V. 内科関連疾患

傍腫瘍性神経症候群

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ガイドラインの現況

近年，ヨーロッパ神経学会と Paraneoplastic Neurological Syndrome Euronetwork による傍腫瘍性神経症候群（paraneoplastic neurological syndrome：PNS）の診断基準が提唱されている[1,2]．PNS の一群に相当する多発性筋炎や重症筋無力症，Lambert-Eaton 筋無力症候群は別項を参照されたい．

どういう疾患・病態か

1 疾患概念・病態機序

PNS は担癌患者の神経障害のうち，直接浸潤や転移，栄養・代謝障害，治療の副作用，易感染状態によらないものであり「腫瘍の遠隔効果」として知られる．広義には，腫瘍が産生する抗体，ホルモン，サイトカイン，播種性血管内凝固による神経障害も含めることもある．近年，免疫学的機序が推測され，腫瘍に対する免疫反応が自己神経組織を傷害していると考えられている．その根拠として，神経傷害部のリンパ球浸潤，血液・髄液中に検出される腫瘍と神経組織の共通抗原に対する自己抗体（抗腫瘍／神経抗体），神経抗原に対する細胞障害性 T 細胞の存在，などが指摘されている．PNS では，抗腫瘍／神経抗体の種類と神経症候，腫瘍の間に一定の傾向がある．

2 典型的な神経症候

PNS の神経症候は**表 1，2** のように多彩である．一般に急性または亜急性で進行性であるが，慢性例や自然軽快する例もある．脳幹や自律神経の障害により，急速に呼吸や循環障害に至り，転帰不良例もある．診療上，典型的な神経症候，随伴しやすい抗腫瘍／神経抗体，合併しやすい腫瘍の理解は重要である．

1. 辺縁系脳炎（paraneoplastic limbic encephalitis：PLE）

急性または亜急性の記銘力障害，幻覚・うつ・性格変化などの精神症状，痙攣，意識障害がみられる．髄液検査は軽度のリンパ球増多，蛋白増加を認める．頭部 MRI の T2 強調画像で側頭葉内側に高信号病変を認める．脳波は側頭葉起源のてんかん波形を認める．合併腫瘍は，肺癌，精巣癌，乳癌が多く，Hodgkin 病，奇形腫，胸腺腫もみられる．抗 Hu 抗体，抗 Ma2 抗体や CV2，CRMP-5，amphiphysin，VGKC，NMDAR に対する抗体の報告がある．

2. 亜急性小脳変性症（paraneoplastic cerebellar degeneration：PCD）

急性または亜急性で進行性の失調，眼振がみられ，ときにオプソクローヌスやミオクローヌス，記銘力障害，錐体路徴候，感覚障害もみられる．合併腫瘍は，女性で卵巣癌などの婦人科系癌，乳癌が多く，それぞれ抗 Yo 抗体，抗 Ri 抗体がみられる．男性では肺癌が多く，抗 Hu 抗体や VGCC，CV2，CRMP

田中優司、犬塚　貴　傍腫瘍性神経症候群　神経疾患・診療ガイドライン（鈴木則宏編）p157-163, 2009, 総合医学社　東京．

表1　傍腫瘍性神経症候群の典型的な神経症候と腫瘍，抗神経抗体

神経症候	症　候	腫　瘍	抗神経抗体
Lambert-Eaton 筋無力症候群	易疲労性，下肢筋力低下．筋電図で高頻度刺激による漸増現象	肺小細胞癌，ときに胃癌，胸腺腫	抗 P/Q，N，L-VGCC（α1，β-subunit），抗シナプトタグミン，抗 amphiphysin，抗 CRMP-5
亜急性小脳変性症（PCD）	亜急性発症・進行性の四肢・体幹・言語の失調	卵巣癌などの婦人科系の癌，乳癌	抗 Yo（pcd17/cdr62），cdr34
		Hodgkin リンパ腫	抗 Tr，抗 mGluR1
		肺小細胞癌	抗 Hu（HuD，HuC，Hel-N1），抗 VGCC，抗 CV2，抗 CRMP-5
		乳癌	抗 Ri（Nova1，Nova2）
	and/or 脳幹脳炎	乳癌，大腸癌，唾液腺癌	抗 Ta（Ma1，Ma2）
傍腫瘍性脳脊髄炎／感覚性ニューロパチー	辺縁系脳炎，脳脊髄炎，小脳炎，脳幹脳炎，感覚性失調，感覚／運動性ニューロパチー	肺小細胞癌，稀に前立腺癌，胃癌，乳癌	抗 Hu（HuD，HuC，Hel-N1），抗 CV2，抗 amphiphysin，抗 CRMP-5
	辺縁系脳炎 and/or 脳幹脳炎	精巣癌 奇形腫	抗 Ta（Ma2） 抗 NMDA
傍腫瘍性オプソクローヌス／ミオクローヌス	不随意・不規則な衝動性眼球運動，ミオクローヌス，失調	乳癌（腺癌）	抗 Ri（Nova1）
傍腫瘍性網膜変性症	羞明，夜盲，進行性視力低下，リング状暗点	肺小細胞癌，婦人科系癌	抗 recoverin，抗 Hsc70
		黒色腫	抗網膜双極細胞
Stiff-man（person）症候群	発作性有痛性筋痙攣．筋硬直自律神経症状，筋電図で持続性放電	大腸癌，肺癌，Hodgkin リンパ腫	抗 GAD，抗 gephyrin
		乳癌	抗 amphiphysin
ニューロミオトニア	筋痙攣，仮性ミオトニア，ミオキミア	胸腺腫，肺小細胞癌，Hodgkin リンパ腫	抗 VGKC，抗 CRMP-5

抗 Yo 抗体，抗 Ri 抗体陽性者はほとんど女性である．　（文献3を参照して作成）

-5 の抗体がみられる．Hodgkin リンパ腫で抗 Tr 抗体や抗 mGluR1 抗体の報告がある．

3. 傍腫瘍性脳脊髄炎

辺縁系脳炎，脳幹脳炎，小脳炎，脊髄炎，Sensory neuronopathy（感覚性ニューロン症）が単独または組合せてみられる．合併腫瘍は肺癌が多く，前立腺癌，胃癌，乳癌もある．抗 Hu 抗体が多く，CV2，amphiphysin，CRMP-5 に対する抗体の報告もある．辺縁系脳炎や脳幹脳炎をきたし，精巣癌を伴う症例では抗 Ta 抗体の報告がある．

4. 傍腫瘍性オプソクローヌス／ミオクローヌス

不随意で不規則なオプソクローヌスやミオクローヌスがみられ，ときに失調，めまい，記銘力障害もみられる．合併腫瘍は乳癌が多く，抗 Ri 抗体がみられる．

5. 亜急性感覚性ニューロン症

亜急性に進行性の感覚性失調がみられ，四肢のしびれ，疼痛が多巣性にみられる．運動障害，自律神経障害を伴うこともある．後根神経節が主座である．合併腫瘍は肺癌が多く，乳癌，卵巣癌，肉腫，Hodgkin リンパ腫もみられる．抗 Hu 抗体が多く，CV2，CRMP-5 に対する抗体の報告もある．

6. Lambert-Eaton 筋無力症候群

易疲労性，下肢の筋力低下，腱反射低下と運動負荷直後の正常化，ときに脳神経障害，自律神経障害もみられる．筋電図は高頻度刺激による漸増現象を認める．合併腫瘍は

表 2　傍腫瘍性神経症候群の神経症候

"Classical syndrome" (PNS として認知度の高い病型)	"Non-classical syndrome" (PNS として生じうる病型)
中枢神経系の神経症候	
Encephalomyelitis	Brainstem encephalitis
Limbic encephalitis	Optic neuritis
Subacute cerebellar degeneration	Cancer associated retinopathy
Opsoclonus-myoclonus	Melanoma associated retinopathy
	Stiff person syndrome
	Necrotising myelopathy
	Motor neuron diseases
末梢神経系の神経症候	
Subacute sensory neuronopathy	Acute sensorimotor neuropathy
Chronic gastrointestinal pseudo-obstruction	Guillain-Barré syndrome
	Brachial neuritis
	Subacute/chronic sensorimotor
	Neuropathies
	Neuropathy and paraproteinaemia
	Neuropathy with vasculitis
	Acute pandysautonomia
神経筋接合部・筋の症候	
Lambert-Eaton myasthenic syndrome	Myasthenia gravis
Dermatomyositis	Acquired neuromyotonia
	Acute necrotising myopathy

（文献 1，2 より引用）

肺癌が多く，胃癌，胸腺腫もみられる．抗 VGCC 抗体が多く，シナプトタグミン，amphiphysin，CRMP-5 の抗体に対する報告もある．

抗腫瘍 / 神経抗体（表 3）

診断ガイドラインでは，抗腫瘍 / 神経抗体のうち「抗 Hu 抗体，抗 Yo 抗体，抗 CV2 抗体，抗 Ri 抗体，抗 Ma2 抗体，抗 amphihysin 抗体」を "well characterized"，「抗 Tr 抗体，抗 ANNA3 抗体，抗 PCA2 抗体，抗 Zic4 抗体，抗 mGluR1 抗体」を "partially characterized" に分類している．

PNS の診断上，抗腫瘍 / 神経抗体は重要な軸であり，腫瘍の局在や組織型などを推測できる．

一方，既知の抗腫瘍 / 神経抗体が陰性の例もありうる．

治療に必要な検査と診断

診断アプローチ

PNS の神経症候は，表 1，2 のように多彩であるが，PNS 以外でも起こり得る．したがって第一に PNS 以外の原因を鑑別する．

PNS を疑う場合，原因腫瘍を検索する．腫瘍が極めて小さいこともあり，約半数では腫瘍の発見が困難である．最初の検索で腫瘍が発見されない場合には経時的に検索する．関連の乏しい腫瘍が発見された場合は重複の可能性を考え検索する．また抗腫瘍 / 神経抗体を検索する．

