Original Paper

European Neurology

Eur Neurol 2014;71:42–48
DOI: 10.1159/000353982

Received: February 22, 2013
Accepted: June 23, 2013
Published online: November 27, 2013

Immunopathological Significance of Ovarian Teratoma in Patients with Anti-N-Methyl-D-Aspartate Receptor Encephalitis

Emi Tabata Masanori Masuda Makoto Eriguchi Masatoshi Yokoyama
Yoshiyuki Takahashi Keiko Tanaka Motohiro Yukitake Etsuo Horikawa
Hideo Hara

Division of Neurology, Department of Internal Medicine, Saga University Faculty of Medicine, Saga, Japan

Key Words
Anti-N-methyl-D-aspartate receptor encephalitis · Ovarian teratoma · Immunopathological study · Limbic encephalitis · Paraneoplastic syndrome

Abstract
Background: The clinical importance of ovarian teratoma in anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis has been established, however investigations of ovarian teratoma in patients with anti-NMDAR encephalitis remain limited. ***Objective:*** To clarify differences in NMDAR distribution and lymphocyte infiltration in ovarian teratoma between patients with and without anti-NMDAR encephalitis. ***Methods:*** Participants initially comprised 26 patients with ovarian teratomas. NMDAR distribution and lymphocyte infiltration in ovarian teratomas were examined using immunopathological techniques. Clinical, laboratory, and radiological data were compared between patients showing the features of encephalitis. Anti-NMDAR antibodies in the serum and cerebrospinal fluid were also measured in encephalitis patients. ***Results:*** Neuronal tissues were obtained from ovarian teratomas in 22 patients (after excluding 4 patients who did not satisfy the inclusion criteria), and the presence of NMDA receptor subunits was revealed in all patients. Lymphocyte infiltration was more frequent in the encephalitis group (n = 3) than in the non-encephalitis group. In particular, dense B-lymphocyte infiltration near neural tissues was observed in the encephalitis group. ***Conclusions:*** Differences in lymphocyte infiltration in ovarian teratomas between anti-NMDAR encephalitis and non-encephalitis patients suggest the immunological importance of the ovarian teratoma as the site of antigen presentation in anti-NMDAR encephalitis.

In recent years, anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis has been receiving attention due to its clinical characteristics such as female predominance, limbic encephalitis-like clinical features, and the presence of an ovarian teratoma in over 50% of cases [1, 2]. Interestingly, this encephalitis has been associated with the antibody (Ab) against NMDAR subtypes 1 and 2B (NR1/NR2) heteromers of NMDAR in the serum and cerebrospinal fluid (CSF) [1, 3]. Several clinical features, such as responsiveness to immunotherapy and surgical teratoma removal and the presence of anti-NMDAR Abs in the serum and CSF, strongly suggest that autoimmunity may be central to the pathogenesis of anti-NMDAR encephalitis [4–6]. Although the relationship between anti-NMDAR encephalitis and ovarian teratomas has been considered, histological investigations of ovarian

KARGER

E-Mail karger@karger.com
www.karger.com/ene

0014–3022/14/0712–0042$39.50/0

Dr. Emi Tabata
Division of Neurology, Department of Internal Medicine
Saga University Faculty of Medicine
5-1-1 Nabeshima, Saga 849-8501 (Japan)
E-Mail e6315@cc.saga-u.ac.jp

teratoma in anti-NMDAR encephalitis remain limited [5, 19].

We performed immunohistological analysis of ovarian teratomas from anti-NMDAR encephalitis patients and non-encephalitis controls to clarify differences in NMDAR distribution and lymphocyte infiltration.

Methods

Standard Protocol Approvals and Patient Consent

Prior to the initiation of this study, informed consent was obtained from each patient following a clear explanation of its purposes and methods. Ethics approval for this study was granted by the Saga University Ethics Committee.

