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.

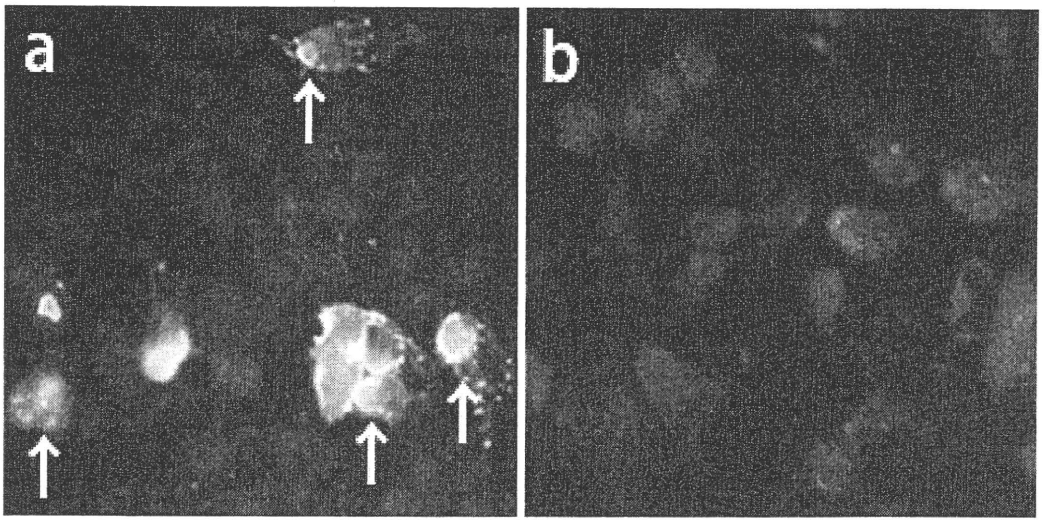

Figure 1. Immunohistochemical demonstration of antibodies against NMDAR. The serum of the patient showing positive immunoreactivity against heteromers of NR1 and NR2B subunits of NMDAR. a: serum of the patient, b: serum of a control patient without this disorder. Arrows indicate positively stained HEK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