診断ガイドライン

表 4，5 のような PNS の診断基準が提唱されている[1,2]．PNS 以外を除外したうえで，診断チャート（**図 1**）をもとに系統的診断を

表3　傍腫瘍性神経症候群の抗腫瘍／神経抗体

Antibody	No of patients reported	Identified by more than one laboratory	Paraneoplastic neurological syndrome	Tumours	% of antibody positive patients without cancer * (number of patients studied)	Frequency in c patients withou PNS (number studied)
Well characterised onconeuronal antibodies						
Anti-Hu (ANNA1)	> 600	Yes	Encephalomyelitis ; sensory neuronopathy ; chronic gastrointestinal pseudo-obstruction ; paraneoplastic cerebellar degeneration (PCD) ; limbic encephalitis	Small cell lung cancer (SCLC)	2% (200)	16% (196 SC (1% with titres similar to those patients with PNS)
Anti-Yo (PCA1)	> 200	Yes	Paraneoplastic cerebellar degeneration	Ovary, breast	2% (125)	1% (107)
Anti-CV2 (CRMP5)	> 100	Yes	Encephalomyelitis ; chorea ; sensory neuronopathy ; sensorimotor neuropathy ; chronic gastrointestinal pseudo-obstruction ; paraneoplastic cerebellar degeneration ; limbic encephalitis	SCLC thymoma	4% (47)	9% (74 SCLC
Anti-Ri (ANNA2)	61	Yes	Brainstem encephalitis	Breast, SCLC	3% (61)	4% (181 ovari cancer)
Anti-Ma2 (Ta)	55	Yes	Limbic/diencephalic encephalitis ; brainstem encephalitis/PCD	Testicular, lung	4% (55)	0% (350)
Anti-amphiphysin	20	Yes	Stiff person syndrome ; various syndromes	Breast, SCLC	5% (20)	0 % (25gynae cologicalcancer 1% (146SCLC
Partially characterised onconeuronal antibodies						
Anti-Tr (PCA-Tr)	28	Yes	Paraneoplastic cerebellar degeneration	Hodgkin's disease	11% (28)	0% (30)
ANNA3	11	No	Various syndromes	SCLC	9% (11)	0% (58)
PCA2	9	No	Various syndromes	SCLC	0% (8)	2% (58)
Anti-Zic4	8	No	Paraneoplastic cerebellar Degeneration	SCLC	12% (8)	16% (74)
Anti-mGluR1	2	No	Paraneoplastic cerebellar Degeneration	Hodgkin's disease	50% (2)	?

（文献1，2より

進める．

治療の実際

基本的な治療方針

1．原因となる腫瘍そのものに対する治療

それぞれのガイドラインに沿う．

2．介在している免疫反応に対する治療

抗腫瘍／神経抗体の除去，炎症過程の抑制，抗体産生の抑制などを目的に，血液浄化療法，ステロイド，免疫抑制剤，免疫グロブリ量療法（IVIg）などを単独または組合せ施行する．

現時点では十分なエビデンスはないが，体が一義的な病因である場合には，抗免疫法が有効である．それ以外の場合にも，早に免疫療法を施行すれば有効性が期待で禁忌，副作用に十分に注意する．

3．神経症候に対する対症療法

必要に応じて進める．

表 4　傍腫瘍性神経症候群の診断基準

＊確実例（definite）

① “classical” の神経症候の存在と，神経症候の出現の 5 年以内の悪性腫瘍の存在．

② “non-classical” の神経症候の存在と，抗腫瘍療法で消失もしくは有意に改善したもの．（免疫療法は併用していないもの，また自然寛解のある症候を除いたもの）

③ “non-classical” の神経症候の存在と，抗腫瘍 / 神経抗体の陽性所見と，神経症候の出現の 5 年以内の悪性腫瘍の存在．

④ “classical” もしくは “non-classical” の神経症候の存在と，特異性の高い（well characterized）抗腫瘍 / 神経抗体（anti-Hu, Yo, CV2, Ri, Ma2, amphihysin）の陽性所見と，悪性腫瘍が発見されていないもの．

＊疑い例（possible）

① “classical” の神経症候の存在と，抗腫瘍 / 神経抗体の陰性所見と，悪性腫瘍が未発見であっても潜在する腫瘍がある危険性が高いもの．

② “classical” もしくは “non-classical” の神経症候の存在と，ある程度特異性の高い（partially characterized）抗腫瘍 / 神経抗体の陽性所見と，悪性腫瘍が発見されていないもの．

③ “non-classical” の神経症候の存在と，抗腫瘍 / 神経抗体の陰性所見と，診断の 2 年以内の悪性腫瘍の存在．

（文献 1，2 より引用）

表 5　抗腫瘍 / 神経抗体を持たない典型的神経徴候（classical syndrome）で，腫瘍の併発を示唆する因子

典型的傍腫瘍性神経症候群	腫瘍の併発を示唆する因子
Limbic encephalitis（PLE）	免疫療法の効果が乏しい抗 VGKC 抗体陰性症例
Subacute cerebellar degeneration（PCD）	抗 VGCC 抗体陽性例 LEMS 合併例
Opsoclonus-myoclonus syndrome	5 歳以下の症例 40 歳以上の症例 免疫療法の効果が乏しい症例
Sensory neuronopathy（SSN）	40 歳以上で喫煙歴のある症例
Lambert-Eaton myasthenic syndrome（LEMS）	40 歳以上で喫煙歴のある症例
Dermatomyositis	40 歳以上の症例

LEMS: Lambert-Eaton myasthenic syndrome
VGKC: voltage gated potassium channel
VGCC: voltage gated calcium channel

Classical syndrome の治療方針

1. 辺縁系脳炎

- **抗腫瘍治療**：早期治療による改善例や進行停止例がある．
- **抗免疫療法**：IVIg，血漿交換，ステロイドの有効例がある．抗 Ma2 抗体陽性例では良好である．
- **対症治療**：精神症状に抗精神病薬，痙攣に抗痙攣薬．

2. 亜急性小脳変性症

- **抗腫瘍治療**：外科治療による改善例や進行停止例がある．
- **抗免疫療法**：早期の IVIg の有効例が散見される．血漿交換は一部に改善例がみられる．ステロイド，免疫抑制剤の効果は乏しい．抗 Tr 抗体を伴う Hodgkin 病では改善例がある．抗 Yo 抗体を伴う卵巣癌は効果が乏しい．
- **対症治療**：歩行，言語・嚥下リハビリテーション．

3. 傍腫瘍性オプソクローヌス / ミオクローヌス

- **抗腫瘍治療**：改善例があるが，多くは治療抵抗性である．

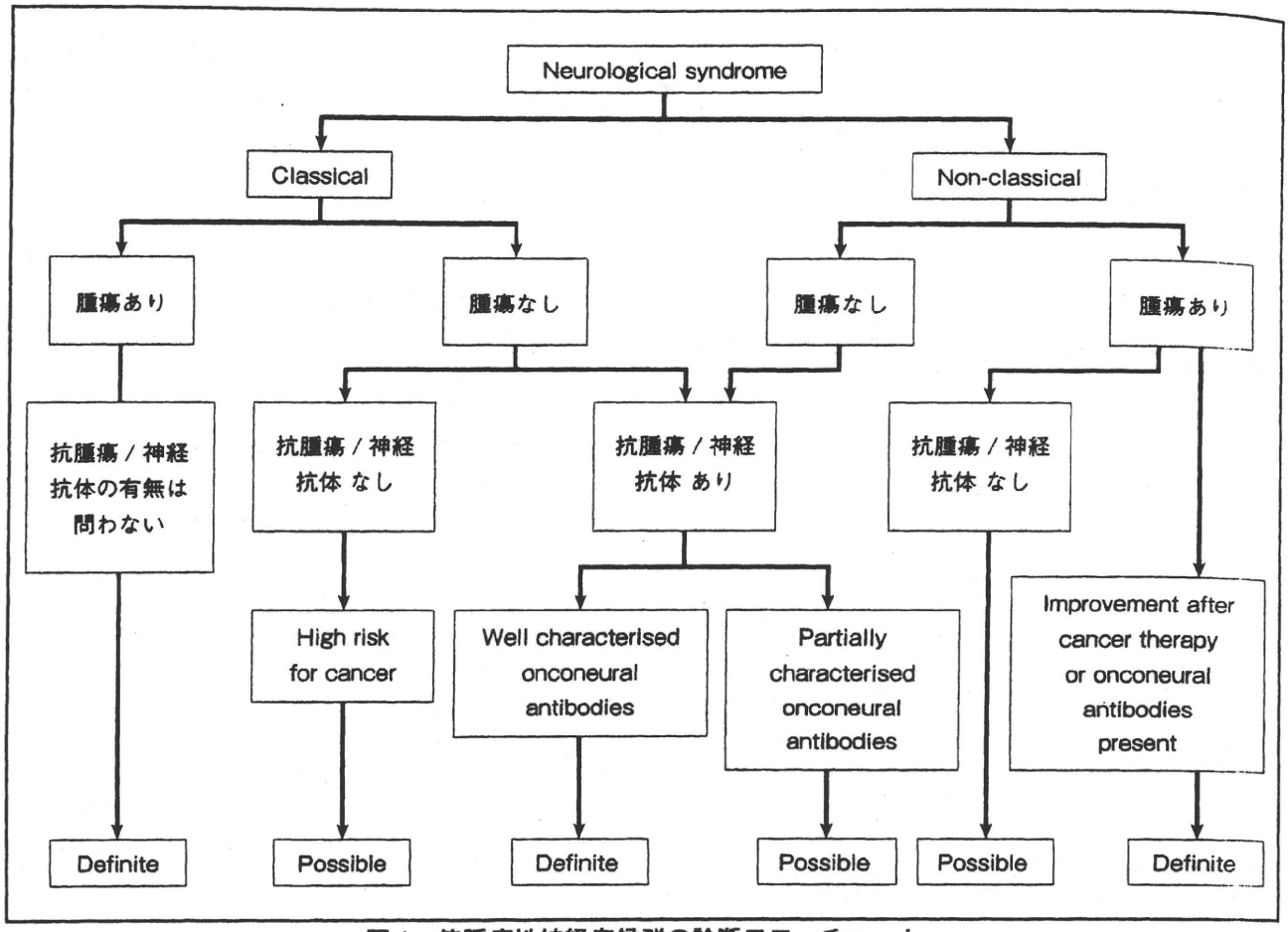

図1　傍腫瘍性神経症候群の診断フローチャート

●**抗免疫療法**：ステロイド，IVIg，免疫抑制剤による改善例があるが，一般に治療抵抗性である．protein A カラムによる血漿浄化療法の改善例がある．神経芽腫に伴う小児例ではステロイドやIVIgに反応性である．