Patients and Controls

Patients with ovarian teratomas hospitalized at Saga University Hospital between January 2004 and February 2010 were investigated in this study. All clinical data were obtained from Saga University Hospital medical records, and all formalin-fixed paraffin-embedded ovarian teratoma tissue blocks were obtained from the Department of Pathology, Saga University Faculty of Medicine. The clinical and laboratory parameters of patients with encephalitis in this study were as follows: age; symptoms; disease severity; laboratory data (thyroid functions, anti-thyroglobulin Abs, antinuclear Abs, anti-DNA Abs, Abs against several viruses including herpes simplex virus (HSV), CSF cell count and protein, immunoglobulin G in the CSF, and polymerase chain reaction testing for HSV DNA in CSF); radiological examinations, and electroencephalography. Exclusion criteria were as follows: an ovarian teratoma containing no neuronal tissue, and inappropriate tissue conditions for this study.

Detection of the Anti-NMDAR Antibody in the Serum and CSF

Methods for detecting the anti-NMDAR Ab have been described previously [8]. In brief, cDNA encoding NR1 and NR2B was ligated into expression vectors and transfected into human embryonic kidney (HEK)-293 cells in medium containing 10 mM MK-801 using Lipofectamine (Invitrogen, Carlsbad, Calif., USA). Twelve hours after transfection, HEK-293 cells were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4) for 20 min. After non-specific binding was blocked with 10% goat serum in phosphate-buffered saline, these cells were incubated with patient sera (1:40) or CSF (1:2) overnight at 4°C and then with fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin G (BD Biosciences, San Jose, Calif., USA) for 30 min at room temperature. SlowFade gold anti-fade reagent (Molecular Probes, Inc., Eugene, Oreg., USA) was applied to the slides and staining was observed under fluorescence microscopy.

Immunohistochemical Study of Ovarian Teratoma

Sections cut from formalin-fixed paraffin-embedded tissue blocks were used. The primary Abs used were NR1-C2 (dilution 1:100; Frontier Institute, Hokkaido, Japan), NR2B (dilution 1:100; Frontier Institute), SMI-31 (dilution 1:500; Convance, Emeryville, Calif., USA), ionized calcium-binding adaptor molecule 1 (IBA-1) (dilution 1:200; Abcam, Cambridge, Mass., USA), glial fibrillary acidic protein (GFAP) (dilution 1:100; Dako Cytomation, Glostrup, Denmark), Neurofilaments (dilution 1:100; Dako Cytomation), CD3 (prediluted; Nichirei Biosciences, Tokyo, Japan), CD4 (dilution 1:20; Nichirei Biosciences), CD8 (dilution 1:100; Dako Cytomation), and CD20 (dilution 1:100; Dako Cytomation). Slides were microwave-heated in ethylenediaminetetraacetic acid (pH 8) for antigen retrieval. The Envision+® System (Dako Cytomation) was used for the secondary Ab. Slides were visualized using diaminobenzidine tetrahydrochloride and nuclei were counterstained with hematoxylin. The Autostainer plus® automatic stainer (Dako Cytomation) was used to stain all Abs [2, 6]. The degree of staining for NMDA receptor Abs (NR1 and NR2B) was graded as follows: 0, no staining; focal (+), <30% cell staining; patchy (++), 31–60% cell staining, and diffuse (+++), >60% cell staining. To estimate the number of lymphocytes, a standard 3-point scoring system was used: low (–), intermediate (+), or high (++). The immunohistological results were independently scored by one pathologist and two neurologists.

Results

Twenty-six patients with ovarian teratomas were included in this study. These patients were divided into two groups: encephalitis group, 3 encephalitis patients with ovarian teratomas, and non-encephalitis group, 23 ovarian teratoma patients with no evidence of encephalitis. Four patients in the non-encephalitis group were excluded due to a lack of neural tissues in the ovarian teratoma (n = 2) or because of an insufficient sample state (n = 2).

Clinical Characteristics of Patients with Encephalitis

The mean age of the encephalitis group was 24.3 years (range 18–33) and that of the non-encephalitis group was 30.3 years (range 18–49). Clinical, laboratory, and radiological characteristics in the encephalitis group are shown in table 1.

Briefly, 2 of the 3 patients (cases 1 and 2) in the encephalitis group exhibited the typical clinical symptoms of anti-NMDAR encephalitis, including initial psychosis, subsequent central hypoventilation, intractable seizures, dysautonomia, and prominent orofacial dyskinesia. In contrast, 1 patient (case 3) exhibited psychosis mimicking limbic encephalitis, but never showed central hypoventilation, seizure, or orofacial dyskinesia. The presence of anti-neuronal Abs against NR1/NR2 heteromers of NMDAR was confirmed in both the serum and CSF; therefore, 3 patients in the encephalitis group were diagnosed with anti-NMDAR encephalitis. In contrast, ovarian teratoma patients in the non-encephalitis group showed no neurological or psychological symptoms according to their medical records.

