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Expression of *N*-Methyl-D-Aspartate Receptor Subunits in the Bovine Ovum: Ova as a Potential Source of Autoantigens Causing Anti-NMDAR Encephalitis

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Autoimmune synaptic encephalitis is characterized by the presence of autoantibodies against synaptic constituent receptors and manifests as neurological and psychiatric disorders. Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is such an autoimmune disorder that predominantly affects young women. It is associated with antibodies against the extracellular region of the NR1 subunit of postsynaptic NMDAR. Each NMDAR functions as a heterotetrameric complex that is composed of four subunits, including NR1 and NR2A, NR2B, or NR2C. Importantly, ovarian teratoma is a typical complication of anti-NMDAR encephalitis in female patients and may contain antigenic neural tissue; however, antigenic sites remain unknown in female patients without ovarian teratoma. The purpose of this study was to investigate the expression of NMDARs in the ovum. We detected NR1 and NR2B immunoreactivity in protein fractions extracted from the bovine ovary and ova by SDS-polyacrylamide gel electrophoresis and immunoblotting analysis. Immunoprecipitates digested with trypsin were analyzed by reverse phase liquid chromatography coupled to tandem mass spectrometry. We obtained the following five peptides: SPFGRFK and KNLQDR, which are consistent with partial sequences of human NR1, and GVEDALVSLK, QPTVAGAPK, and NEVMSSK, which correspond to those of NR2A, NR2B and NR2C, respectively. Immunocytochemical analysis revealed that the bovine ovum was stained with the immunoglobulin G purified from the serum of a patient with anti-NMDAR encephalitis. Taken together, we propose that the normal ovum expresses NMDARs that have strong affinity for the disease-specific IgG. The presence of NMDARs in ova may help explain why young females without ovarian teratomas are also affected by anti-NMDAR encephalitis.

Keywords: glutamate receptor; limbic encephalitis; *N*-methyl-D-aspartate receptor; oocyte; ovarian teratoma
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Introduction

Paraneoplastic and non-paraneoplastic autoimmune encephalitis are characterized by the presence of autoantibodies against neuronal surface membrane antigens (Lancaster et al. 2011). Autoimmune synaptic encephalitis has recently been described as a brain disease in humans that leads to neurological and psychiatric disorders through autoantibodies against synaptic constituent receptor proteins (Moscato et al. 2010). The representative disorder is anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis (Dalmau et al. 2007). The target molecules in anti-NMDAR encephalitis are NMDARs, which are ionotropic glutamate receptors (GluRs) located on the surface of postsynaptic membranes. NMDARs are heterotetrameric complexes composed of four subunits derived from three related families, NR1, NR2, and NR3 subunits, which include seven subtypes: NR1, NR2A, NR2B, NR2C, NR2D, NR3A,