●**対症治療**：オプソクローヌス，眼振，動揺視に，抗痙攣薬，バクロフェン，プロプラノロール，ミオクローヌスにクロナゼパム．

4. 亜急性感覚性ニューロン症

●**抗腫瘍治療**：外科治療による進行停止例があるが，一般に予後不良である．

●**抗免疫療法**：ステロイドは一般に無効である．血漿交換，IVIgの有効例がある．抗Hu抗体を伴う症例では免疫抑制剤の有効例がある．早期治療による有効例，進行停止例がある．

●**対症治療**：しびれ，疼痛に，carbamazepineなど抗痙攣薬，amitriptylineなど抗うつ薬．起立性低血圧に自律神経調節薬，症状によって心ペースメーカーを検討する．

5. Lambert-Eaton 筋無力症候群

●**抗腫瘍治療**：多くは改善を認める．

●**抗免疫療法**：ステロイド，azathioprineの免疫抑制剤，血漿交換，IVIgが行われる．腫瘍の非合併例では血漿交換，免疫抑制剤の併用の有効例がある．

●**対症治療**：3,4-diaminopyridine（3,4-DAP）は電位依存性カリウムチャンネル阻害剤であり，神経終末での活動電位を高いままに維持しCa^{2+}の流入を増やしシナプス小胞からのアセチルコリン遊

離促進作用がある（本邦では医薬品として入手できない）．抗コリンエステラーゼ阻害薬が使用される．

処　方　例

副腎皮質ステロイド

処方　プレドニゾロン　1 mg/kg/日
経口 または 点滴静注
＊パルス療法は，メチルプレドニゾロン　1,000 mg/日の点滴静注を3日間．

免疫グロブリン大量療法

処方　免疫グロブリン　400 mg/kg/日
点滴静注　5日間

血液浄化療法

血漿交換では1回の血漿処理量は2,000～4,000 mL（40～50 mL/kg）で多くは3,000 mL前後．免疫吸着では1回の血漿処理量は1,500～2,000 mL（40 mL/kg）で多くは2,000 mL前後．置換液は通常，5%ヒトアルブミン製剤または新鮮凍結血漿を用いる．血液流量は最大で100 mL/分，血漿処理流量は最大で25 mL/分で行う．処理回数は通常は1週間に2～3回，状況により翌週に2～3回を追加する．経過により月1回の維持治療を進める．

専門医に紹介するタイミング

PNSでは早期診断が重要である．疑いをもった時点で専門医受診を薦める．

専門医からのワンポイントアドバイス

- 早期診断が重要である．同時に悪性腫瘍の検索が重要である．
- 神経症候が腫瘍発見に先行することが多く，潜在する腫瘍の存在を示す重要な症候である．

文　献

1) Graus F, Delattre JY, Antoine JC et al：Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 75：1135–1140, 2004
2) Vedeler CA, Antoine JC, Giometto B et al：Paraneoplastic Neurological Syndrome Euronetwork. Management of paraneoplastic neurological syndromes：report of an EFNS Task Force. Eur J Neurol 13：682–690, 2006
3) 犬塚　貴：傍腫瘍性神経症候群の分子機構．老年医学update2004–05，日本老年医学会雑誌編集員会 編．メジカルレビュー社，pp174–181, 2004

Acta Neurol Scand 2010: 121: 251–256 DOI: 10.1111/j.1600-0404.2009.01198.x

ACTA NEUROLOGICA
SCANDINAVICA

Acute encephalitis with refractory, repetitive partial seizures (AERRPS): a peculiar form of childhood encephalitis

Sakuma H, Awaya Y, Shiomi M, Yamanouchi H, Takahashi Y, Saito Y, Sugai K, Sasaki M. Acute encephalitis with refractory, repetitive partial seizures (AERRPS): a peculiar form of childhood encephalitis. Acta Neurol Scand: 2010: 121: 251–256.

Objective –We conducted a nationwide multicenter study in Japan to elucidate the clinical and laboratory characteristics of acute encephalitis with refractory, repetitive partial seizures (AERRPS). *Materials and methods* – Clinical and laboratory features, treatment, and outcome were assessed using a structured questionnaire. *Results* –Twenty-nine children were enrolled in the study. Refractory and repetitive partial seizures accompanied by fever were the cardinal clinical features. Partial seizures consisted principally of eye deviation or facial twitching, being periodically repeated during the acute phase. These seizures were refractory to conventional anticonvulsants and were only suppressed by high-dose intravenous barbiturate administration. Rhythmic activities on electroencephalography and non-specific cerebral atrophy on neuroimaging were common. Serum or cerebrospinal antibodies against GluRε2 were positive in six patients. General prognosis was unfavorable due to intractable epilepsy and cognitive deficits. *Conclusion* – Based on the peculiar and homogenous features, AERRPS can be regarded as a distinct clinical entity.

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Key words: AERRPS; epilepsy; status epilepticus

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Accepted for publication March 11, 2009

Introduction

Acute encephalitis/encephalopathy in children is often associated with seizures, and status epilepticus (SE) is one of the presenting symptoms. In most cases, seizures appear transiently during the acute phase and can be controlled by standard therapy. However, 2.7–6.7% of the central nervous system (CNS) infection results in the evolution of epilepsy (1). This 'post-encephalitic' epilepsy is characterized by intractable partial seizures associated with mesial temporal sclerosis and cognitive impairment (2). Post-encephalitic epilepsy is observed after a latent period of several months or years (3).

In contrast, in some cases with intractable epilepsy following encephalitis exists, without any latent period. This population was first recognized by Awaya et al. in 1986, and has been subsequently reported in Japan as 'acute encephalitis with refractory, repetitive partial seizures (AERRPS)' (4, 5). AERRPS has three cardinal features in common: (i) acute encephalitis of unknown origin, without underlying developmental delay or prior unprovoked seizures; (ii) presenting with repetitive and refractory partial seizures during the acute phase, referred to as 'refractory partial SE', which is followed by, (iii) continuous transition to intractable epilepsy without a latent period. It is distinct from encephalitis or acute neurological insults of known origin (shown in Table 1). AERRPS has not been perceived in Western countries, although it is widely recognized in Japan. Moreover, systematic analyses with regard to detailed clinical characteristics of AERRPS have not been performed.

In the present study, we describe the clinical characteristics of 29 patients with AERRPS based on the data of the first nationwide multicenter study conducted in Japan. These cases have some

Table 1 Differential diagnosis of AERRPS

Viral encephalitis and virus-associated encephalopathy
Herpes simplex encephalitis
Japanese encephalitis
Acute necrotizing encephalopathy
Acute encephalopathy with late reduced diffusion
Acute infantile encephalopathy
Predominating over frontal lobe
Acute limbic encephalitis
Paraneoplastic
Non-paraneoplastic
Anti-VGKC antibody associated
Anti-NMDA-R antibody associated
Miscellaneous
Metabolic encephalopathy
Organic aciduria
Urea cycle disorder
Fatty acid oxygenation disorder
Mitochondrial disease
Epilepsy
Rasmussen's encephalitis
Migrating partial seizures in infancy
Other known acute neurological insults

peculiar features in common; such as acute onset of extremely refractory and repetitive partial seizures, presumed autoimmune inflammatory pathomechanism, and poor neurological outcomes.

Patients and methods

Among acute encephalitis of unknown origin in childhood, AERRPS was defined as those fulfilling three aforementioned features. Acute neurological illnesses of known origin (shown in Table 1) were extensively studied and thus were excluded.

This is a retrospective, multicenter, questionnaire-based study. First, we inquired from principal medical institutions whether or not they had experience on cases identical to or analogous to AERRPS. Of 85 institutions consulted, 22 of them responded to us. Then, we sent a detailed multiple-choice-based questionnaire to these institutions, and they agreed to participate in our study. The items in the questionnaire included patient profiles, precipitating factors before onset, seizure types and their duration, concomitant neurological symptoms, blood and cerebrospinal fluid (CSF) studies, electrophysiological and neuroradiological findings, treatments and their efficacy, and outcomes.

There were 39 cases collected from 22 hospitals between 1990 and 2006. After a careful review of the cases, 10 were concluded not to be identical to AERRPS and thus excluded for the following reasons: one had no partial seizure; four required neither intravenous barbiturates nor benzodiazepines to alleviate seizures; two did not develop subsequent epilepsy; three were diagnosed as acute limbic encephalitis; and one had insufficient medical records. Consequently, 29 patients fulfilled the criteria for AERRPS and were included in the study. Tables S1–S3 detail the clinical characteristics and investigations of the patients with AERRPS. Clinical details of Patients 22–24 have been reported previously (6). Case reports of patients 7, 9, 12, 14, 29, and 25 have also been published elsewhere in Japanese journals. Because of the retrospective nature of the study, written informed consent was not always acquired.

Seizures were classified according to the revised clinical and electroencephalographic classification of epileptic seizures from the Commission on Classification and Terminology of the International League Against Epilepsy (7). Routine blood and CSF studies, electroencephalography (EEG) and magnetic resonance (MR) imaging were performed in all patients. Serum and CSF antibodies against glutamate receptor epsilon 2 subunit (GluRε2) were analyzed as previously described (8). With respect to the efficacy of treatment, we used the following scale according to the reduction in seizure frequency: complete (seizure free), excellent (seizure reduction of 75% or more), good (50–75% reduction), and poor (50% or less reduction).

Results

Patient profiles

Among the 29 patients (M:F = 19:10), the age of disease onset ranged from 1 to 14 years (6.8 ± 4.0). Fourteen patients were at their school age. The family histories of the patients were unremarkable. Four patients had experienced febrile convulsions but none had underlying epilepsies or other neurological abnormalities.

Precipitating factors

Out of the 29 patients, 26 bore evidence of antecedent febrile illness before the onset of neurological symptom. The latency between antecedent fever and the onset of neurological symptoms was 4.9 ± 2.3 days (range: 2–10 days).

Characteristics in the acute phase

The acute phase was defined as the period during which patients presented with persistent fever or necessitated continuous anticonvulsant infusion to suppress seizures or both as described below. The duration of this phase ranged from 15 to 312 days.

Clinical manifestations – All 29 patients presented with fever at the onset of neurological symptoms, as well as throughout the acute phase. High-grade fever was observed in 23 patients (higher than 39°C), which was persistent in most cases. Intermittent fever concomitant with aggravation of seizures was also present.

The initial neurological manifestations were seizures in 20 patients, altered consciousness in seven patients, visual agnosia in one patient, and unidentified in one patient, whereas the manifestations in one patient could not be identified.