Table 1. Clinical, laboratory, and radiological characteristics of patients with anti-NMDAR encephalitis

	Case 1 (severe group)	Case 2 (severe group)	Case 3 (mild group)
Age, years	24	34	18
Duration between disease onset to ovarian teratoma resection	70 days (hyperkinetic phase)	17 days (hyperkinetic phase)	20 days (psychotic phase)
Mechanical respiratory assistance	+	+	–
CSF findings			
Cells/ml	114	37	7
Protein, mg/dl	218	27	13
Anti-NMDAR antibody			
Serum	+	+	+
CSF	+	+	+
Abnormal head MRI findings	T2 hyperintensity (cerebrum)	T2 hyperintensity (cerebrum and cerebellum)	T2 hyperintensity (cerebellum)
Treatment			
Ovarian teratoma resection	+	+	+
Corticosteroids	+	+	+
Intravenous immunoglobulin	+	+	–
Plasma exchange	+	+	–

Histopathological and Immunohistochemical Findings in Ovarian Teratoma

Neural tissues were demonstrated in all teratoma samples from the encephalitis and non-encephalitis groups according to cell morphology and varying degrees of immunostaining for anti-SMI-31 Ab (a neuron-specific marker), anti-GFAP Ab (an astrocyte-specific marker), and anti-IBA-1 Ab (a microglia-specific marker) (fig. 1a–d, 2a–d). The presence of NMDAR in neuronal tissues was also revealed. In the encephalitis group, all neuronal tissues showed positive staining by anti-NR1 and anti-NR2 Ab with (++) intensity (fig. 1e, f). In contrast, the intensity of staining for anti-NR1 and anti-NR2 Ab-positive neural tissues in ovarian teratomas in the non-encephalitis group varied from (–) to (+++) (fig. 2e, f). Immunohistochemical data for neuronal tissue staining and the presence of NMDAR in the encephalitis and non-encephalitis groups are summarized in table 2.

Inflammatory cell infiltration around neural tissues was also observed in both the encephalitis group (n = 3) and non-encephalitis group (n = 2). Interestingly, inflammatory cell infiltration was observed in only 2 of 19 patients in the non-encephalitis group, whereas all 3 patients in the encephalitis group showed varying degrees of inflammatory cell infiltration. Both CD4-positive T lymphocytes and CD8-positive T lymphocytes were observed close to neural tissues (fig. 2g, h), and a predominance of CD4+ T-lymphocyte infiltration was observed in 4 of the 5 patients showing inflammatory cell infiltration (80%; all 3 patients in the encephalitis group and 1 of 2 patients in the non-encephalitis group) (fig. 2g, h). The presence of CD20-positive B lymphocytes was also observed around neural tissues in teratomas in the encephalitis and non-encephalitis groups. The state of B-lymphocyte infiltration was markedly denser in the encephalitis group than in the non-encephalitis group (fig. 1i, 2i). In addition, B-lymphocyte infiltration appeared to be adjacent to the site of NR1- and NR2B-positive neuronal tissues. This characteristic B-lymphocyte infiltration was observed among patients showing typical clinical features such as initial psychosis, subsequent central hypoventilation, intractable seizures, dysautonomia, and prominent orofacial dyskinesia. Immunohistochemical data for lymphocyte infiltration in the encephalitis and non-encephalitis groups are summarized in table 3.

Discussion

Immunotherapies such as intravenous methylprednisolone pulse therapy, intravenous immunoglobulin administration, and plasma exchange are well recognized as first-line treatments for anti-NMDAR encephalitis in the acute clinical phase. These therapeutic strategies strongly suggest that immunological pathogenesis lies in anti-NMDAR encephalitis. In addition, concerning anti-NMDAR en-