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and NR3B (Nakanishi 1992; Cull-Candy et al. 2001; Furukawa et al. 2005). The NR1 subunit contains a glycine-binding site (Kuryatov et al. 1994; Hirai et al. 1996; Furukawa et al. 2005), while the NR2 subunit contains a (S)-glutamate-binding site (Laube et al. 1997; Anson et al. 1998; Furukawa et al. 2005). The well-characterized glutamate- and glycine-responsive NMDAR requires both NR1 and NR2 subunits. Recent studies have reported that functional NMDARs generally form as heterotetramers of two NR1 and two NR2 subunits (for example, NR1/NR1/NR2A/NR2A, NR1/NR1/NR2B/NR2B, NR1/NR1/NR2A/NR2B and NR1/NR1/NR2C/NR2C) (Laube et al. 1998; Furukawa et al. 2005). Assembly of the receptor is thought to proceed via a "dimer-of-dimer" mechanism that can respond to functional and spatiotemporal diversities of NMDARs (Kutsuwada et al. 1992; Watanabe et al. 1992; Schorge and Colquhoun 2003; Papadakis et al. 2004; Furukawa et al. 2005; Schüler et al. 2008). The NR2D subunit is expressed only during prenatal development (Monyer et al. 1994). Although NR3 subunit can complex with NR1 subunit to form a glycine-responsive excitatory receptor, it does not bind glutamate (Chatterton et al. 2002). Initially, anti-NMDAR encephalitis, which is potentially lethal, manifests clinically as a range of psychiatric symptoms, and subsequently as intractable seizure disorders, dyskinesia, autonomic instability or hypoventilation (Dalmau et al. 2007, 2008; Iizuka et al. 2008). An anti-neural antibody for the NR1/NR2 heteromer of NMDARs has been identified as a disease-specific hallmark (Vitaliani et al. 2005; Dalmau et al. 2008). Another feature of anti-NMDAR encephalitis is that it predominantly affects women of reproductive age (Kamei et al. 2009), and is often accompanied by ovarian teratoma (Dalmau et al. 2007, 2008). Mature- and immature-appearing neurons in ovarian teratoma ectopically express NMDARs (Sansing et al. 2007; Seki et al. 2008; Tüzün et al. 2009); this is thought to contribute to the production of anti-NMDAR antibodies. Therefore, anti-NMDAR encephalitis has been considered to be a type of paraneoplastic encephalitis (Vitaliani et al. 2005; Dalmau et al. 2007). However, based on recent reports and the rapidly increasing number of patients with anti-NMDAR encephalitis (Prüss et al. 2010; Dalmau et al. 2011), it has become clear that this encephalitis also affects children and infants (Florance et al. 2009; Hacohen et al. 2013). Furthermore, among the female patients with anti-NMDAR encephalitis, only about half were associated with ovarian teratoma (Irani et al. 2010; Titulaer et al. 2013). Additionally, about 11% of 577 patients enrolled in a multi-institutional cohort observation study (Titulaer et al. 2013) were male (Eker et al. 2008), and the vast majority of these male patients were found to have no underlying neoplasm (Novillo-López et al. 2008; Wong-Kisiel et al. 2010; Tojo et al. 2011). Thus, the mechanism underlying the production of anti-NMDAR antibodies remains unclear.

In our previous immunohistochemical studies (Tachibana et al. 2010, 2013), we reported the expression of an NR2B epitope in the normal human ovary, but failed to show the expression of NR1 and NR2A epitopes. Demonstrating the presence of NMDARs is necessary to provide evidence that normal ovaries contain NR1, NR2A and NR2B, the three major epitopes of NMDAR. However, we do not want to use human ovaries and ova in this study from an ethical point of view. Instead, we attempted immunocytochemical and proteomic identification of NMDAR subunits in a post-mortem bovine ovary and degenerated ova. Amino acid sequences compared between human and bovine NMDARs show 89-96% homology (The UniProt Consortium 2015), suggesting that bovine and human NMDARs have the same function (Zimin et al. 2009). Immunoglobulin G (IgG) purified from the serum of a patient with anti-NMDAR encephalitis has also been shown to bind strongly to the cell membrane of bovine ova. Based on the above, in the pathogenesis of young females with anti-NMDAR encephalitis and no concomitant ovarian teratoma, the normal ovary may play an important role in initiating NMDAR-related antigen-antibody production in areas outside of the brain.

Materials and Methods

The cattle used in the study all died of natural causes before autopsy; all autopsies were performed by an experienced veterinary pathologist (Y.U.). This study was carried out in accordance with the guidelines approved by the Animal Research Committee of Azabu University, and all study procedures strictly adhered to Japanese law. An experienced veterinarian (Y.K.) collected the unfertilized or degenerated bovine ova, the use of which was approved by the Ethical Committee of the Nagano Public Animal Industry Station. The study protocol was approved by the Ethical Committee of Shinshu University School of Medicine. Human brain samples used in this study had already been obtained in a previous study (Tachibana et al. 2013).

Immunohistochemical staining

Paraffin-embedded ovarian tissue blocks were collected from five autopsied cows (age range, 4 to 5 years). Serial sections were stained by an automated immunohistochemistry system (Ventana XT; Ventana Medical System, Tucson, AZ). We used the following primary antibodies: anti-NR1 antibody (AB 1516, Chemicon, Temecula, CA, ×100); anti-NR2A antibody (clone A12W, Upstate Biotechnology, Lake Placid, NY, ×50); and anti-NR2B antibody (Frontier Institute Co., Hokkaido, Japan, ×50). For the application of anti-NR2A and anti-NR2B antibodies, deparaffinized sections in 0.01 M citrate buffer at pH 6.0 were pre-treated by microwave (for 7 min) and autoclave (121°C for 20 min), respectively. Both pre-treatments were employed for antigen retrieval in formalin-fixed and paraffin-embedded tissues (Shi et al. 1991; Shi et al. 2001). Positive control sections were prepared from human temporal lobe and cerebellum, and negative control sections were treated in the same way, except that the primary antibodies were replaced with normal bovine serum.