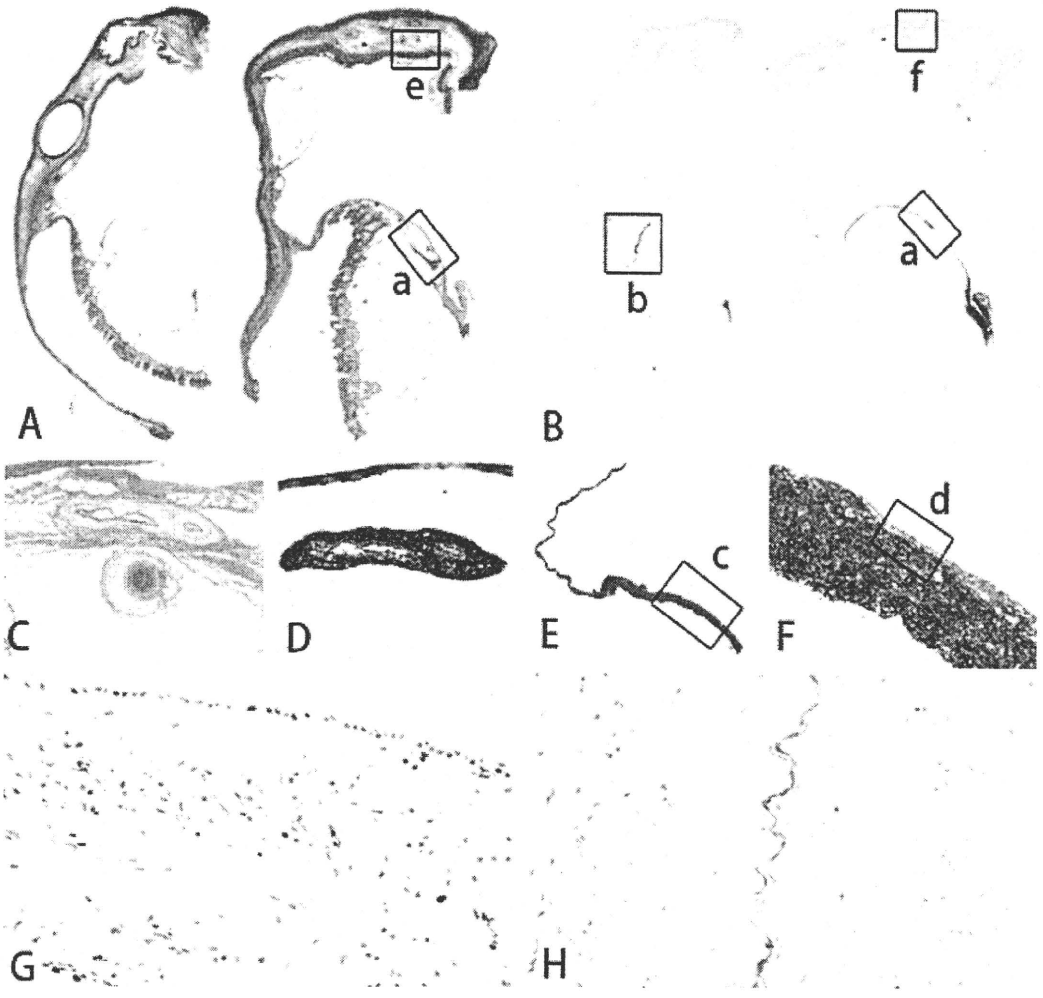

Figure 2. Histopathology of ovarian teratoma. A&B: Low magnification of the tumor. A: Hematoxylin and Eosin staining (×3), B: Immunoperoxidase staining with anti-GFAP antibody (×3). GFAP-positive immunoreactivity is seen in small localized areas of the tumor. C and D are higher magnifications of the 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-