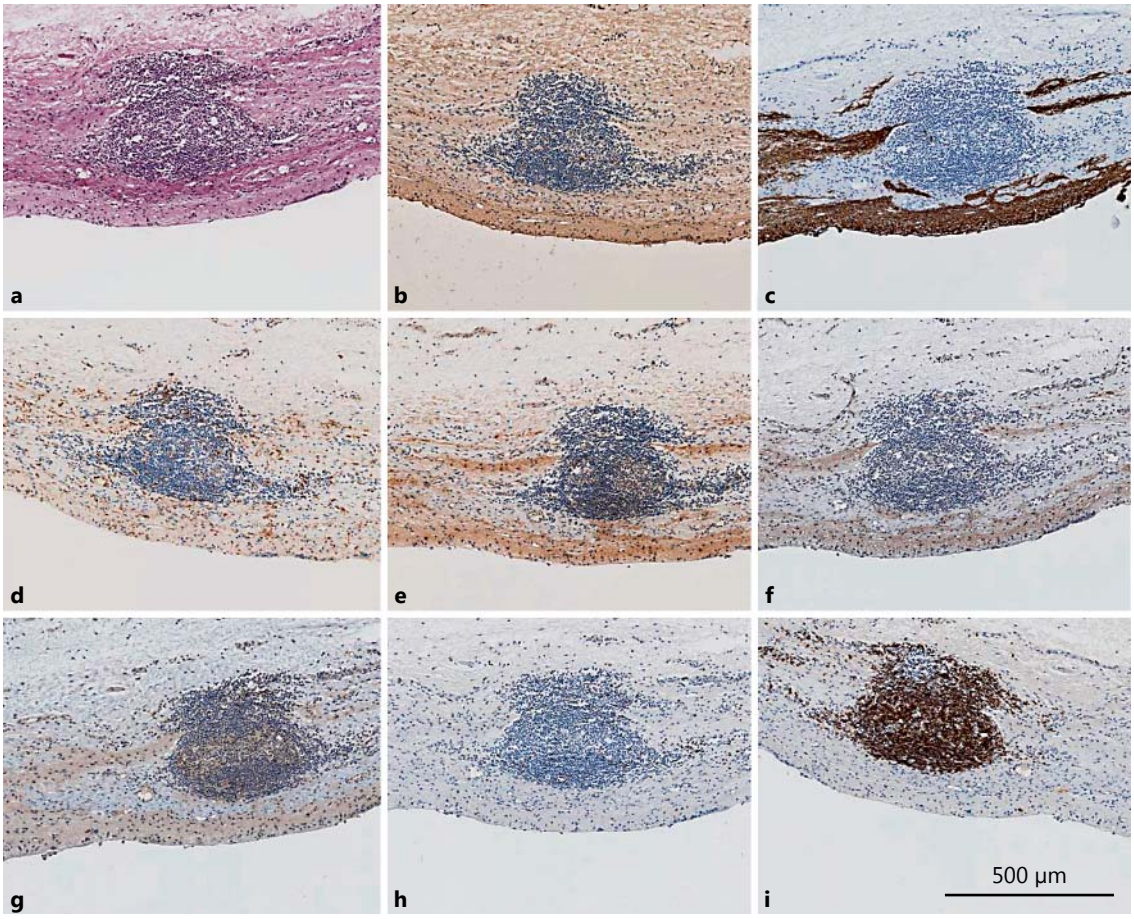

Fig. 1. Histopathological and immunohistochemical findings in ovarian teratomas with encephalitis (case 1). Neuronal tissues in the ovarian teratoma were demonstrated based on cell morphology (**a**). Immunostainings using anti-SMI-31 Ab (**b**), anti-GFAP Ab (**c**), and anti-IBA-1 Ab (**d**) confirmed the presence of several neuronal compartments in neuronal tissues. The presence of NMDAR in neuronal tissues was also demonstrated using anti-NR1 (**e**) and anti-NR2 Ab (**f**). Lymphocyte infiltration was revealed around neuronal tissues. CD4+ T lymphocytes (**g**) and CD8+ T lymphocytes (**h**) were observed around neuronal components. CD20-positive B lymphocytes were also observed around neuronal tissues with dense infiltration in the encephalitis group (**i**).

cephalitis complicated by ovarian teratoma, there have been reports that resection of the teratoma resulted in rapid and marked improvements in the condition, suggesting an involvement of ovarian teratoma in immune responses [7–12]. Ovarian teratoma appears to contribute to the pathogenesis of anti-NMDAR encephalitis [13–15]. Recent studies have shown that neuronal tissues in ovarian teratomas in anti-NMDAR encephalitis patients expressed NR2B and/or NR2A, and B- and T-lymphocyte infiltration has also been reported in the ovarian teratoma [2, 8]. In general, however, information on inflammatory cell infiltration in ovarian teratomas in patients with anti-NMDAR encephalitis is limited, and all except one report have described findings in the clinical recovery phase.

