ribos.P Abs to penetrate and induce apoptosis in Jurkett T cells [20]. Another group demonstrated the interaction of anti-Ribos.P Abs with a different antigen, a neuronal surface P antigen, located on the surface of hippocampal neurons. This interaction lead to impaired memory in mice accompanied by mice brain cell apoptosis, presumably by impairing glutamatergic synaptic transmission and neuronal plasticity [21, 22].

In the present study, we demonstrate the binding and penetration of human anti-Ribos.P Abs into rat hippocampal cells and human differentiated neuroblastoma neuronal cells, coupled with protein synthesis inhibition. Moreover, by exposing human anti-Ribos.P Abs to a high-content human brain protein array generated from a cDNA library we found the neuronal growth-associated protein 43 (GAP43) molecule to be a target for Anti-Ribos.P Abs.

Materials and methods

Antibodies

Human anti-Ribos.P Abs were affinity purified from a patient with NPSLE using a column composed of rabbit liver ribosomes, as previously described. Ribosomes were prepared by a published method from a freshly prepared rat liver [23]. The rat ribosome preparation precipitated strongly with anti-ribosomal P0-positive sera by gel diffusion. Rat ribosome solution (11 mg of protein/ml) was coupled to cyanogen bromide-activated Sepharose according to the manufacturer’s instructions (Pharmacia, Uppsala, Sweden). Sepharose (11 μg) was mixed with 10 mg of ribosomal protein in 12.5 ml of coupling buffer. Fifty-six percent of the added ribosomal protein was coupled to Sepharose. As control IgG, we used commercial IgG (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA, USA). Mouse anti-Ribos.P monoclonal Ab. had been generated by us previously [11].

Cell cultures

Primary hippocampal cells cultures were prepared from rat fetal hippocampus originating from pregnant rats. Dissected hippocampal tissue was digested with trypsin (1 mg/ml) for 10 min at 37°C. Cells were grown in neurobasal medium.

Human neuronal cell culture was prepared by differentiation of the human neuroblastoma cell line SH-SY-5Y with 10 μM retinoic acid over 10 days. Microtubule-associated-protein-2 served as a neuronal marker. Human umbilical vein endothelial cell primary cultures were purchased from Lonza (Lonza, Walkersville, MD, USA).

Screening a human brain high-content protein array

Based on a human fetal brain cDNA library (hEx1) expressing 37200 human proteins, we generated a protein array on a polyvinylidene fluoride membrane, which eventually became a chip containing a set of over 10,000 non-redundant human proteins for antibody screening and serum profiling. The screening of high-content human protein arrays (Imagenes, Berlin, Germany) was conducted as described previously [24, 25]. Briefly, after removal of desiccated bacterial colonies and blocking, the anti-Ribos.P antibody (10 μg/ml) was diluted 1:20 in 2% (w/v) bovine serum albumin (BSA) Tris-Buffered Saline (TBS)-Tween and was incubated on the high-content protein
Anti-ribosomal-P autoantibodies penetrate neurons

Fig. 1 Penetration of human and mouse anti-Ribos.P Abs into neuronal cells (confocal microscope analyses)

Human anti-Ribos.P-FITC Abs bind to human retinoic acid-differentiated neuroblastoma SH-SY-5Y cells at 10 min (A). One hour later, the binding signal was significantly reduced (B) (A and B ×100 magnification). Human anti-Ribos.P-FITC Abs bind first to the cellular surface (C); this was confirmed by staining of the cytoskeleton with Phalloidin (D), and DAPI nuclei staining (E). (F) shows merged image (C-E ×40).
arrays (hEx1) at a final concentration of 0.5 μg/ml in 2% (w/v) BSA TBS-Tween. After 20 h incubation at room temperature with slow agitation/rocking, the arrays were washed three times for 30 min each in TBS-Tween. The secondary antibody used was an alkaline-phosphatase conjugated anti-IgG (Sigma, Aldrich) diluted at a 1:5000 factor in 50 ml in 2% (w/v) BSA TBS-Tween and incubated for 1 h at room temperature. The arrays were then washed and equilibrated in AttoPhos buffer (100 mM Tris-HCL pH 9.5, 1 mM magnesium chloride) for 10 min, and then transferred to a 1:40 dilution of AttoPhos substrate (Roche) in AttoPhos buffer, incubated in the dark for 5 min and image-captured using the Fuji imager LAS 3000. The protein expression vector used for the cDNA library was pQE30NST (GenBankTM accession number AF074376) and the cDNA library was transformed into Escherichia coli strain SCS1 Stratagene [24]. The cDNA/protein expressing E. coli clones identified as positives on the high-content protein arrays following screening by the anti-Ribos.P antibody were identified using Visual Grid software (GPC biotech) and were sent for DNA sequencing. Sequence Analysis of Expression Clones-cDNA inserts were PCR-amplified and tag-sequenced as described previously. The sequences were searched against public databases (National Center for Biotechnology Information). GAP43 was expressed in 1 ml E. coli cultures in deep-well microtitre plates. The protein was extracted from the culture and purified.