Seizures were constantly the most prominent and significant manifestation of AERRPS (Table 2). Partial seizure was invariable, and in the vast majority of cases, it was the predominant seizure type: Most commonly, these seizures took the form of eye deviation (69%) and hemifacial twitching (62%), with an occasional development to ipsilateral clonic seizure (48%). Autonomic manifestations such as apneic spell were not uncommon (28%). Seizures usually lasted 1–5 min (83%) and terminated spontaneously, but often occurred in clusters without recovery of consciousness during the interval periods. Within a week, they increased in frequency despite treatment and became full-blown, being periodically repeated every 5–10 min in 14 cases (48%).

Impairment of consciousness was also common. Other neurological symptoms included psychiatric and movement disorders, and memory impairment.

Table 2 Clinical features and laboratory findings

	No. of patients
Acute symptoms	
Seizure	
Simple partial	1
Complex partial	25
Secondary generalized	24
Generalized tonic–clonic	8
Tonic	8
Myoclonic	4
Impairment of consciousness	24
Psychiatric disorders	9
Movement disorders	12
Memory impairment	8
Laboratory findings	
Blood	
High ferritin	4/4 (221–2,370 mg/dl)
Positive anti-GluRε2 Ab	6/9
CSF	
Pleocytosis (>5/mm^3)	19/29 (6–25/mm^3)
High protein concentration (>45 mg/dl)	5/29 (46–85 mg/dl)
High neopterin	4/4 (246–1,154 nmol/ml)
High neuron specific enolase	2/6 (33–34 mg/dl)
Positive anti-GluRε2 Ab	5/9

Ab, antibody.

EEG findings – A total of 71 EEGs was studied (1–289 days of admission). During the first 14 days of neurological illness, pretreatment EEGs consisted principally of high-voltage slow background activity (7/9, 78%). At the later stage, all 29 patients developed interictal epileptiform discharges with a variety of spatial distribution. Multiple independent foci were observed in 15 patients (54%). Seven patients (24%) were found to have epileptic foci that migrated during the acute phase. Ictal discharges were recorded in 24 patients. They typically began with localized rhythmic activities consisting of spikes or sharp waves and progressively involved the adjacent areas, thus leading to secondary generalization. Ictal discharges disappeared spontaneously within a few minutes and then reappeared, being periodically repeated every 5–10 min.

Neuroimaging – Magnetic resonance imaging (MRI) was examined at least once in all patients. Those performed within 7 days after onset (14 cases) revealed mild brain edema in two patients but were otherwise normal. Subsequently, six patients showed hippocampal or amygdaloid hyperintensities on fluid-attenuated inversion recovery (FLAIR) without the evolution of epileptic foci at the corresponding area. Abnormal symmetrical T2 hyperintensity in the periventricular white matter and claustrum were found in four and two patients, respectively.

Laboratory examinations – The routine blood cell count and biochemistry, as well as blood levels of glucose, ammonia, and lactate were generally unremarkable. The data on inflammatory and autoimmune markers are summarized in Table 2. Plasma amino acid and urinary organic acid revealed no abnormalities. Extensive virological studies were also conducted. Herpes simplex virus was serologically negative or had remotely infected 27 patients who were examined. Serological studies for cytomegalovirus (n = 16), Epstein–Barr virus (n = 20), and human herpes virus 6 (n = 11) showed no serial elevation of antiviral titer. Viral cultures from CSF or throat swabs in 14 patients were all negative.

Treatment – Intravenous barbiturates were administered in 22 patients, of whom 13 showed complete, three excellent, and four good responses, while none were poor. Pentobarbital was most frequently used (15 patients), followed by thiopental (five) and thyamiral (four). The effective and maximal doses of pentobarbital were 4.22 ± 1.82 and 4.98 ± 2.06 mg/kg/h, respectively. The EEG backgrounds when seizure had been suppressed were high voltage slow wave in one, burst-suppres-

sion pattern in 14, and complete suppression in three. The duration of barbiturate infusion ranged from 4 to 312 days (52.3 ± 72.6 days).

Benzodiazepines, mainly midazolam, were administrated in 25 patients, of whom three showed complete, five good, and 17 poor effects. The maximal dose of midazolam was 0.47 ± 0.33 mg/kg/h. Diazepam was used in bolus injection successfully in five of 12 patients, which were only temporarily effective. The efficacy of intravenous lidocaine (1.5–6 mg/kg/h) and phenytoin (5–25 mg/kg/day) were limited (8% and 30%, respectively), transient, and incomplete.

Immunomodulatory treatments were challenged in some cases. Twelve patients were treated with corticosteroids mostly by intravenous methylprednisolone, of whom two were effective. Intravenous immunogloburin (IVIG) in 13 patients did not result to any improvement. One patient underwent plasma exchange, which was unsuccessful.

Chronic phase

Course and prognosis

Two of the 29 patients dropped out, and therefore, the remaining 27 patients received follow-up, with a mean period of 60.9 months (ranging from 8 to 194 months). All patients had residual epilepsy. As defined in the diagnostic criteria, all patients showed continuous evolution from encephalitis to residual epilepsy without a latent period. The types of seizures were essentially the same as those in the acute phase except for scarce secondary generalization. Most patients had residual cognitive impairment. Intelligence quotients (IQs), measured using the Wisconsin Intelligence Scale for Children-III (WISC-III), were less than 70 in 16 patients and below 20 in 10 patients. All patients who had the antibody against GluRε2 were found to have residual cognitive impairment. Other residual neurological deficits included memory impairment (15%), autistic tendency (22%), hyperkinetism (15%), learning disability (15%), personality change (15%), and emotional instability (22%). One patient suddenly died of unknown cause 9 years after onset. Serial MRI scanning revealed diffuse brain atrophy after a month or more. Hippocampal or amygdaloid signal abnormalities remained unchanged in four of six patients.

Discussion

The clinical entity of AERRPS arose in 1986, when Awaya et al. described five cases of 'peculiar onset post-encephalitic epilepsy' (9). In his investigation into post-encephalitic epilepsy, he found a novel subtype of epilepsy characterized by refractory partial seizures persisting from the onset of encephalitis to the convalescent phase. Meanwhile, in 1989, Shiomi advocated a subgroup of encephalitis characterized by very frequent seizures that can be suppressed only by intravenous barbiturates. It should be noted that Awaya defined this illness as post-encephalitic 'epilepsy' and therefore, discussed mainly its epileptogenesis (4), while Shiomi classified it as a subtype of 'encephalitis' and put emphasis on the symptoms in the acute phase. These two clinical entities shared some characteristics: acute onset of illness, very frequent partial seizures, extremely refractory trait of seizures, inconspicuous switchover from encephalitis to subsequent epilepsy, and residual cognitive impairment. In 2001, we proposed the term AERRPS to integrate the characteristics of these entities (5). To date, more than 30 cases compatible to this condition have been accumulated in Japan (4).

We report the first multicenter collaborative study on acute encephalitis with refractory, repetitive partial seizures (AERRPS) to clarify its clinical features. The definitive features became evident as a result of this study. Several clinical aspects that seem to be characteristics of AERRPS are vital for the diagnosis, and these are listed in the diagnostic criteria (Table 3). EEG findings in AERRPS, particularly ictal recordings, are of diagnostic significance. The repetitive EEG pattern of ictal discharges has been described (6). In

Table 3 Diagnostic criteria for AERRPS

Mandatory:

1. Acute onset of seizures or consciousness impairment, in the absence of underlying developmental delay or prior unprovoked seizures
2. Extraordinarily frequent and refractory partial seizures, referred to as 'refractory partial SE': The patients usually show partial seizures characterized by eye deviation and facial twitching which repeat within an interval of 30 minutes or less, and necessitate long-term anesthesia (2 weeks or more) with intravenous barbiturates or benzodiazepines to attain burst-suppression coma on EEG
3. Continuous switchover to refractory epilepsy without a latent period

Supportive findings:

1. Antecedent febrile illness, which occurs 2–10 days before the onset of neurological symptoms
2. Persistent fever during the acute phase of illness
3. CSF findings: mild pleocytosis or slight increase in protein (inflammatory markers such as IL-6 or neopterin may be upregulated) or both
4. EEG: slow background during the acute phase and multifocal spikes during the chronic phase (ictal EEG reveals variable foci of epileptiform discharges and frequent secondary generalization)
5. MRI: no specific abnormalities except for occasional T2/FLAIR hyperintese signal of mesial temporal lobe
6. Profound neurological sequelae: cognitive deterioration, psychiatric disorders, and memory impairment, as well as occasional motor disability

contrast, MRI is not always helpful in establishing diagnosis because there are no specific neuroradiological abnormalities in AERRPS. Hyperintensities in limbic structures on MRI, which were observed in six patients in our series, are presumably due to refractory SE. This finding is consistent with a recent report on a Taiwanese series (10).

Clinical entities resembling AERRPS have also been reported elsewhere outside Japan. Kramer et al. (11) reported five cases of refractory SE which is presumably caused by encephalitis of unknown origin. In their report, they identified the preceding febrile illness, persistent seizures despite induced burst suppression coma, and negative results for the cause of seizures, as common features. They postulated that this severe refractory type of SE is due to relatively mild encephalitis. Mikaeloff et al. (12) reported 14 cases of 'devastating epileptic encephalopathy in school-age children', which is characterized by prolonged SE following non-specific febrile illness without any latent period. These cases are slightly different from AERRPS because they are limited to the cases with school-age onset and perisylvian involvement. These facts clearly demonstrate that the clinical entities similar to AERRPS are distributed all over the world.

Limbic encephalitis (LE) is characterized by limbic seizures, short-term memory loss, and psychiatric symptoms (13). Recent studies have revealed that acute non-paraneoplastic LE is related to the anti-voltage-gated potassium channel antibody (14, 15) or antibodies that reacted with the *N*-methyl-D-aspartate receptor (16, 17). AERRPS can be distinguished from LE for the following reasons. First, psychiatric disorders and memory impairment are uncommon and seldom present as initial predominating features in AERRPS. Second, no case has been described in which AERRPS was presumably caused by a neoplasm. Third, AERRPS, but not LE, shows a uniformly unfavorable outcome with neurological sequelae. Nevertheless, there is a report of an atypical clinical presentation of paraneoplastic LE with pharmacoresistant epilepsy lacking memory loss or psychiatric symptoms (18). Hence, there is a possibility of some overlap between AERRPS and LE.