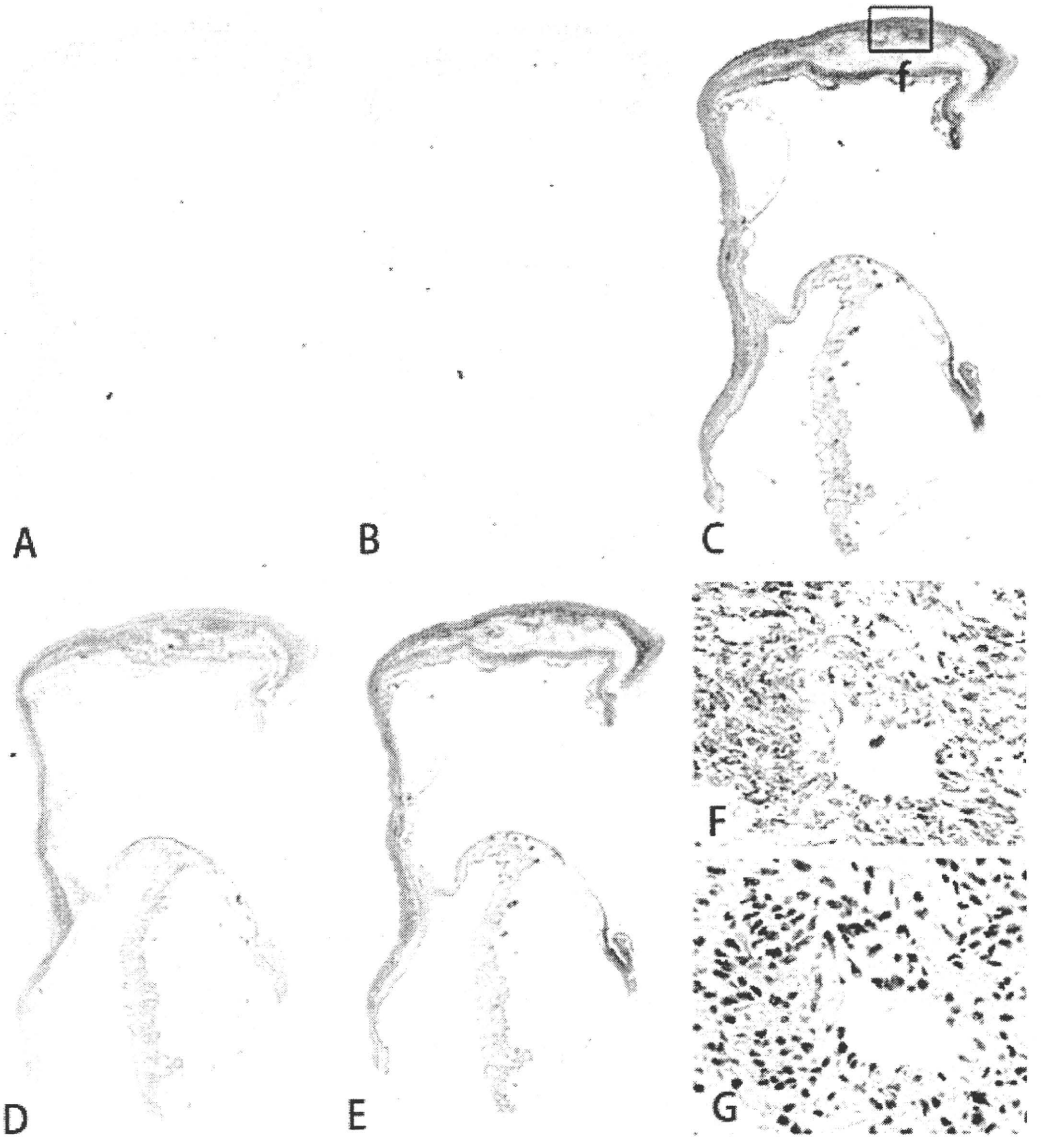

Figure 3. Immunohistochemical expression of NMDAR epitopes within ovarian teratoma. A: anti-NR1, B: anti-NR2A, C: anti-NR2B, D: anti-GluR1, E: anti-GluR 2/3, F&G: Higher magnification of the framed area "f". Neither anti-NR1 nor NR2 antibody is immunoreactive for the ovarian tumor, while anti-NR2B, GluR1 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