Immunofluorescence staining

The collected bovine ova were mounted on glass slides, air-dried, fixed in cold acetone for 10 min at 4°C, and then serially incubated with 10% goat serum for 30 min with the commercially avail-

able anti-NR1 or anti-NR2B antibodies (Frontier Institute Co., ×100) for 1 h at room temperature. After applying fluorescein isothiocyanate-conjugated anti-rabbit IgG (Dako Japan, Tokyo, Japan, ×100) for 30 min at room temperature, SlowFade Gold antifade reagent (Molecular Probes, Eugene, OR) was applied, and the staining was observed under a fluorescence microscope (Zeiss AxioVision, Zeiss, Oberkochen, Germany).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis

Fresh bovine ovarian tissue (1 g) removed from a 5-year-old autopsied cow was cut into small pieces and homogenized in 10 mM PBS containing 0.01% sodium azide. The 938 bovine ova collected were homogenized and sonicated in the same solution. After centrifugation at 100,000 × g for 30 min at 4°C, the supernatants were discarded. This operation was repeated, and the resultant pellets were homogenized in 20 mM phosphate (pH 7.2) containing 500 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 0.02% sodium azide, and protease inhibitor cocktail (RIPA), and were then centrifuged at 100,000 × g for 30 min at 4°C. These supernatant fractions were dissolved in 0.2 M Tris-HCl (pH 6.8) containing 1% SDS, 2% mercaptoethanol, 40% glycerol and 0.04% CBB G-250. For SDS-PAGE, we used Mini-PROTEAN Precast gel (4-20%; Bio-Rad, Hercules, CA) and TGX Precast gel (Any kD; Bio-Rad). Immunoblotting was carried out according to a previously described method (Kume and Kametani 2006), and bs-2030R (anti-NR1), bs3507R (anti-NR2A), bs3307R (anti-NR2B) and bs0323R (anti-NR2C) were purchased from Bioss Inc. (Woburn, MA). These antibodies were used according to commercially recommended dilutions.

Extraction of NMDAR-related proteins from bovine ova, and analysis by reverse phase liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

The 300 bovine ova were homogenized and sonicated in 10 mM PBS containing 0.01% sodium azide. The final supernatant fractions, which were used for the next immunoprecipitation, were obtained by the method previously mentioned. A capturing antibody to each NMDAR subunit was added to the supernatant fraction. Anti-NR1 clone 54.1 (Merck Millipore, Billerica, MA) and anti-NR2A, NR2B, and anti-NR1-2C (Frontier Institute Co.) were used according to commercially recommended dilutions. The mixtures were then incubated for 16 h at 4°C. Next, we added 20 μL of UltraLink Immobilized Protein A/G beads (Thermo Fisher Scientific Inc., Waltham, MA) to each mixture. After incubation for 2 h, the beads were washed four times with PBS.

Immunoprecipitates were extracted from the beads with 0.5% TFA/30% acetonitrile as described in a previous study (Kametani 2004). Dried extracts were dissolved in 50 mM TEAB containing 10% acetonitrile and sonicated. Next, trypsin solution (trypsin final concentration: 200 ng/mL) was added, and the mixture was incubated at 37°C for 20 h, as described elsewhere (Kametani and Ikeda 2013). The digests were dried and then dissolved in 0.1% formic acid containing 2% acetonitrile. After centrifugation at 20,000 × g for 5 min, the digests were subjected to a DiNa HPLC system fitted with an automatic sampler (KYA Technologies Corporation, Tokyo, Japan). A Develosil ODS-HG-5 reverse-phase capillary column (0.075 mm × 150 mm; Nomura Chemical Co., Ltd., Seto, Japan) was used at a flow rate of 300 nL/min with a 2-80% linear gradient of acetonitrile for 60 min. Eluted peptides were directly detected with an ion trap mass spectrometer (Velos Pro; Thermo Fisher Scientific Inc.) at a spray voltage of 1.9 kV and a collision energy of 35%. The mass acquisition method consisted of one full MS survey scan followed by an MS/MS scan of the most abundant precursor ions from the survey scan. Dynamic exclusion for the MS/MS was set to 30 seconds. The data were analyzed with Mascot software (Matrix Science Inc., Boston, MA).