The process of epileptogenicity in AERRPS is not well understood. Epilepsy secondary to encephalitis is reported to occur after a latent period of 3.82 ± 3.7 years (1). In contrast, there is no definite seizure-free period in AERRPS. We hypothesize that extraordinary epileptogenicity prolongs seizures even after the acute phase and therefore makes the initiation of epilepsy inconspicuous.

The etiology of AERRPS remains to be clarified. In the present study, several lines of evidence supported the hypothesis that some of AERRPS are associated with CNS inflammation. First, unlike epilepsy, neurological manifestations are preceded by febrile illness and are accompanied by persistent fever. Second, the levels of neopterin, which are known to be up-regulated in macrophage activation syndrome, are elevated in CSF; however, others seem to be irrelevant to the inflammatory process. Not all the patients showed increased mononuclear cells or elevated CSF protein concentration. In the previous reports, Kramer et al. maintained an 'inflammatory' theory (11), whereas Mikaeloff et al. objected to this (12). Taken together, it is possible that AERRPS and similar disorders are caused by multiple etiologies with a common clinical phenotype.

The involvement of the inflammatory process permits us to speculate that a specific infectious agent causes AERRPS; however, this is unlikely because extensive viral studies were all negative. Another possibility is an autoimmune mechanism. It is intriguing that the serum or CSF of some patients with AERRPS was positive for antibodies against GluRε2. Ito et al. first reported a patient with AERRPS who had this antibody (19). This antibody is not specific to AERRPS but is found in patients with various neurological diseases, including intractable epilepsy, Rasmussen encephalitis, and other forms of encephalitis (8). However, the early appearance (0–20 days after onset) of antibodies against GluRε2 in CSF suggests that GluR autoimmunity contributes to the onset of encephalitis (20).

In conclusion, a novel clinical syndrome designated AERRPS is characterized by definite hallmarks. AERRPS is currently defined solely by its clinical characteristics, and thus further investigation into its pathomechanisms is necessary.

Acknowledgements

We thank Drs Shihoko Kimura-Ohba, Takahito Inoue, Sawa Yasumoto, Katsuya Yamamoto, Masaru Takayanagi, Jun Tohyama, Kimio Minagawa, Kazuhiro Haginoya, Eiji Hattori, Shinichi Hirabayashi, Akihisa Okumura, Tamami Yano, Kenji Yokochi, Rie Miyata, Hisashi Kawashima, Masahiro Kikuchi, Susumu Miyake, Mana Kurihara, Takeshi Miyamoto, Akimitsu Watanabe and Toshihiko Nishida, for providing clinical information of their patients. This work was supported by a research grant for nervous and mental disorders from the Ministry of Health, Labour and Welfare, Japan (grant number 17A-11).

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical features.

Table S2. Laboratory, EEG, and MRI findings.
Table S3. Treatment and Outcome.

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References

1. Marks DA, Kim J, Spencer DD, Spencer SS. Characteristics of intractable seizures following meningitis and encephalitis. Neurology 1992;**42**:1513–8.
2. Chen YJ, Fang PC, Chow JC. Clinical characteristics and prognostic factors of postencephalitic epilepsy in children. J Child Neurol 2006;**21**:1047–51.
3. Trinka E, Dubeau F, Andermann F et al. Clinical findings, imaging characteristics and outcome in catastrophic post-encephalitic epilepsy. Epileptic Disord 2000;**2**:153–62.
4. Awaya Y, Fukuyama Y, Hayashi K, Osawa M. Acute non-herpetic encephalitis with severe refractory status epilepticus – its overwhelming ictogenicity, epileptogenicity, long-term prognosis and review of the literature. No To Hattatsu 2007;**39**:138–44. (in Japanese).
5. Sakuma H, Fukumizu M, Kohyama J. Efficacy of anticonvulsants on acute encephalitis with refractory, repetitive partial seizures (AERRPS). No To Hattatsu 2001;**33**: 385–90. (in Japanese).
6. Saito Y, Maegaki Y, Okamoto R et al. Acute encephalitis with refractory, repetitive partial seizures: Case reports of this unusual post-encephalitic epilepsy. Brain Dev 2007;**29**: 147–56.
7. The Commission on Classification and Terminology of the International League against Epilepsy. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 1981;**22**:489–501.
8. Takahashi Y, Mori H, Mishina M et al. Autoantibodies and cell-mediated autoimmunity to NMDA-type Glu-Repsilon2 in patients with Rasmussen's encephalitis and chronic progressive epilepsia partialis continua. Epilepsia 2005;**46**:152–8.
9. Awaya Y, Fukuyama Y. Epilepsy sequelae of acute encephalitis or encephalopathy (3rd report). Jpn J Psychiatr Neurol 1986;**40**:385–7.
10. Shyu CS, Lee HF, Chi CS, Chen CH. Acute encephalitis with refractory, repetitive partial seizures. Brain Dev 2008;**30**:356–61.
11. Kramer U, Shorer Z, Ben-zeev B, Lerman-sagie T, Goldberg-stern H, Lahat E. Severe refractory status epilepticus owing to presumed encephalitis. J Child Neurol 2005;**20**: 184–7.
12. Mikaeloff Y, Jambaque I, Hertz-pannier L et al. Devastating epileptic encephalopathy in school-aged children (DESC): a pseudo encephalitis. Epilepsy Res 2006;**69**:67–79.
13. Tuzun E, Dalmau J. Limbic encephalitis and variants: classification, diagnosis and treatment. Neurologist 2007; **13**:261–71.
14. Vincent A, Buckley C, Schott JM et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain 2004;**127**:701–12.
15. Pozo-rosich P, Clover L, Saiz A, Vincent A, Graus F. Voltage-gated potassium channel antibodies in limbic encephalitis. Ann Neurol 2003;**54**:530–3.
16. Ances BM, Vitaliani R, Taylor RA et al. Treatment-responsive limbic encephalitis identified by neuropil antibodies: MRI and PET correlates. Brain 2005;**128**: 1764–77.
17. Dalmau J, Tuzun E, Wu HY et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;**61**:25–36.
18. Kerling F, Blumcke I, Stefan H. Pitfalls in diagnosing limbic encephalitis – a case report. Acta Neurol Scand 2008;**118**:339–42.
19. Ito H, Mori K, Toda Y, Sugimoto M, Takahashi Y, Kuroda Y. A case of acute encephalitis with refractory, repetitive partial seizures, presenting autoantibody to glutamate receptor Gluepsilon2. Brain Dev 2005;**27**:531–4.
20. Takahashi Y. Infections as causative factors of epilepsy. Future Neurol 2006;**1**:291–302.

INTERNAL MEDICINE

□ CASE REPORT □

Expression of Various Glutamate Receptors Including *N*-Methyl-D-Aspartate Receptor (NMDAR) in an Ovarian Teratoma Removed from a Young Woman with Anti-NMDAR Encephalitis

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Abstract

A 21-year-old woman developed psychiatric symptoms, progressive unresponsiveness, generalized seizures, severe dyskinesia, marked fluctuation of blood pressure, and hypersalivation after a flu-like episode. Anti-glutamate receptor (GluR)ε2 and anti-*N*-methyl-D-aspartate receptor (NMDAR) antibodies were positive in both her serum and CSF. After she recovered five months later she underwent surgery to remove a right ovarian teratoma. Immunohistochemical examinations of her teratoma disclosed abundant expression of various GluRs including NR2B subunit of NMDAR, GluR1, and GluR2/3. These immunoreactivities of GluRs were seen not only in small areas of neural tissue identified as anti-glial fibrillary acidic protein (GFAP)-immunoreactive areas but also in other large areas of undifferentiated neuroepithelial tissue without GFAP immunoreactivity. Our findings strongly support the recent idea that neural elements in ovarian teratoma play an important role in the production of antibodies to NMDARs in anti-NMDAR encephalitis. Additionally, the study of control ovaries clearly showed NR2B-related immunoreactivity in the cytoplasm of oocytes, indicating that the normal ovary itself has expression of NMDARs. This finding might provide a clue to understand the pathogenesis of this disease in female patients without ovarian teratoma.

Key words: limbic encephalitis, paraneoplastic syndrome, ovarian teratoma, glutamate receptor, *N*-methyl-D-aspartate receptor (NMDAR)

(Inter Med 49: 2167-2173, 2010)
(DOI: 10.2169/internalmedicine.49.4069)

Introduction

A unique limbic encephalitis that predominantly affects young females and exhibits various manifestations including initial psychiatric symptoms, and subsequent central hypoventilation, intractable seizures, dysautonomia and prominent orofacial dyskinesia has been noted (1-3). Recently a causative relationship between such encephalitis and ovarian teratoma has been proposed (4-6) and in patients with this disorder a new anti-neural antibody for the NR1/NR2 heteromers of *N*-methyl-D-aspartate receptor (NMDAR) (NMDAR complex composed of NR1+NR2A or NR2B) has been identified as a disease-specific hallmark (2). This disease is, therefore, called anti-NMDAR encephalitis.

Ionotropic glutamate receptors (GluRs) are subdivided into three major subtypes: *N*-methyl-D-aspartate (NMDA)-type, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type and kainate (KA)-type (7). NMDA-type GluRs (NMDARs) have heterotetramer complex structures composed of NMDAR subunits (8). NMDAR subunits have the two nomenclatures from rats and mice, and NR1, NR2

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A, and NR2B in rat-derived nomenclature have almost homologous sequences with GluRζ1, GluRε1, and GluRε2 in mice-derived nomenclature, respectively.

In this study we examined the immunohistochemical expression of GluRs in the ovarian teratoma obtained from a young woman with anti-NMDAR encephalitis, and showed the characteristics of GluR expression in the tumor.