**Immunohistochemistry**

**Anti-Ribos.P Abs binding to neuronal cell cultures**

Human anti-Ribos.P Abs or control human IgG (10–100 μg/ml) or mouse anti microtubule-associated protein (MAP-2 Abs (Jackson)) were added to the retinoic acid (Sigma) differentiated neuroblastoma cells or rat hippocampal cells. The cells were incubated with different antibodies on glass slides in 24-well plates for 0.5 h, 1 h, 2 h, 4 h at 37°C with 5% CO₂. In order to track the antibodies by confocal microscopy-analysis, the anti-Ribos.P Abs and control IgG were conjugated to fluorescein isothiocyanate (FITC) using a FluoroTag FITC Conjugation Kit (Sigma) according to the manufacturer’s instructions. Briefly, 1 mg of Immunoglobulin was incubated at a ratio of 5:1 with FITC in carbonate buffer pH 9 for 2 h at room temperature. Sephadex G-25M was used for gel-filtration of the unconjugated fluorescein. The elution of the labelled protein was done with PBS. The monolayer cells were washed with PBS and fixed with 4% formaldehyde (EMS Inc. Hatfield, PA, USA) in PO₄ buffer. F-actin was probed by 100 nM Phalloidin-Rodomin (Molecular Probes Inc. Eugene, OR, USA), whereas nuclei were stained with 4’,6-diamidino-2-phenylindole. Anti-Ribos.P Abs binding to mouse brain sections

Mouse brain cryosections (30–50 μm) were exposed to human anti-Ribos.P Abs (10 μg/ml PBS) ± pre-incubated with GAP43 (10 μg/ml) (Sigma) or control human IgG (10 μg/ml PBS) overnight at 4°C. Following washing and blocking with 5% horse serum, anti-human-IgG conjugated to Dy549 (Jackson) was added. Counterstaining was performed with 4’,6-diamidino-2-phenylindole.
performed with Hoechst. The experimental procedures were approved by the Israeli Ministry of Health Animal Welfare Committee.

Proliferation assay

The rate of neuronal cell proliferation was measured using a colorimetric assay and Bromodeoxyuridine incorporation. CHEMICON’s bromodeoxyuridine cell proliferation assay kit (Chemicon by Merck–Millipor, Darmstadt, Germany) was used according to the manufacturer’s instructions.

Hippocampal cells exposure to anti-Ribos.P Abs and immunoblotting

Rat hippocampal cells grown at 70% confluence were starved for 8 h in primary neuron growth medium supplemented with 2% fetal calf serum. Human anti-Ribos.P Abs or mouse anti-Ribos.P mAbs, or human control IgG or mouse control IgG were applied to the cultures at 20 μg/ml for 1–4 h. Next, the cells were lysed in lysis buffer [20 mM Tris–HCl (pH 7.5), 1% SDS, protease inhibitor cocktail and 0.2 mM phenylmethanesulfonyl fluoride (all the reagents were from Sigma)]. The protein content of the lysates was measured using the BCATM protein assay kit (Thermo Scientific, Rockford, IL, USA). The protein lysates were mixed with Laemmli sample buffer containing β-mercaptoethanol, boiled for 5 min at 95 °C and loaded on 10% SDS-PAGE gel. This was followed by the standard transfer procedure to polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 10% skim milk overnight at 4 °C. The following rabbit Abs (Santa Cruz Biotechnology Inc. Santa Cruz, CA, USA) were used for immunoblot analyses: Akt (1:500), phospho-Akt (ser 473 1:100), extracellular signal-regulated kinase (ERK) (1:1000), phospho-ERK (1:1000). Following 4 h incubation at room temperature, the membrane was probed for 1 h with anti-rabbit-IgG-HRP or GAPDH-HRP (1:200). Finally, the proteins were visualized by chemiluminescence (Santa Cruz) using Kodak BioMax film. Between each exposure to a specific Ab, the membranes were stripped.