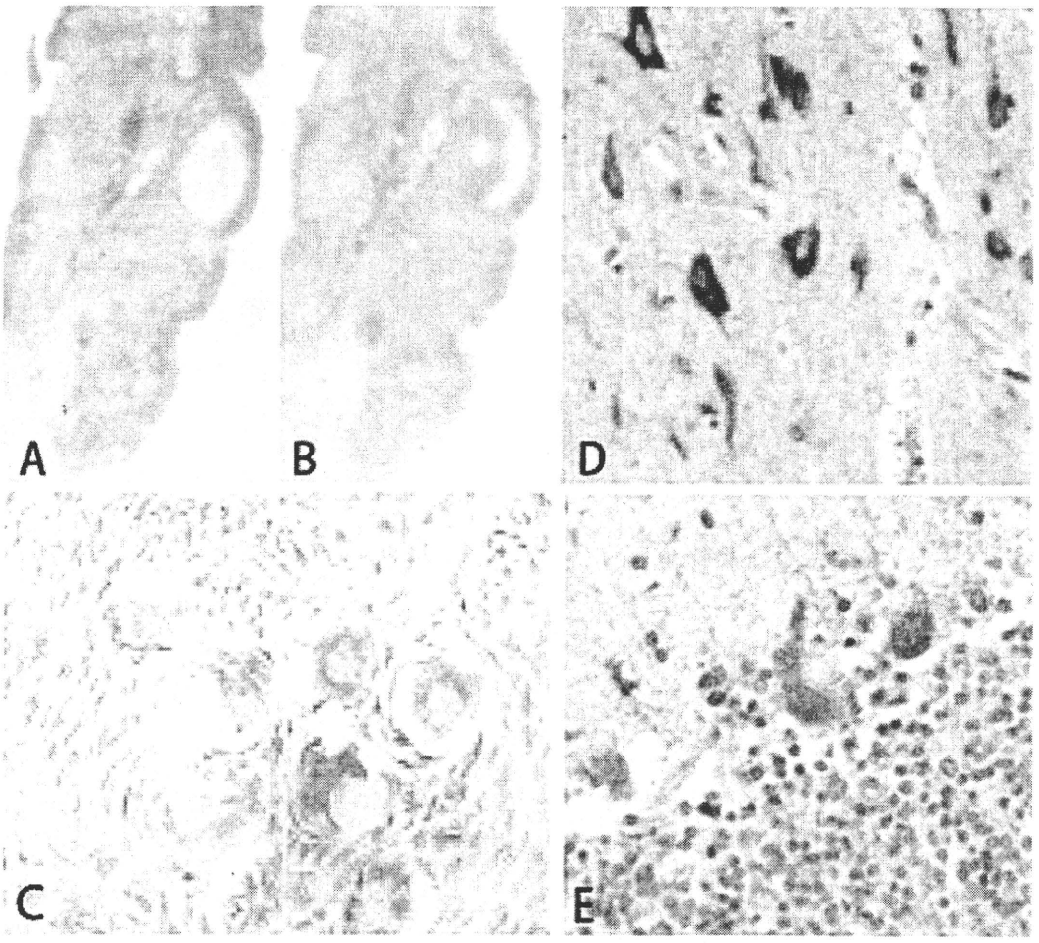

Figure 4. Immunohistochemical findings of controls. A&B: Low magnification of the control ovary obtained from a 21-year-old woman. A: Immunoperoxidase staining with anti-GFAP antibody (×2). No immunoreactivity is seen. B&C: Immunoperoxidase staining with anti-NR2B antibody. Some areas show slightly positive 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

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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.

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BRIEF COMMUNICATION

*HLA-B*1511* is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients

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Summary

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but life-threatening severe cutaneous adverse reactions. Recently, strong associations of *HLA-B*1502* with carbamazepine-induced SJS/TEN have been found in Han Chinese patients. These associations have been confirmed in several Asian populations, excluding Japanese. SJS patients carrying *HLA-B*1508*, *HLA-B*1511*, or *HLA-B*1521*, which are members of the HLA-B75 type along with *HLA-B*1502*, were detected in studies in India and Thailand. In the current study, we genotyped the *HLA-B* locus from 14 Japanese typical and atypical SJS/TEN patients in whom carbamazepine was considered to be involved in the onset of adverse reactions. Although there were no *HLA-B*1502* carriers, four patients had *HLA-B*1511*. Our data suggest that *HLA-B*1511*, a member of *HLA-B75*, is a risk factor for carbamazepine-induced SJS/TEN in Japanese.

KEY WORDS: HLA-B*1502, HLA-B75, Serotype.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe adverse drug reactions (ADRs) with mucosal and cutaneous disorders, and often are accompanied by high fever and systemic complications. Although incidence is low, SJS and TEN are life-threatening and their mortalities are estimated at 5% and 30%, respectively. On the basis of summarized spontaneous reports of severe ADRs to the Ministry of Health, Labor and Welfare (MHLW) from 2006 to 2008, the incidence of SJS/TEN in Japan can be calculated as 3.4 patients per million per year (approximately 430 cases annually), and major causative drugs are allopurinol and carbamazepine.

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As for carbamazepine-induced SJS/TEN, involvement of *HLA-B*1502* in Han Chinese SJS/TEN patients has been reported (Chung et al., 2004), and has been confirmed in Asians in Hong Kong (Man et al., 2007), Europe (Lonjou et al., 2006), Thailand (Locharernkul et al., 2008), and India (Mehta et al., 2009). However, no association between *HLA-B*1502* and carbamazepine-related SJS/TEN was detected in our previous study with seven Japanese SJS/TEN patients (Kaniwa et al., 2008). Therefore, we extended the investigation to explore other biomarkers in Japanese SJS/TEN patients who were administered carbamazepine.

Methods

Patients

The ethics committee of each participating institute of the JSAR (Japan Severe Adverse Reactions) research group approved this study. Written informed consent was obtained from each patient. Fifteen unrelated Japanese patients who were prescribed carbamazepine before the onset of SJS/TEN were recruited from participating institutes or through

a nationwide blood sampling network in Japan operated by the National Institute of Health Sciences in cooperation with the MHLW and the Federation of Pharmaceutical Manufacturers' Association of Japan. Patient characteristics are summarized in Table 1. Seven patients were included in our previous report (Kaniwa et al., 2008), and two patients were in another study (Ikeda et al., 2009). Twelve patients were diagnosed as definite SJS or TEN and three patients were diagnosed as probable SJS due to atypical or mild symptoms by the JSAR research group experts. This diagnosis was based on criteria proposed by Bastuji-Garin et al. (1993) using a standardized case report form including medicinal records, disease progress, and involvement of systemic complications as well as treatment. Severity of ocular complication was scored as follows: 0, no involvement; 1, only hyperemia of bulbar and palpebral conjunctiva; 2, pseudomembrane formation; 3, defect of conjunctival or corneal epithelia.