Purification of IgG and its affinity to bovine ova

For preparation of IgG, serum (2 mL) from a patient or a control was incubated with 1 mL of a bio-spin chromatography column (Bio-Rad) of protein A/G-Sepharose beads for 30 min at 4°C. After washing with PBS three times, the samples were eluted with 100 mM glycine (pH 2.5) and neutralized with Tris-HCl (pH 8.0) dialyzed against PBS, freeze-dried, and then reconstituted with PBS as 1 mg/mL solutions and adjusted to pH 7.4 before use. Each IgG preparation was tested for antibody reactivity by staining NMDAR NR1 and NR2B co-transfected HEK cells (Tachibana et al. 2010; Tojo et al. 2011).

The purified patient or control's serum IgG was diluted 1:100 and applied onto glass slides mounted with bovine ova. The affinity of purified IgG to these ova was observed under an immunofluorescence microscope using similar methods to those of immunofluorescence staining previously described.

Results

Immunohistochemical and immunofluorescence analysis

The anti-NR2B antibody showed positive immunoreactivity in all five bovine ovarian tissues examined. Coarse granular immunoreactivity was seen in the cytoplasm of oocytes in primordial follicles (Fig. 1A). By immunofluorescence staining, positive immunoreactivity with an anti-NR2B antibody was seen in the cytoplasm of isolated bovine ovum (Fig. 1B), but not detectable with anti-NR1 (Fig. 1C) or NR2A antibody (not shown). No significant immunoreactivity was observed in negative controls (not shown).

SDS-PAGE and immunoblotting analysis

Immunoprecipitates obtained from a bovine ovary showed two clear bands: an anti-NR1 immunoreactive protein of about 110 kDa (Fig. 2A) and an anti-NR2B immunoreactive protein of about 180 kDa (Fig. 2B). Similarly, immunoprecipitates extracted from the collected bovine ova revealed anti-NR1 and anti-NR2B immunoreactive proteins (Fig. 2C and D).

Proteomic analysis findings

Five different peptides were identified in the extracts obtained from membrane-associated fractions of the collected bovine ova (Figs. 3 and 4A-D), corresponding to the following partial sequences of NMDAR: SPFGRFK and KNLQDR to 593-599 and 869-874 of NR1; and GVEDALVSLK, QPTVAGAPK, and NEVMSSK to 339-348 of NR2A, 1421-1429 of NR2B, and 817-823 of NR2C, respectively.

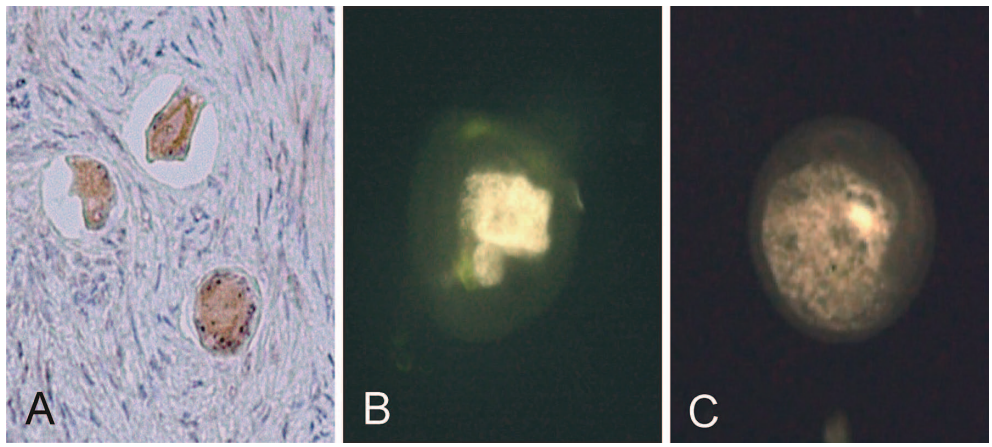

Fig. 1. Immunohistochemical and immunofluorescence expression of NMDAR-related epitope in the bovine ovary and ovum.
(A) An ovary removed from a 5-year-old cow contains primordial follicles with a single layer of flattened follicular cells. The cytoplasm of these oocytes in primordial follicles shows positive immunoreactivity for anti-NR2B antibody. Immunoperoxidase staining, ×200. (B) Immunofluorescence findings in the ovum show positive immunoreactivity with an anti-NR2B antibody. Immunofluorescence staining, ×450. (C) No reactivity is seen with an anti-NR1 antibody. Immunofluorescence staining, ×450.