Statistical analysis

The Shapiro–Wilk test was performed to evaluate the normality of the data, followed by unpaired Student’s t test to identify differences between groups. The analyses were done using SPSS 17.0 statistical software. Results were expressed as mean (±S.D.), and P < 0.05 was considered significant.

Results

Human anti-Ribos.P Abs penetrate neuronal cells

We used two types of cultured neuronal cells: rat primary hippocampal cells and retinoic acid–differentiated SH-SY-5Y neuroblastoma cells. HUVEC cells were used as control cells. Human affinity purified anti-Ribos.P Abs from an NPSLE patient and control IgG were applied to the cultures. After 10 min of incubation, human anti-Ribos.P-FITC Abs demonstrated direct binding to human neuronal cells (Fig. 1A and C). This binding decreased within 1 h (Fig. 1B). Fig. 1D demonstrates F-actin stained by Phalloidin, while Fig. 1E shows nuclei stained by 4′,6-diamidino-2-phenylindole. We raised the possibility that anti-Ribos.P may also penetrate the cells. Therefore, the intracellular localization of anti-Ribos.P Abs was evaluated after 30 min of incubation, with a confocal microscope analysing in-depth 0.5-μm-thick sections.

Fig. 3 Mouse anti-Ribos.P-FITC monoclonal Abs’ penetration into rat hippocampal cell

Mouse anti-Ribos.P-FITC monoclonal Abs bind to rat hippocampal neuronal cells at 0.5 h (A and C), merged with f-actin and nuclei (B and D). Thirty minutes later, penetration is demonstrated (E and G) merged (F and H). C, D, G and H are cross-sectional pictures. Notice in H the presence of anti-Ribos.P Abs in the cytoplasm and close to the nucleus. Control commercial mouse IgG-FITC did not show any specific binding (I merged J). Green: mouse anti-Ribos.P IgG detected with anti-mouse IgG labelled with FITC; blue: DAPI staining of the nuclei; red: f-actin-cytoskeleton stain by Phalloidin.
The confocal microscope demonstrated the penetration of anti-Ribos.P Abs into human retinoic acid differentiated neuroblastoma SH-SY-5Y cells, (Fig. 2A). Intracellular presence of the immunoglobulin deposits was detected deep in the cell, mainly in sections C through E. Data presented in Fig. 2J-R demonstrate the penetration of human anti-Ribos.P-FITC Abs into cultured rat hippocampal cells within 30 min. Enhanced signal of anti-Ribos.P-FITC was noticed in Fig. 2L. For a positive control, we used mouse anti-Ribos.P-FITC monoclonal antibodies (mAbs) directed to the last 22 amino acids of the carboxyl-terminal site of the 60S ribosomal subunit. These Abs bound and penetrated into the rat hippocampal cells during the 30 min of incubation (Fig. 3). GAP43 is a target protein for human anti-Ribos.P Abs on neuronal cells.

The confocal microscope demonstrated the penetration of anti-Ribos.P Abs into human retinoic acid differentiated neuroblastoma SH-SY-5Y cells, (Fig. 2A). Intracellular presence of the immunoglobulin deposits was detected deep in the cell, mainly in sections C through E. Data presented in Fig. 2J-R demonstrate the penetration of human anti-Ribos.P-FITC Abs into cultured rat hippocampal cells within 30 min. Enhanced signal of anti-Ribos.P-FITC was noticed in Fig. 2L. For a positive control, we used mouse anti-Ribos.P-FITC monoclonal antibodies (mAbs) directed to the last 22 amino acids of the carboxyl-terminal site of the 60S ribosomal subunit. These Abs bound and penetrated into the rat hippocampal cells during the 30 min of incubation (Fig. 3). GAP43 is a target protein for human anti-Ribos.P Abs on neuronal cells.