Similar to our results, Dabner et al. [19] reported pathological differences in ovarian teratomas between patients with anti-NMDAR encephalitis and non-encephalitis controls. Diffuse lymphoplasmacytic infiltrates were observed within the neurological matrix of ovarian teratomas in patients with anti-NMDAR encephalitis. Our study revealed the presence of neuronal tissues in ovarian teratomas in the encephalitis and non-encephalitis groups. NR1 and NR2B, as subunits of NMDAR, were also detected in neuronal tissues in both groups, which suggests that the presence of NMDAR itself is not a specific finding for anti-NMDAR encephalitis with ovarian teratoma. In addition, denser inflammatory cell infiltration around neural tissues in ovarian teratomas was observed in the encephalitis group.

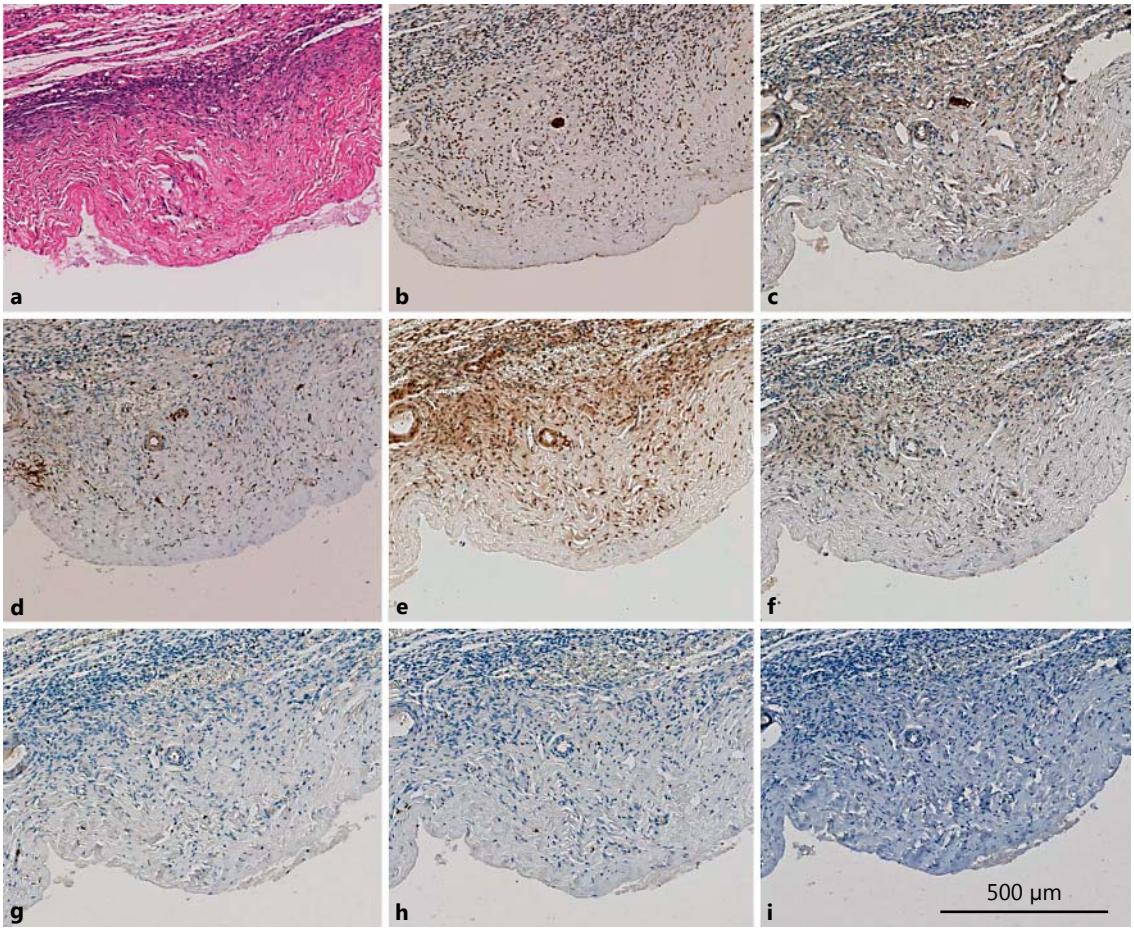

Fig. 2. Histopathological and immunohistochemical findings in ovarian teratomas with non-encephalitis. Neuronal tissues in the ovarian teratoma were demonstrated based on cell morphology (**a**). Immunostaining using anti-SMI-31 Ab (**b**), anti-GFAP Ab (**c**), and anti-IBA-1 Ab (**d**) confirmed the presence of several neuronal compartments in neuronal tissues. The presence of NMDAR in neuronal tissues was also demonstrated using anti-NR1 (**e**) and anti-NR2 Ab (**f**). Lymphocyte infiltration was revealed around neuronal tissues. CD4+ T lymphocytes (**g**) and CD8+ T lymphocytes (**h**) were observed around neuronal components. CD20-positive B lymphocytes were found around neuronal tissues with no or slight infiltration in the non-encephalitis group (**i**).

Furthermore, another study showed NR2B-related immunoreactivity in the cytoplasm of oocytes in normal ovaries. Taking these findings together, the presence of NMDAR itself is necessary, but not a sufficient condition in anti-NMDAR encephalitis. However, these results do not deny the importance of neuronal elements in an ovarian teratoma in the immunopathogenesis of anti-NMDAR encephalitis because differences in lymphocyte infiltration were also observed between ovarian teratomas in the encephalitis and non-encephalitis groups in this study.