Case Report

A 21-year-old woman developed orthostatic fainting, appetite-loss and insomnia after a flu-like episode. During the subsequent two weeks, a progressive psychiatric state with emotional instability and abnormal behavior appeared. At admission she showed confusion and agitation, but physical and neurological findings were unremarkable. Routine laboratory data were normal except for an increased number of leukocytes in peripheral blood (12,900/μL). Brain MRI showed no specific findings, but lumbar puncture revealed lymphocytic pleocytosis (124 cells /μL) with normal glucose and protein concentrations. Bacterial and viral studies, including PCR for herpes simplex virus, were all negative. She soon started to experience recurrent generalized tonic-clonic seizures, severe dyskinesia in face and arms, hyperthermia, marked fluctuation of blood pressure, and hypersalivation. The symptoms were not relieved by methylprednisolone pulse therapy (1 g/day for 3 days) and subsequent intravenous administration of immunoglobulin (400 mg/kg/day for 5 days), and her convulsions were unresponsive to conventional anti-epileptic drugs. She was finally treated with intravenous administration of thiopental sodium (100 mg/hour) and mechanical ventilation. After two months she was released from mechanical ventilation, and her symptoms gradually subsided. Five months after admission, her cognitive function had recovered, and a pelvic CT disclosed a right ovarian tumor of 52 mm in diameter. She underwent unilateral salpingo-oophorectomy, and a mature teratoma was found. After this operation she returned to her university.

Materials and Methods

Analysis of anti-neural antibodies in serum and CSF

Using GluRε2-cDNA from mice and immunoblotting technique, IgG and IgM-antibodies to whole molecules of GluRε2 (NR2B) were examined (9). Recombinant B18 cells expressing cDNA of GluRε2 and non-recombinant A1 cells were cultured for 48 hours with doxycycline (1 μg/mL). Supernatants of cell extracts were subjected to SDS-PAGE, and the gels were transferred to nitrocellulose membranes. Each membrane was cut into 20 strips after overnight blocking with the blocking buffer (0.02 M Tris HCl, 0.16 M NaCl, 0.05% bovine serum albumin). The strips of B18 and A1 were reacted with patient serum (diluted 20-fold with blocking buffer) or CSF (diluted 15-fold with blocking buffer) for 48 hours at 4℃, and were stained by alkaline phosphatase-labeled second antibodies (IgG or IgM) (Jackson ImmunoResearch, West Grove, Philadelphia, PA, U.S.A.). The presence of antibodies against GluRε2 was judged by a positively stained band with molecular size around 180 kDa, which was found only on the B18 strip and not on the A1 strip.

Detection of anti-NMDAR antibody was carried out as follows: cDNAs encoding NR1 (GluN1) and NR2B (GluN2 B) (Gene Bank accession number NM-008169, NM-008170, NM-008171, respectively) were ligated into the expression vectors and transfected into human embryonic kidney (HEK) 293 cells in the media containing 10 μM MK-801 using Lipofectamine (Invitrogen). Twelve hours after transfection, HEK-293 cells were fixed in 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS, pH 7.4) for 20 minutes. After non-specific binding was blocked with 10% goat serum in PBS, these cells were incubated with patient sera (1:40) or cerebrospinal fluid (1:2) overnight at 4℃ and then with FITC-conjugated rabbit anti-human IgG (BD Biosciences) for 30 min at room temperature. *SlowFade* gold anti-fade reagent (Molecular Probes) was applied to the slides and the staining was observed under a fluorescence microscope. NMDAR expression on the cell surface was confirmed with the rabbit antibodies against each of the NMDAR subunits, NR1, NR2A and NR2B.

Immunohistochemical examination of ovarian tumor

Serial sections were prepared from a formalin-fixed, paraffin-embedded block of the ovarian teratoma and a Ventana XT automated immunohistochemistry system (Ventana Medical Systems, AZ) was employed. The primary antibodies used, dilutions, and the pretreatment procedures were as follows: anti-glial fibrillary acidic protein (GFAP) (Ventana, AZ, without dilution), anti-phosphorylated neurofilament (SMI-31, Sternberger Monoclonals, Baltimore, MD, ×2000), anti-human synaptophysin (Dako, Glostrup, Denmark, × 100), anti-NR 1 (AB1516, Chemicon, Temecula, CA, ×100), anti-NR2A (clone A12W, Upstate, Lake Placid, NY, ×50, microwave treatment in citrate buffer), anti-NR2B (Zymed, South San Francisco, CA, ×50), anti-GluR 1 (AB1504, Chemicon, Temecula, CA, ×5, microwave in citrate buffer), and anti-GluR 2 / 3 (AB1506, Chemicon, Temecula, CA, × 10, microwave in citrate buffer). GluR1 and GluR2/3 are pharmacologically classified into the AMPA type. Positive control sections were prepared from blocks including two ovarian tissues obtained from 21- and 29-year-old females at autopsy, and human temporal lobe and cerebellum. Negative control sections were treated in the same way except that the primary antibodies were replaced with normal bovine serum.

Prior to the study, detailed informed consent was obtained from the patient following a clear explanation of the purpose of the study. Our study protocol was approved by the local ethics committee.

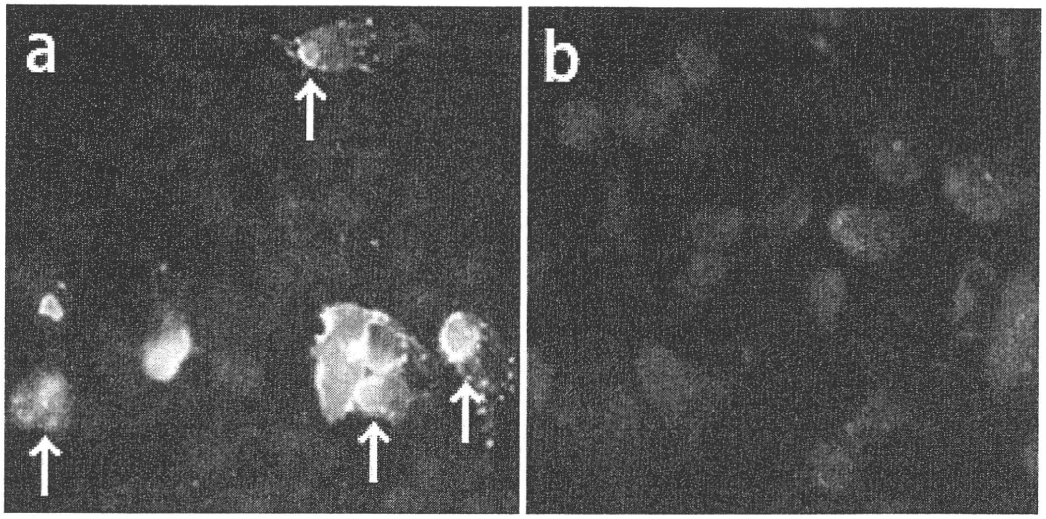

Figure 1. Immunohistochemical demonstration of antibodies against NMDAR. The serum of the patient showing positive immunoreactivity against heteromers of NR1 and NR2B subunits of NMDAR. a: serum of the patient, b: serum of a control patient without this disorder. Arrows indicate positively stained HEK cells. Immunofluorescence staining (×400).

Results

Detection of anti-neural antibodies in the serum and CSF of our patient

IgM-antibodies to whole molecules of GluRε2 were detected in serum obtained on day 50, and IgG-antibodies to whole molecules of GluRε2 were seen in CSF taken the same day. Both serum and CSF specifically reacted with HEK-293 cells expressing heteromers of NR1/NR2B (Fig. 1).

Histopathological and immunohistochemical findings of ovarian tumor

The capsular layers in this cystic tumor contained squamous epithelium, exocrine and sebaceous glands, hair follicles, fat, and neural tissues (Fig. 2-A and C). GAFP-immunoreactive areas showed a band-like or small dot-like distribution in the mural tumor tissues (Fig. 2-B and D), and among them many fibrous structures that were positively stained by an anti-phosphorylated neurofilament antibody were seen (Fig. 2-E, F and G). Other neural tissues were widely distributed in the areas with no expression of GFAP: they contained many small cells that were labeled by anti-phosphorylated neurofilament and anti-synaptophysin antibodies, and some of them showed epithelial pseudo-rosette formation (Fig. 3-F and G). A small number of anti-phosphorylated neurofilament antibody-positive fibrous structures were also observed in these areas with no immunoreactivity for GFAP (Fig. 2-A, B and H). Extensive areas of this tumor, including both mature neural tissues with expression of GFAP and immature neuroepithelial tissues without expression of GFAP, were specifically immunolabeled by anti-NR 2B and GluR 1, and GluR 2/3 antibodies (Fig. 3-C, D and E), while anti-NR1 and NR2A antibodies did not produce any significant immunoreactivities (Fig. 3-A and B). In two normal appearing ovaries anti-GFAP, anti-phosphorylated neurofilament, anti-synaptophysin, anti-NR1 and NR2A antibodies showed no immunoreactivities (Fig. 4-A), but anti-NR 2B, GluR 1 and GluR 2/3 antibodies produced faint immunoreactivity: this was most clearly seen on the sections stained by an anti-NR 2B antibody (Fig. 4-B), where the cytoplasm of oocytes was specifically immunolabeled (Fig. 4-C). Neurons and astrocytes in positive control sections were immunoreactive for some or all anti-NR2B, GluR1 and GluR2/3 antibodies (Fig. 4-D and E). However, no significant immunoreactivity was observed in negative controls.

