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

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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 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 the 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.

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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 P05388] [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 naive 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 anti-depressant 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 ability of anti-Ribos.P Abs to bind to neuronal cells of different cell types, including neuroblastoma cells [18, 19]. 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 Jurkat 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 leads to impaired memory in mice accompanied by mice brain cell apoptosis, presumably by impairing glutamatergic synaptic transmission and neural 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 saline-perfused 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 11 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 were cultured 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 polystyrene micro array plate, 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 (Imagenses, 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 array. The array was washed with Tris-buffered saline (TBS) Tween. After washing, a secondary antibody was added to the array, and the array was washed again with TBS-Tween.
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 were 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 cell cultures or rat hippocampal cells. The cells were incubated with the 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 9.5 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 0.5 ml/well for 10 min. Following washing with 0.1% Triton X-100 in PO4 buffer, the cultures were blocked for 1 h with 5% horse serum, 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 done with 4’,6-diamidino-2-phenylindole.

**Fig. 2 Human anti-Ribos.P Abs penetration into neuronal cells in vitro**

The intracellular localization of anti-Ribos.P-FITC Abs was followed for 30 min by microscopic sections (0.5-μm in-depth intervals) in two different kinds of cells: human retinoic acid-differentiated neuroblastoma SH-SY-5Y cells (A and G) and rat hippocampal cells (J–P). The intracellular presence of the immunoglobulin deposits was detected mainly in the C, D and E sections (SH-SY-5Y cells) and in the L, M, N and O sections (Rat hippocampal cells). Pictures H and Q show the F-actin-cytoskeleton (Phalloidin staining) and the nucleus (DAPI staining). Pictures I and R illustrate the 3D staining. The human anti-Ribos.P-FITC IgG Abs were affinity-purified from neuropsychiatric lupus patients and labelled with FITC.
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 BCA™ 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
The confocal microscope demonstrated the penetration of anti-Ribos.P Abs into human retinoic acid/C150-differentiated neuroblastoma SH-SY-5Y cells, (Fig. 2A/I). 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/O. 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). Fig. 3A shows the binding (B, merge) and Fig. 3E the penetration (F, merge). The penetration was confirmed by colocalization of anti-Ribos.P-FITC Abs in a cross-section to be in the cytoplasm and close to the nucleus, as depicted in Fig. 3H. Control commercial mouse IgG-FITC did not show any specific binding, as shown in Fig. 3I (J, merge). The specificity of anti-Ribos.P Abs binding was tested by exposure of the HUVEC to human-anti-Ribos.P-FITC Abs, which showed binding, but not penetration into the cells after 2 h incubation time (Fig. 4A and B). In addition, the control IgG did not bind to HUVEC cells (Fig. 4C and D).

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 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. 5A. 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).

Anti-ribosomal-P autoantibodies penetrate neurons

Fig. 5 GAP43 inhibits the binding of anti-Ribos.P Abs to ribosomal proteins in vitro (A) and to brain sections ex vivo (B–I).

GAP43 and recombinant ribosomal-P (rRibos-P) each inhibit the binding of anti-Ribos.P Abs to the protein rRibos.P, whereas l-2glycoprotein-I (l2GPI) did not inhibit this binding. Control IgG with GAP43 did not bind to rRibos.P. The data are mean (±S.D.) of three repeated experiments (A). GAP43 inhibited human anti-Ribos.P Abs binding to brain sections, as illustrated by immunohistochemistry. The sections represent the CA3-hippocampus (B and C), dentate gyrus (D and E), CA1 hippocampus (F and G) and amygdala (H and I). On the left column (B, D, F and H), anti-ribos.P Abs bind to the areas mentioned, while GAP43 inhibited this binding, right column (C, E, G and I). The nucleus was identified using Hoechst staining, whereas the binding of human anti-Ribos.P IgG was done using Goat-anti-human IgG 549 conjugated.

Anti-ribosomal-P Abs dephosphorylate Erk and Akt signalling 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 dephosphorylate Akt by 48%, whereas mouse anti-Ribos.P mAbs dephosphorylate 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, 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 Fc 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. GAP43 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...
Anti-ribosomal-P autoantibodies penetrate neurons

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-Ribos.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 other 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-idiotypic anti-Ribo.P Abs are part of an idiotypic network that regulates anti-Ribo.P Ab expression, as well as pathogenicity in mice and humans [15, 46]. These findings are similar to other anti-idiotypic 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-idiotypic 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 Ab penetration, followed by decreased hippocampal cell proliferation, as well as past findings about the ability of Anti-Ribo.Ps to induce depression in mice [15] and about altered GAP43 expression in the rat helplessness 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.

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Shaye Kivity et al.