In contrast with the ubiquitous presence of NMDAR in the ovary with or without encephalitis, the frequency and mode of lymphocyte infiltration differed markedly between the encephalitis and non-encephalitis groups. In particular, dense infiltration of CD20+ B lymphocytes around NR1- and NR2B-positive neuronal tissues represents a unique finding because this mode of infiltration was only observed in patients in the encephalitis group. This characteristic B-lymphocyte infiltration supports the immunopathogenesis of anti-NMDAR encephalitis against neuronal elements in ovarian teratomas, and differences in B-lymphocyte reactions against neuronal elements such as NMDAR could depend on some genetically predisposed individuals. Therefore, an ovarian teratoma may represent a site of antigen presentation for anti-NMDAR encephalitis patients with ovarian teratomas.

Table 2. Histopathological and immunohistochemical findings of ovarian teratoma in patients with anti-NMDAR encephalitis and non-encephalitis group

	Encephalitis group (n = 3)	Non-encephalitis group (n = 19)
Neuronal tissues staining, %		
SMI-31	100	100
GFAP	100	100
IBA-1	100	100
NMDAR staining, n (%)		
NR1 no stain (–)	0 (0)	1 (5)
Focal (+)	0 (0)	4 (18)
Patchy (++)	3 (100)	5 (25)
Diffuse (+++)	0 (0)	9 (45)
NR2B no stain (–)	0 (0)	5 (26)
Focal (+)	0 (0)	7 (42)
Patchy (++)	3 (100)	3 (16)
Diffuse (+++)	0 (0)	3 (16)
Lymphocyte infiltration, %	3 (100)	2 (10)

Table 3. Lymphocyte infiltration around neuronal tissues in the ovarian teratoma in patients with or without anti-NMDAR encephalitis

	T lymphocyte				B lymphocyte CD20
	CD3	CD4	CD8		
Encephalitis group					
Case 1	(+)	(+)	(+)	CD4 predominance	(++)
Case 2	(+)	(+)	(+)	CD4 predominance	(++)
Case 3	(+)	(+)	(+)	CD4 predominance	(+)
Non-encephalitis					
Group 1	(+)	(+)	(+)	CD4 = CD8	(+)
Group 2	(+)	(+)	(+)	CD4 predominance	(+)

(+) = Intermediate lymphocytes infiltration; (++) = highly lymphocytes infiltration.

Further investigations, such as differences in HLA constellations or microRNA expression profiles, are needed to clarify individual differences in genetic backgrounds.