Discussion

Limbic encephalitis is a disorder ascribed to diverse causes, and several antibodies against neural surface antigens were identified in autoimmune or paraneoplastic limbic encephalitis (10). In Japan, young female patients with acute non-herpetic limbic encephalitis have been studied as acute juvenile female non-herpetic encephalitis (AJFNHE) (3). Neurological manifestations in these Japanese females consist of prominent psychiatric symptoms, seizures, dysautonomia, and involuntary movements, and the vast majority of them have a history of prodromal flu-like symptoms. This clinical picture of AJFNHE closely resembles that of the recently proposed anti-NMDAR encephalitis (1, 2), where antibodies to NR1/NR2 heteromers of NMDAR play an important role (4-6). Although antibodies against GluRε2 (NR2 B) have been frequently found in the sera and CSF of patients with AJFNHE (3), they have been also detected in patients with Rasmussen's syndrome and epilepsia partialis continua (EPC) (8). The presence of antibodies against

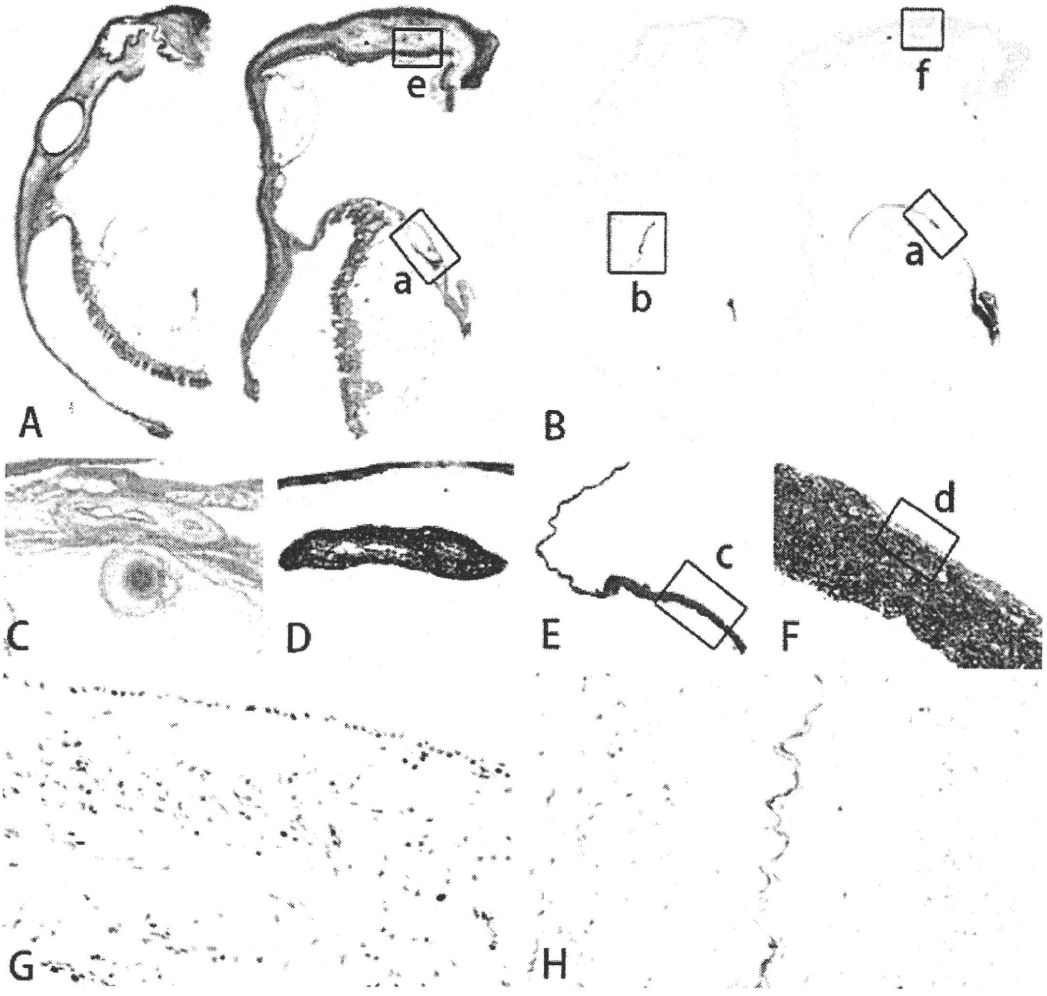

Figure 2. Histopathology of ovarian teratoma. A&B: Low magnification of the tumor. A: Hematoxylin and Eosin staining (×3), B: Immunoperoxidase staining with anti-GFAP antibody (×3). GFAP-positive immunoreactivity is seen in small localized areas of the tumor. C and D are higher magnifications of the framed area "a". The presence of hair follicle and neural tissue is noted and the latter is strongly immunolabeled by anti-GFAP antibody. C: Hematoxylin and Eosin staining (×50), D: Immunoperoxidase staining with anti-GFAP antibody (×60). E: Higher magnification of the framed area "b". F: Higher magnification of the framed area "c". Band-like distribution of neural tissue on the tumor can be identified as GFAP-immunoreactive area. E (×10), F (×70). G: Higher magnification of the framed area "d". Many anti-phosphorylated neurofilament antibody immunoreactive fibrous structures (possibly axons or dendrites) are seen in this area. Immunoperoxidase staining with anti-phosphorylated neurofilament antibody (×220). H: Higher magnification of the framed area "e". An anti-phosphorylated neurofilament antibody immunoreactive axon-like structure is visible. Immunoperoxidase staining with anti-phosphorylated neurofilament antibody (×250).

GluRε2 in patients with anti-NMDAR encephalitis is now recognized to be less specific for the disease (11, 12).

In a large series of patients with anti-NMDAR encephalitis more than half of them were reported to have ovarian teratoma (2) and thus, the pathogenetic significance of ovarian teratoma in this encephalitis has been investigated. Dalmau et al reported that all five tumors obtained from the diseased patients showed mature- and immature-appearing neurons with expression of NR2B and /or NR2A (5), which suggests that ectopically expressed NMDARs in ovarian teratoma contribute to the production of antibodies to NMDARs. A patient with this disorder who promptly recovered after early removal of an ovarian teratoma has been reported (13). In the pathogenesis of anti-NMDAR encephalitis the antibody immune-response is thought to be more relevant than cytotoxic T-cell mechanisms (14): patients' NMDAR antibodies cause a specific, titer-dependent, and reversible decrease in NMDAR surface density and synaptic localization, especially in hippocampus (15). The loss of this subtype of GluRs eliminates NMDAR-mediated synaptic function, resulting in the learning, memory, and other behavioral deficits seen in patients with anti-NMDAR-

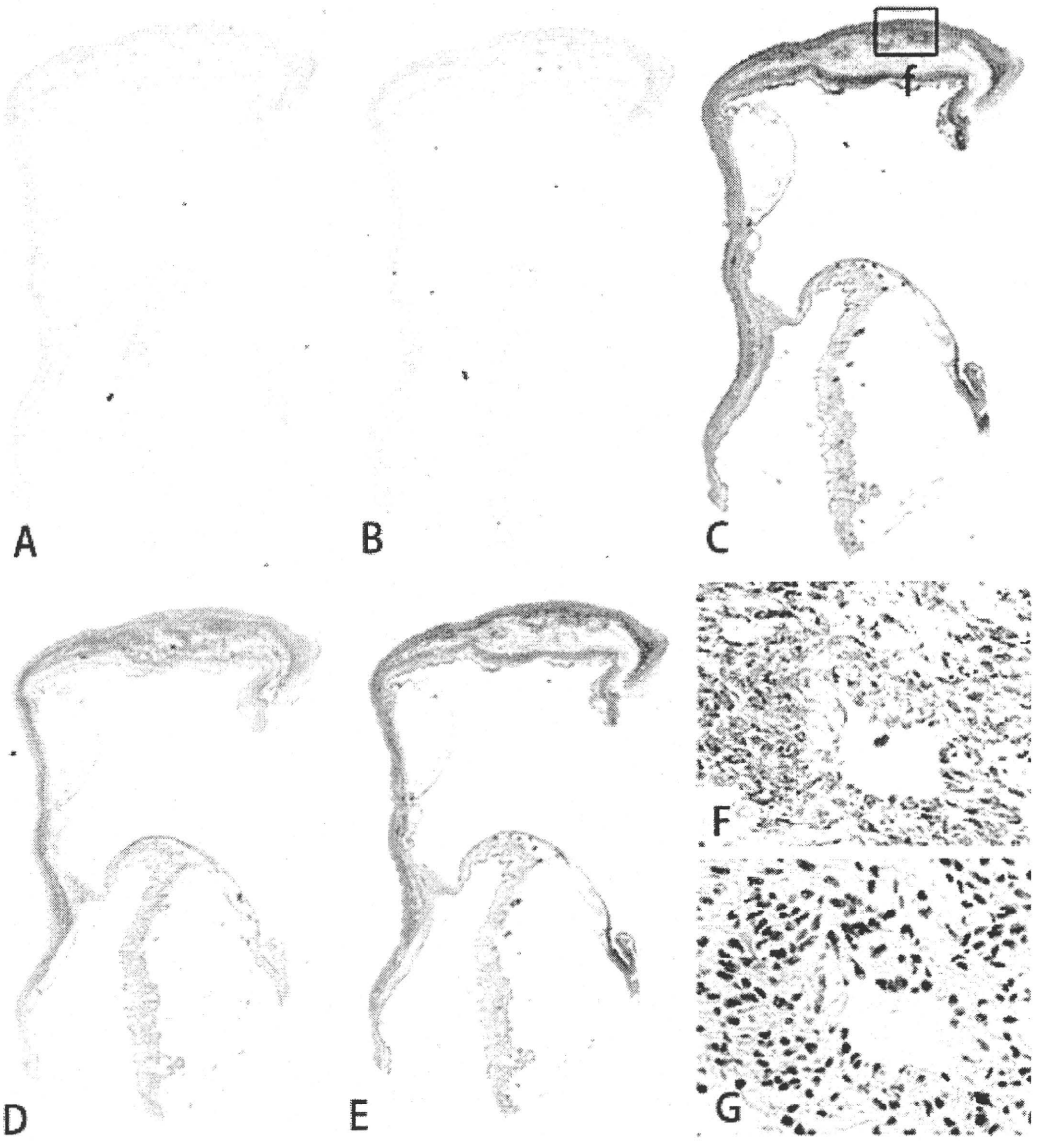

Figure 3. Immunohistochemical expression of NMDAR epitopes within ovarian teratoma. A: anti-NR1, B: anti-NR2A, C: anti-NR2B, D: anti-GluR1, E: anti-GluR 2/3, F&G: Higher magnification of the framed area "f". Neither anti-NR1 nor NR2 antibody is immunoreactive for the ovarian tumor, while anti-NR2B, GluR1 and 2/3 antibodies disclose strong immunoreactivity on an extensive area of this tumor. It is notable that the framed area "f" is not immunolabled by anti-GFAP antibody (see Fig. 1-B) but that this part is apparently immunoreactive for all the anti-NR2B, GluR 1 and GluR2/3 antibodies. This area consists of many small cells, some of which contribute to the pseudo-rosette formation. The vast majority of these small cells are immunolabeled by both anti-phosphorylated neurofilament (F) and anti-human synaptophysin antibodies (G). Immunoperoxidase staining (A to E, ×3; F&G, ×250).

encephalitis. Thus, immunosuppressive therapy including corticosteroid, plasma exchange and intravenous immunoglobulin has been used for the treatment of this disease (2). Recently rituximab, an anti-CD20 monoclonal antibody, is expected to expedite the recovery of the patients with this disease (16, 17).