To find out which protein(s) can be recognized by the human anti-Ribos.P Abs, we incubated the Abs against a high-content human protein array derived from a human brain cDNA library. The most frequent proteins were ribosomal-P0 and the axonal membrane protein: neuronal GAP43. We verified these findings by testing the capacity of GAP43 to inhibit human anti-Ribos.P Abs binding to the recombinant ribosomal-P0. The inhibition assay (Fig. 5A) showed that GAP43 attenuated the binding of human anti-Ribos.P Abs to recombinant ribosomal-P0 (rRibos.P) (38 KDa) by 84 ± 7% (P < 0.001) at a concentration of 40 μg/ml, whereas rRibosomal-P0 decreased the binding by 96 ± 8% (P < 0.001) at the same concentration. Control IgG pre-incubated with rRibosomal-P0 + GAP43 caused inhibition of anti-Ribos.P Abs binding to rRibos.P by 8 ± 2%. β-2-glycoprotein-I (45 KDa) was used as a control inhibitor and had no significant effect on the anti-Ribos.P Abs binding to rRibos.P (P > 0.05).
Likewise, human-anti-Ribos.P Abs binding was tested ex vivo on brain sections from C3H-naïve mice, and the results are shown in Fig. 5. Human Anti-Ribos.P Abs bound mouse brain in different brain regions; in particular hippocampus CA3 and CA1, dentate gyrus and amygdala (Fig. 5B, D, F and H), whereas GAP43 prevented the human anti-Ribos.P antibody binding in the same areas (Fig. 5C, E, G and I). Human control IgG did not bind the brain sections (data not shown).

**Human anti-Ribos.P Abs inhibit the proliferation of neuronal cells**

We demonstrated the penetration of human anti-Ribos.P Abs into rat hippocampal cells and into human neuroblastoma differentiated neuronal cells. We therefore assessed the effect of the anti-Ribos.P Abs inside the neuronal cells. We found that anti-Ribos.P Abs inhibit the proliferation of neuronal cells in a dose-dependent manner (Fig. 6A). At 20 μg/ml, human anti-Ribos.P Abs inhibited the proliferation of the neuronal cells by 69 ± 7%, while control IgG had no effect on the neuronal cell proliferation activity (inhibition of 6 ± 2%) at the same Ab concentration (P < 0.001). Moreover, pre-incubation of human anti-Ribos.P Abs with GAP43, reduced the inhibitory properties of anti-Ribos.P by 7.6 times (P < 0.001). No significant difference in the percentage of inhibition of proliferation was noticed between the control IgG and anti-Ribos.P Abs pre-incubated with GAP43 (P > 0.05).

**Ribos.P Abs dephosphorylate Erk and Akt signaling pathways**

Following inhibition of hippocampal cell proliferation by anti-Ribos.P Abs, we studied the involvement of Akt and Erk/MAPK expression (which are related to the survival and cell function) upon treatment with anti-Ribos.P. In vitro exposure of the neuronal cells to human or mouse anti-Ribos.P Abs resulted in significant dephosphorylation of Erk and Akt (Fig. 6B and D). As illustrated in Fig. 6B, human anti-Ribos.P Abs dephosphorylated Akt by 48%, whereas mouse anti-Ribos.P mAbs dephosphorylated Akt by 52%, P < 0.0003 in comparison with exposure to the relevant control IgG. Likewise, significant dephosphorylation of Erk/MAPK was documented when the cells were exposed to human and mouse anti-Ribos.P Abs, P < 0.001.

**Discussion**

In this study we demonstrated the ability of anti-Ribos.P Abs to penetrate, in vitro, live human neuroblastoma differentiated neuronal cells, and to penetrate primary rat hippocampal cultured cells. Furthermore, an in-depth analysis using a confocal microscope demonstrated anti-Ribos.P Abs (human and mouse) in the cytoplasm and near the nucleus of live neuronal cells. In contrast, binding of anti-Riboso.P Abs, without penetration, was demonstrated after exposure to HUVEC cells. In addition, human anti-Ribos.P Abs screened against a high-content human brain protein array generated from a cDNA library [24, 25] revealed that the neuronal GAP43 was a target.
molecule for these Abs. Cross-reactivity between ribosomal-P0 and GAP43 was proven by an ELISA inhibition assay.

Since the first report that anti-ribonucleoprotein IgG can penetrate a living cell via Fcγ receptor [26], many other autoantibodies with different specificities/activities have been reported to penetrate cells through various pathways [18, 20, 27, 28]. For example, several natural anti-DNA Abs translocate across the plasma membrane and localize in the nucleus of mammalian cells, inducing caspase-mediated apoptosis through catalytic hydrolysis of DNA [27]. Anti-Rib.P Abs have been shown to cause cell dysfunction, following penetration of several cell lines in vitro [20, 28]. However, the membrane molecule through which the anti-Ribos.P penetrates has not yet been deciphered. In our current study, screening the anti-Ribos.P Abs against a high-content human brain protein array generated from a human brain cDNA library revealed that neuron growth protein GAP43 is bound by the studied immunoglobulins.