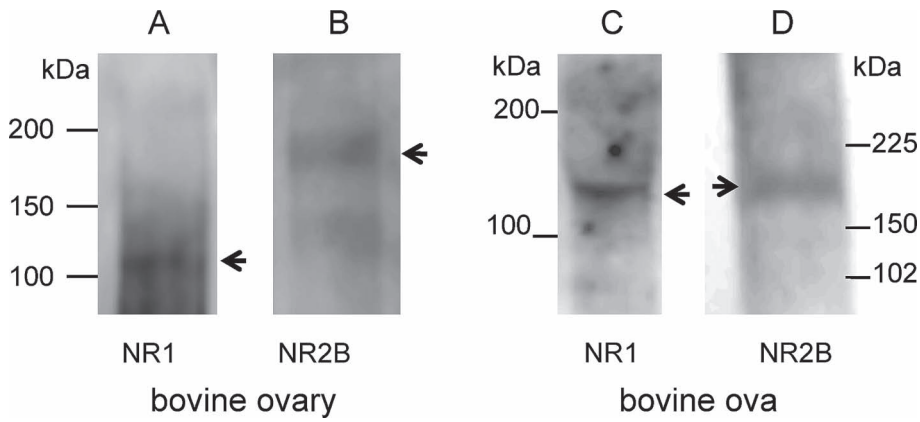

Fig. 2. Immunoblot analysis of NMDAR-related proteins obtained from the bovine ovary and ova.
Proteins were extracted from 1 g ovary and 938 collected ova. Immunoprecipitates were separated on any kD TXG Precast gel (ovary) and 4-20% Precast gels (ova), electroblotted, and then labeled with anti-NMDAR antibodies.
(A) An anti-NR1 immunoreactive band (arrow) from the ovary. (B) An anti-NR2B immunoreactive band (arrow) from the same tissue. (C) An anti-NR1 immunoreactive band (arrow). (D) An anti-NR2B immunoreactive band (arrow) from collected ova.

Application of patient's IgG to ova

Immunocytochemical analysis showed that IgG purified from the serum of a patient with anti-NMDAR encephalitis strongly bound to the surface of the ovum; however, no reaction was observed with control human IgG (Fig. 5).

Discussion

NMDARs are thought to play an important role in synaptic plasticity, synaptogenesis, excitotoxicity, memory acquisition and learning in the central nervous system (Cull-Candy et al. 2001; Cao et al. 2011). The NMDARs in the hippocampus or cortex are most frequently composed of NR1 associated with NR2A or NR2B subunits (Wenthold et al. 2003; Groc et al. 2006), and seem to be related to learning and memory (Abe et al. 2004). In anti-NMDAR encephalitis, anti-neural antibodies cause selective and reversible capping and internalization of surface NMDARs in the brain, leading to severe dysfunction of neocortical memory, learning, and cognitive abilities (Hughes et al. 2010; Dalmau et al. 2011); however, it remains controversial which subunit is more important in the pathogenesis of anti-NMDAR encephalitis. Gleichman et al. (2012) and Amrutkar et al. (2012) emphasized that a small region (N386/G369) within an extracellular N-terminal domain of the NR1 subunit was crucial for the development of immunoreactivity and the subsequent dysfunction of NMDARs. Mikasova et al. (2012) reported that anti-NMDAR antibodies produced differential effects on the two main NR2A- and NR2B-containing subtypes. These effects involve a rapid lateral dispersal of NR2A-containing NMDARs out