In the present study we immunohistochemically examined an ovarian teratoma removed from a young female patient with antibodies to GluRε2 (NR2B) and NR1/NR2 heteromers. In mural tissues of the tumor, well-differentiated neural tissues with GFAP-immunoreactivity showed expression of GluRs including NR2B, GluR1, and GluR2/3. Additionally, immature neuronal tissues without GFAP-immunoreactivity also showed expression of these GluRs. The most notable finding in this study is that the former tissue was very limited within the tumor but the latter was more extensively distributed than previously recognized. Although NR1 and NR2A epitopes were undetectable in our teratoma tissues, the lack of immunoreactivity for NR1 might be attributed to technical reasons (11), because expression of NR2B, which is one subunit of NR1/NR2 heteromers in the NMDAR complex, was clearly seen in the

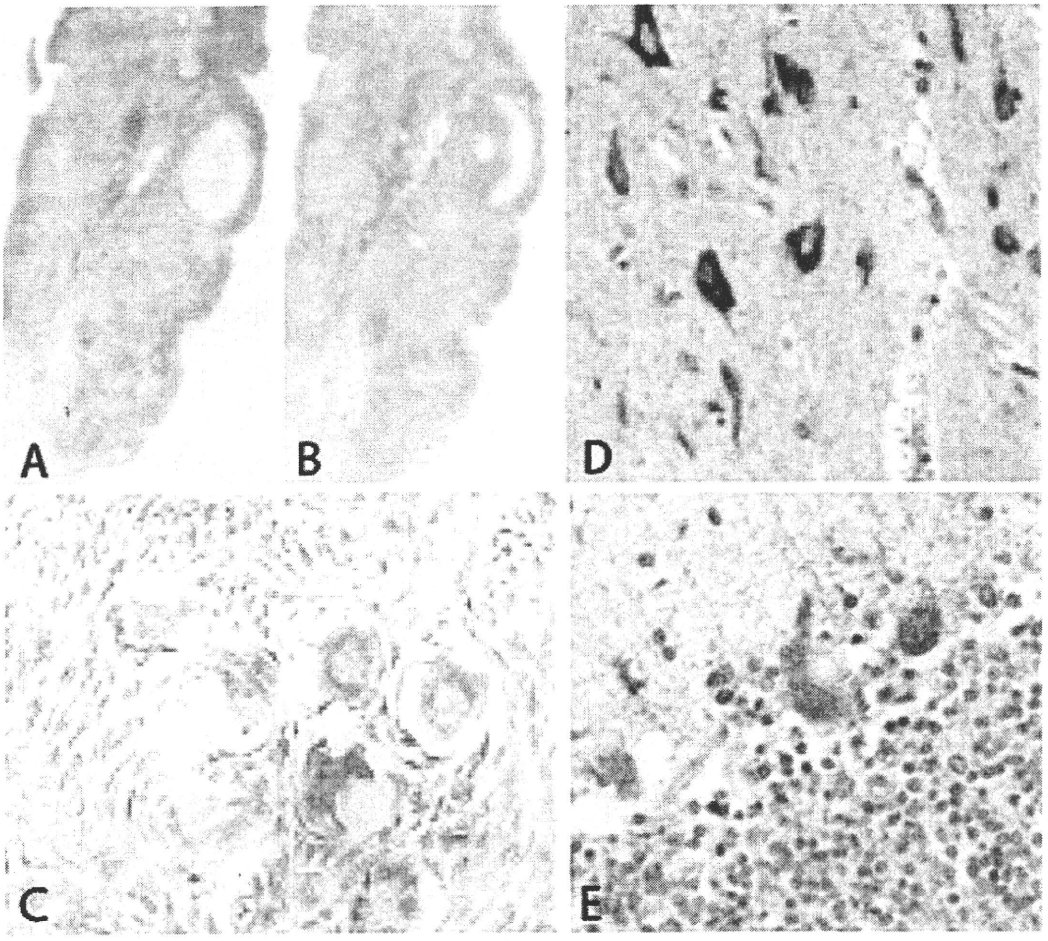

Figure 4. Immunohistochemical findings of controls. A&B: Low magnification of the control ovary obtained from a 21-year-old woman. A: Immunoperoxidase staining with anti-GFAP antibody (×2). No immunoreactivity is seen. B&C: Immunoperoxidase staining with anti-NR2B antibody. Some areas show slightly positive immunoreactivity (B, ×2) and among them the cytoplasm of oocytes is specifically immunolabeled (C, ×200). D: Immunoperoxidase staining of human temporal lobe with anti-NR2B antibody. Neurons and astrocytes are positively stained (×180). E: Immunoperoxidase staining of human cerebellum with anti-GluR 2/3 antibody. Purkinje cells are positively stained (×180).

cell surface of neural tissues examined. On the basis of our immunohistochemical findings ovarian teratoma seems to have abundant expression of various GluR-related epitopes including that of NMDAR, supporting the paradigm whereby the preceding flu-like illness causes inflammation in ovarian teratoma (14), which then leads to the triggering of abnormal antibody production targeting NMDARs (4, 5). Moreover the strong expression of GluR1 and GluR 2/3 (they are pharmacologically classified into AMPA) within the tumor might cause other antibodies against other GluRs than NMDAR: a few anti-AMPA encephalitis cases with antibodies to GluR 1 and GluR 2 were recently reported (18, 19), but they were not accompanied with ovarian teratoma, and their clinical pictures were different from those of the patients with anti-NMDAR encephalitis. The significance of the expression of GluR1 and GluR2/3 within the tumor, therefore, remains undetermined in considering the pathogenesis of autoimmune or paraneoplastic limbic encephalitis.

It is now widely accepted that the presence of ovarian teratoma is a serious predisposing factor for the development of anti-NMDAR encephalitis, but this tumor could not be found in about 40% of adult patients with the disease (2), the number of patients with the latter condition being increasing (20). Raising the recognition of this unique encephalitis has also disclosed that children or adolescents also encounter this disease (21), although the frequency of associated ovarian teratoma in them was much lower in comparison with that in adults (21). Although a few male cases (2, 22, 23) with anti-NMDAR encephalitis including a 20-month-old boy (17) were reported, the vast majority of the patients with this disease are females. The present study has added the possibility that the ovary itself has expression of NMDARs, since NR2B-related immunoreactivity was apparently observed in the cytoplasm of oocytes in control ovaries. The mechanisms that initiate this disorder are still incompletely understood in patients without ovarian teratoma and further studies are required.

Acknowledgement

This work was supported by a grant from the Neuroimmunological Disease Division, the Ministry of Public Health, Labor and Welfare, Japan.

References

1. Sansing LH, Tüzün E, Ko MW, Baccon J, Lynch DR, Dalmau J. A patient with encephalitis associated with NMDA receptor antibodies. Nat Clin Pract Neurol **3**: 291-296, 2007.
2. Dalmau J, Gleichman A, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol **7**: 1091-1098, 2008.
3. Kamei S, Kuzuhara S, Ishihara M, et al. Nationwide survey of acute juvenile female non-herpetic encephalitis in Japan: relationship to anti-N-methyl-D-aspartate receptor encephalitis. Intern Med **48**: 673-679, 2009.
4. Vitaliani R, Mason W, Ances B, Zwerdling T, Jiang Z, Dalmau J. Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. Ann Neurol **58**: 594-604, 2005.
5. Dalmau J, Tüzün E, Wu H-Y, et al. Paraneoplastic *N*-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol **61**: 25-36, 2007.
6. Iizuka T, Sakai F, Ide T, et al. Anti-NMDA receptor encephalitis in Japan. Long-term outcome without tumor removal. Neurology **70**: 504-511, 2008.
7. Bloom FE. Neurotransmission and the central nervous system. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. Brunton LL, Lazo JS, Parker KL, Eds. McGraw-Hill, New York, 2006: 317-340.
8. Groc L, Heine M, Cousins SL, et al. NMDA receptor surface mobility depends on NR2A-2B subunits. Proc Natl Acad Sci USA **103**: 18769-18774, 2006.
9. Takahashi Y, Mori H, Mishina M, et al. Autoantibodies to NMDA receptor in patients with chronic forms of epilepsia partialis continua. Neurology **61**: 891-896, 2003.
10. Bataller L, Leopa KA, Wu GF, Rossi JE, Rosenfeld MR, Dalmau J. Autoimmune limbic encephalitis in 39 patients: immunophenotypes and outcomes. J Neurol Neurosurg Psychiatry **78**: 381-385, 2007.
11. Takahashi Y. Epitope of autoantibodies to *N*-methyl-D-aspartate receptor heteromers in paraneoplastic limbic encephalitis. Ann Neurol **64**: 110-111, 2008.
12. Dalmau J. Reply: epitope of autoantibodies to *N*-methyl-D-aspartate receptor heteromers in paraneoplastic limbic encephalitis. Ann Neurol **64**: 111-112, 2008.
13. Seki M, Suzuki S, Iizuka T, et al. Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. J Neurol Neurosurg Psychiatry **79**: 324-326, 2008.
14. Tzn E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J. Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. Acta Neuropathol **118**: 737-743, 2009.
15. Hughes EG, Peng X, Gleichman AJ, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci **30**: 5866-5875, 2010.
16. Ishiura H, Matsuda S, Higashihara M, et al. Response of anti-NMDA receptor encephalitis without tumor to immunotherapy including rituximab. Neurology **71**: 1921-1922, 2008.
17. Wong-Kisiel LC, Ji T, Renaud DL, et al. Response to immunotherapy in a 20-month-old boy with anti-NMDA receptor encephalitis. Neurology **74**: 1550-1551, 2010.
18. Lai M, Hughes EG, Peng X, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Ann Neurol **65**: 424-434, 2009.
19. Graus F, Boronat A, Xifro X, et al. The expanding clinical profile of anti-AMPA receptor encephalitis. Neurology **74**: 857-859, 2010.
20. Niehusmann P, Dalmau J, Rudlowski C, et al. Diagnostic value of *N*-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. Arch Neurol **66**: 458-464, 2009.
21. Florance NR, Davis RL, Lam C, et al. Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. Ann Neurol **66**: 11-18, 2009.
22. Eker A, Saka E, Dalmau J, et al. Testicular teratoma and anti-1*N*-methyl-D-aspartate receptor-associated encephalitis. J Neurol Neurosurg Psychiatry **79**: 1082-1083, 2008.
23. Novillo-Lopez ME, Rossi J, Dalmau J, Masjuan J. Treatment-responsive encephalitis and NMDA receptor antibodies in man. Neurology **70**: 728-729, 2008.