On the other hand, systemic viral infection or mild inflammation affecting the ovary may create a trigger for NMDAR recognition, or the ovary itself may be important even in anti-NMDAR encephalitis patients without ovarian teratomas as a site of antigen exposure because NMDAR is present even in the ovary itself.

Although the number of patients in this study was small, the results, which showed differences in lymphocyte infiltration in ovarian teratomas between the anti-NMDAR encephalitis and non-encephalitis groups, suggest the importance and contribution of immunological mechanisms involving NMDAR to ovarian teratomas in anti-NMDAR encephalitis.

Acknowledgments

The authors are grateful to members of the Pathology Department in Saga University Faculty of Medicine for their helpful suggestions about ovarian pathology. Immunopathological analysis was contributed by Dr. Masatoshi Yokoyama and measurements of anti-NMDAR Abs in the serum and CSF were contributed by Dr. Yukitoshi Takahashi and Keiko Tanaka. This study was supported in part by grants from the Ministry of Health, Labour and Welfare, Japan.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

1 Dalmau J, Lancaster E, Martinez-Hernandez E: Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. Lancet Neurol 2011;10:63–74.
2 Dalmau J, Tüzün E, Wu HY: Paraneoplastic N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25–36.
3 Benarroch EE: NMDA receptors: recent insights and clinical correlations. Neurology 2011;76:1750–1757.
4 Iizuka T, Sakai F, Ide T: Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. Neurology 2008;70:504–511.
5 Dalmau J, Gleichman AJ, Hughes EG: Anti-NMDA receptor encephalitis: case series and analysis of effects of antibodies. Lancet Neurol 2008;7:1091–1098.
6 Takahashi Y, Mogami Y, Takayama R: Antibodies to glutamate receptor in limbic encephalitis. Brain Nerve 2010;62:827–837.
7 Uchino A, Iizuka T, Urano Y: Pseudo-piano playing motions and nocturnal hypoventilation in anti-NMDA receptor encephalitis: response to prompt tumor removal and immunotherapy. Intern Med 2011;50:627–630.
8 Zhang Q, Tanaka K, Sun P: Suppression of synaptic plasticity by cerebrospinal fluid from anti-NMDA receptor encephalitis patients. Neurobiol Dis 2012;45:610–615.
9 Seki M, Suzuki S, Iizuka T: Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. J Neurol Neurosurg Psychiatry 2008;79:324–326.

10 Vitaliani R, Mason W, Ances B: Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. Ann Neurol 2005;58:594–604.
11 Kleyensteuber B, Ruterbusch V, Bennett J: Limbic encephalitis presenting with seizures, anterograde amnesia, and psychosis in a patient seven weeks status post immature ovarian teratoma removal. Mil Med 2010;175:616–618.
12 Lo JW, Leung EY, Ng BL: Anti-N-methyl-D-aspartate receptor encephalitis in a young woman with an ovarian tumour. Hong Kong Med J 2010;16:313–316.
13 Sonn TS, Merritt DF: Anti-NMDA receptor encephalitis: an adolescent with an ovarian teratoma. J Pediatr Adolesc Gynecol 2010; 23:e141–e144.
14 Tüzün E, Zhou L, Baehring JM: Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. Acta Neuropathol 2009;118:737–743.
15 Kataoka H, Dalmau J, Ueno S: Paraneoplastic encephalitis associated with ovarian teratoma and N-methyl-D-aspartate receptor antibodies. Eur J Neurol 2008;15:e5–e6.
16 Trojanowski JQ, Hickey WF: Human teratomas express differentiated neural antigens. An immunohistochemical study with anti-neurofilament, anti-glial filament, and anti-myelin basic protein monoclonal antibodies. Am J Pathol 1984;115:383–389.
17 Sabin TD, Jednacz JA, Staats PN, Thomas D: Case 26-2008 – a 26-year-old woman with headache and behavioral changes. N Engl J Med 2008;359:842–853.
18 Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y: Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. Neurology 2011;77: 589–593.
19 Dabner M, McCluggage WG, Bundell C, Carr A, Leung Y, Sharma R, Stewart CJ: Ovarian teratoma associated with anti-N-methyl-D-aspartate receptor encephalitis: a report of 5 cases documenting prominent intratumoral lymphoid infiltrates. Int J Gynecol Pathol 2012;31:429–437.