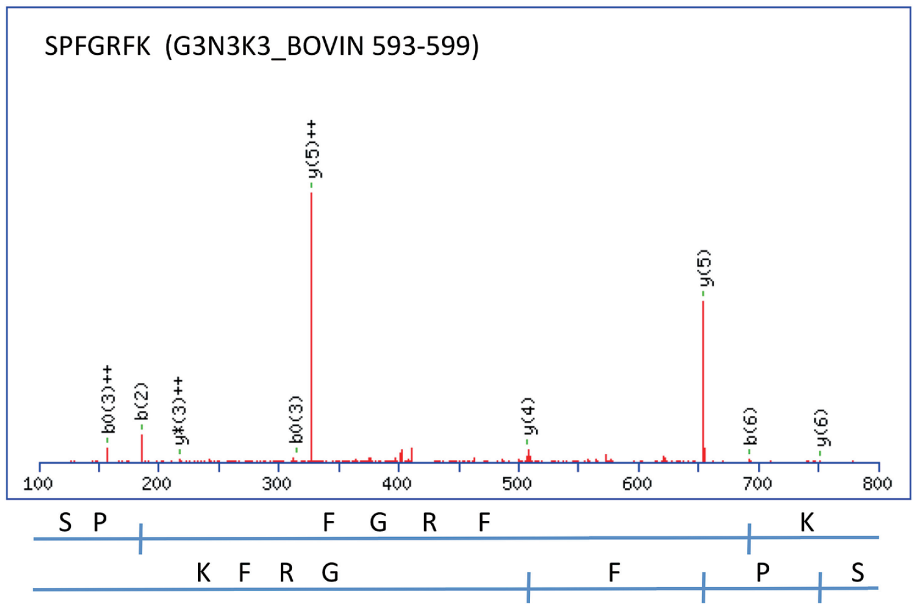

Fig. 3. Identification of NMDAR-related peptides derived from collected bovine ova by LC-MS/MS analysis. A representative MS/MS spectrum indicates a partial sequence of 593-599 of the NR1 subunit.

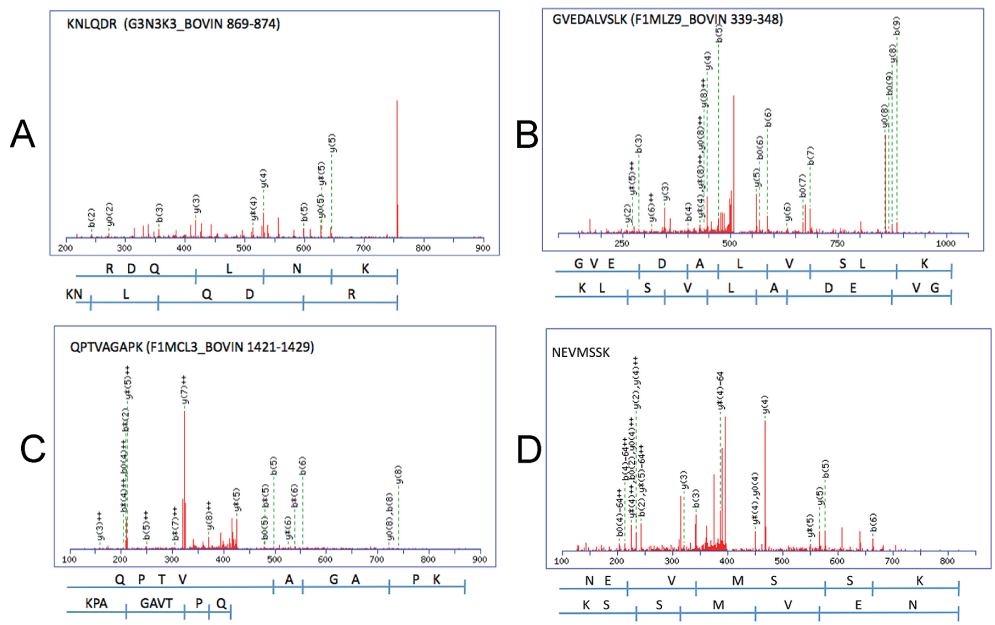

Fig. 4. Identification of remaining NMDAR-related peptides derived from collected bovine ova by LC-MS/MS analysis. (A) MS/MS spectrum of 869-874 of the NR1 subunit. (B) MS/MS spectrum of 339-348 of the NR2A subunit. (C) MS/MS spectrum of 1421-1429 of the NR2B subunit. (D) MS/MS spectrum of 817-823 of the NR2C subunit.

of the synaptic area and complete blockage of synaptic plasticity, and in contrast, a marked reduction of the surface diffusion of NR2B-containing NMDARs in the extrasynaptic area, which leads to their internalization and degradation.