GAP-43 is an abundant protein in axonal growth cones of developing and regenerating neurons, as well as in presynaptic terminals. It is an intrinsic determinant in the establishment and reorganization of synaptic connections and plays an important role in guiding the growth of axons and modulating the formation of new connections. Experimental and neuropathological conditions may alter GAP43 expression [29], and aberrant GAP43 expression may affect axonal growth and neuroplasticity [30]. Our data raise the possibility that anti-Ribos.P Abs may also alter the biological function of GAP43. Experiments investigating learned helplessness in rats, or depression
in monkeys, have demonstrated that hippocampal GAP43 dysfunction may play a role in the pathophysiology of depression [31, 32].

Our results suggested that the penetration of anti-Ribo.P Abs may affect different signal transduction pathways. These pathways may include the MAK kinase or Erk and Akt signalling pathways, which are involved in neurogenesis, self-renewal and proliferation of neuronal cells [33–35]. We found that the anti-Ribo. P Abs attenuated the level of phosphorylated Erk and Akt, leading to an arrest of the hippocampal cells. Notably, in the context of NPSLE and depression, loss of hippocampal neuronal plasticity is related to changes in hippocampal volumes in patients with mood disorders [36]. Several studies showed decreased signalling and activity of Erk and Akt in post-mortem analyses of depressed subjects [37]. Interestingly, treatment with various antidepressants has restored Erk and Akt signalling [37].

It should be mentioned that several autoantibodies have been demonstrated in the serum and cerebrospinal fluid of NPSLE patients, and may play a role in its pathogenesis [38, 39]. Diamond et al. [40, 41] demonstrated that anti-dsDNA Abs can recognize the N-methyl-o-aspartate receptors NR2a and NR2b. The anti-DNA/NR2 Abs were shown to cause hippocampal neuron damage, associated with apoptotic cell death and memory loss in experimental mice [42]. Another example is anti-endothelial cells Abs being associated with psychosis and mood disorders in SLE patients [43]. It is suspected that anti-endothelial cell Abs are pathogenic via several mechanisms, including activation of the endothelial cells, induction of coagulation pathways or induction of apoptosis through the binding of phospholipids or HSPs [44, 45].

Past studies suggest that anti-idiotype anti-Ribo.P Abs are part of an idiotypic network that regulates anti-ribosomal P Ab expression, as well as pathogenicity in mice and humans [15, 46]. These findings are similar to other anti-idiotype studies, such as in experimental antiphospholipid antibody syndrome, which has been treated with anti-β2 glycoprotein-I Abs, preventing thrombosis [47]. Thus, it has been proposed that anti-idiotype Abs may be used in the future to prevent NPSLE symptoms.

A tenable hypothesis regarding the role of anti-Ribo.P Abs in the pathogenesis of NPSLE is based on our current data of intraneuronal anti-Ribo.P Abs penetration, followed by decreased hippocampal cell proliferation, as well as past findings about the activity of anti-Ribo.P Abs to induce depression in mice [15] and about altered GAP43 expression in the ratHelplessness model. The course of events in humans is hypothesized to include perturbation of the BBB, penetration of anti-Ribo.P Abs into the brain, and binding to neurons through GAP43, leading to dysfunction of neuronal cells. Likewise, we propose use of a novel therapeutic soluble GAP43 in order to neutralize anti-Ribo.P Abs, to block their binding to membrane GAP43, preventing their penetration of the intracellular compartment. This has potential as a unique approach to the treatment of NPSLE.

Acknowledgements

This study is a part of Maria-Teresa Arango (MTA) Doctoral Program in Biomedical Sciences, Universidad del Rosario, Bogotá, Colombia, and The Center for Autoimmune Disease Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia. We would like to thank Judy and Stewart Colton from the Colton Foundation for funding this project as part of the doctoral program of MTA.

Funding: This study was supported partially by The Dr Pinchas Borenstein Talpiot Medical Leadership Program 2013; Sheba Medical Center, Tel-Hashomer, Israel and P6 E-ERA European grant on rare autoimmune diseases 4790.

Disclosure statement: D.J.C. is a co-founder and shareholder of Protein AG, Germany. All other authors have declared no conflicts of interest.

References


11 Ben-Ami Shor D, Blank M, Reuter S et al. Anti-ribosomal-P antibodies accelerate lupus glomerulonephritis and induce...


Anti-ribosomal-P autoantibodies penetrate neurons

RETRACTED