Ovarian teratoma was frequently detected in early series of patients with anti-NMDAR encephalitis; therefore, it was initially reported as paraneoplastic autoimmune encephalitis (Vitaliani et al. 2005; Dalmau et al. 2007). However, based on recent studies comprising large numbers of patients with anti-NMDAR encephalitis, we discovered that ovarian teratomas were detectable in only about half of the affected females (Irani et al. 2010; Dalmau et al. 2011; Titulaer et al. 2013). Another feature of anti-NMDAR encephalitis is that the vast majority of the patients are of reproductive age (Kamei et al. 2009; Dalmau et al. 2011; Titulaer et al. 2013). All these points strongly suggest that NMDAR-related antigens are provided from normal tissues outside the brain, which led us to examine normal ovaries.

Ovarian teratoma with NMDAR-related epitopes

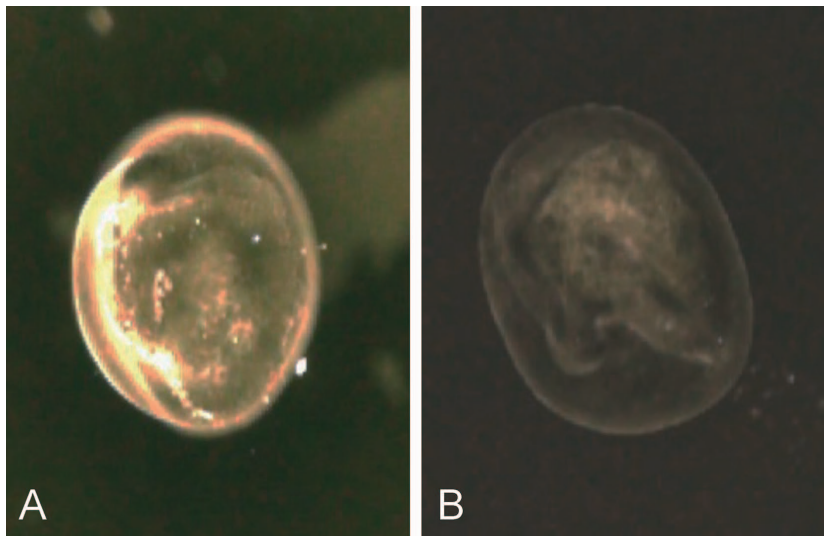

Fig. 5. Immunocytochemical analysis of the affinity of IgG to the cell membrane of a bovine ovum.
(A) Patient's IgG. The patient was a 33-year-old woman who showed psychosis, involuntary movements, convulsions and hypoventilation, and was proven to have a high titer of an anti-NMDAR antibody (×16,000), using a method previously reported (Zhang et al. 2012). (B) Control human IgG. A strong immunofluorescent reaction is seen in an extensive area of cell membrane in the ovum treated with the patient IgG (A), but this reaction is undetectable in that of the ovum treated with the control IgG (B).

develops by neoplastic transformation of an oocyte, and it is thus expected that primordial oocytes have similar epitopes (Monyer et al. 1994), even though epitopes other than NR2B were not found in our previous immunohistochemical studies of paraffin-embedded human ovarian tissue blocks (Tachibana et al. 2010, 2013). In the present study with a bovine ovary and isolated ova, we could only immunohistochemically confirm NR2B epitope expression, and neither NR1 nor NR2A epitope expression was seen in the tissues and/or cells examined. However, in proteomic analysis, we were able to identify major NMDAR-related subunits (NR1, NR2A, NR2B and NR2C) in the cell membrane fraction obtained from collected bovine ova. The discrepancy between immunohistochemical studies and proteomic analysis might be explained by the subunit assembly of NMDAR. This receptor is a heterotetrameric cation channel composed of NR1 and NR2/3 subunits. During synaptic development, the subunit composition of synaptic NMDARs changes from heterodimers containing predominantly NR2B subunits at an early stage to heterodimers containing NR1/NR2B, NR1/NR2A, and NR1/NR2A/NR2B subunits at a mature stage (Monyer et al. 1994; Cull-Candy et al. 2001; Furukawa et al. 2005; Groc et al. 2006). It is possible that the bovine ovary and isolated ova express mainly NR2B subunits as opposed to NR2A and NR2C subunits, which would explain why NR2B epitopes are more easily detectable on immunohistochemical staining (Tachibana et al. 2010, 2013). Recent studies of NMDAR subunit assembly have shown that the NMDAR is unique in its obligatory heterotetrameric structure (Laube et al. 1998; Schorge and Colquhoun 2003; Furukawa et al. 2005; Schüler et al. 2008), which indicates that the assembly of receptor subunits is under critical control. It is also known that, due to the presence of multiple endoplasmic reticulum (ER) signals, NR1 and NR2 subunits cannot be present in the cell membrane when expressed alone in heterogeneous cells. These ER retention signals are only masked when NR1 and NR2 subunits are assembled together and trafficked to the surface of synaptic membrane as functional channels (Hawkins et al. 2004; Cao et al. 2011; Horak et al. 2014). Thus, the existence of an NR2B epitope on the surface of a single cell suggests the co-expression and heteromerical assembly of NR1and NR2 subunits. The discrepancy between immunohistochemical and proteomic analyses of the NR1 subunit might be a result of the antibody we used, which corresponded to the C-terminus of the NR1 subunit. Additionally, the lack of an immunohistochemically detected NR2A epitope may have been due to antibody mismatch. The amino acid length of the bovine NR2A subunit is 1226 (UniProtKB/Swiss-Prot: M5FK77; UniProt), while that of the mouse NR2A subunit is 1464 (UniProtKB/Swiss-Prot: P35436; UniProt). The C-terminal cytoplasmic region of the mouse NR2A subunit was recognized by the anti-NR2A antibody used in our study (amino acid residues 1126-1408). Therefore, it might be difficult to stain bovine ovaries and ova using an anti-NR2A antibody (clone A12W, Upstate, Lake Placid, NY).

NMDAR is a membrane-associated protein that is usually localized at the synapse as well as the extrasynaptic receptor (Gleichman et al. 2012; Mikasova et al. 2012). Although the NMDAR that was isolated from the bovine ovum cell membrane in this study is classified as extrasynaptic, we have shown that IgG purified from the serum of a patient with anti-NMDAR encephalitis, which was previ-

ously demonstrated to have a high titer of antibody against the NR1/NR2 heteromer of NMDAR, strongly bound to the cell membrane of bovine ovum; therefore, this NMDAR seems to be capable of expressing sufficient target antigens. Although the biophysical and pharmacological properties of this ovarian NMDAR remain unclear, our findings indicate that an ovary with abundant oocytes may provide ectopic expression of NMDAR outside of the central nervous system (CNS). It also remains unclear how NMDAR-related antigens in oocytes contact inflammatory cells, including B lymphocytes, and lead to the production of anti-NMDAR antibodies. A prodromal infection, which was reported in 70-90% of patients with anti-NMDAR encephalitis (Dalmau et al. 2011), might become an immunological trigger like in Guillain-Barré syndrome (Tojo et al. 2011). It is thought that antibodies produced within the CNS by activated plasma cells or systematically synthesized antibodies reaching the CNS by blood-brain barrier disruption bind to extracellular epitopes of NMDAR, causing its subsequent dysfunction and internalization (Moscato et al. 2010). However, no information on the non-brain tissues expressing NMDAR-related epitopes in infants, children and adult male patients has been reported.

Intensive immunomodulating therapies are necessary for anti-NMDAR encephalitis. Combination therapy with corticosteroid, plasma exchange, and intravenous administration of immunoglobulin is recommended as the first-line treatment (Dalmau et al. 2011). Recently, rituximab, an anti-CD20 monoclonal antibody, and/or cyclophosphamide, has been increasingly used for intractable patients requiring second-line immunotherapy (Titulaer et al. 2013). Patients without ovarian tumor seem to be more resistant to these therapeutic approaches (Lancaster et al. 2011). When NMDAR-related epitopes in an ovary with abundant oocytes are targeted in patients with anti-NMDAR encephalitis, the ectopic source of this autoantigen cannot be surgically removed. Therefore, more intensive and long-term immunosuppressive treatments might be needed for such patients. The pathogenesis of anti-NMDAR encephalitis is considered to be more complicated than previously thought, and warrants investigation in future studies.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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