HLA-B typing

High-resolution *HLA-B* typing was performed by a sequence-based method using SeCore B Locus Sequencing kit (Invitrogen Corp., Brown Deer, WI, U.S.A.) and an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Genomic DNA (250 ng) was used for PCR amplification and sequencing exons 2, 3, and 4. HLA-B haplotype was estimated with the Assign SBT software (version 3.2.7b; Conexio Genomics, Applecross, WA, Australia).

Statistical analysis

*HLA-B*1511* allele frequency reported by Tanaka et al. was used as the control frequency (Tanaka et al., 1996). Fisher's exact test was conducted using JMP ver. 7.0.1 (SAS Institute Japan, Co., Ltd., Tokyo, Japan) to calculate the odds ratio and its 95% confidence interval (CI).

Results

Demographics, symptomatic state, coadministered drugs with carbamazepine, and *HLA-B* diplotypes of 15 patients are summarized in Table 1. However, Patient 12 was excluded from the following statistical analyses because zonisamide was a more likely causative drug. Involvement of carbamazepine in the onset of SJS/TEN could not be excluded for the remaining 11 definite SJS/TEN patients and three probable SJS patients.

In contrast to data on Han Chinese (Chung et al., 2004) and Thai populations (Locharernkul et al., 2008), *HLA-B*1502* was not detected in this work. However, two patients with definite SJS/TEN and two patients with probable SJS carried *HLA-B*1511*. The allele frequencies of *HLA-B*1511* in the SJS/TEN groups were compared with the allele frequency in a Japanese population reported by Tanaka et al. (1996) (n = 493) instead of that in carbamazepine-tolerant patients, because the incidence of SJS/TEN in Japan is very low (three per million/year). Allele frequencies of *HLA-B*1511* increased significantly in the SJS/TEN group regardless of the exclusion or inclusion of probable SJS patients [0.0909 (2 of 22) and 0.143 (4 of 28), respectively) than in the Japanese population (0.01), and the odds ratios were 9.76 (p = 0.0263, CI 2.01–47.5) and 16.3 (p = 0.0004, CI 4.76–55.6), respectively. No patients with *HLA-B*1511* had severe ocular complications.

Discussion

Recently, *HLA-B*1502* involvement has been reported in carbamazepine-induced SJS/TEN in Southern Asian patients (Chung et al., 2004; Man et al., 2007; Locharernkul et al., 2008; Mehta et al., 2009) and patients of Asian ancestry living in Europe (Lonjou et al., 2006). Although we did not detect SJS/TEN patients receiving carbamazepine who carried *HLA-B*1502*, we did find four patients carrying *HLA-B*1511*. *HLA-B*1511* and *HLA-B*1502* belong to the same HLA-B75 serotype. Other major members of HLA-B75 are *HLA-B*1508, HLA-B*1515,* and *HLA-B*1521*. Mehta et al. (2009) have investigated the association between *HLA-B*1502* and carbamazepine-induced SJS using eight Indian patients. Although in their study most patients (six of eight) did carry *HLA-B*1502*, one patient was homozygous *HLA-B*1508*. Tassaneeyakul et al. (2010)have also performed a case–control study using 42 CBZ-induced SJS/TEN patients and 42 carbamazepine-tolerant controls in a Thai population. In their study, 37 SJS/TEN patients carried *HLA-B*1502* and the very strong association of *HLA-B*1502* with SJS/TEN was again confirmed. Although the statistical significance was not examined, two patients carrying heterozygous *HLA-B*1521* and one patient carrying heterozygous *HLA-B*1511* were detected, suggesting that not only *HLA-B*1502* but also some subfamilies of serotype HLA-B75 are involved in the onset of carbamazepine-induced SJS/TEN.

Allele frequencies of individual HLA genotypes in worldwide populations obtained from various studies are shown at Allelefrequencies.net (Middleton et al., 2003). Table 2 summarizes the population allele frequencies of representative types of HLA-B75 in various ethnic groups. In Han Chinese, Thai and Indians, carriers of *HLA-B*1502*, *HLA-B*1521,* and *HLA-B*1508* are at high risk of carbamazepine-induced SJS/TEN, although *HLA-B*1502* is mainly involved. A comparable allele frequency of *HLA-B*1511* (higher than 3.8%) to that of *HLA-B*1502* in Han Chinese in Beijing has been reported recently by Yang et al. (Yang et al., 2010). Because the allele frequency of *HLA-B*1511* is higher than that of *HLA-B*1502* in Japanese and Koreans, carriers of the former may more easily be detected in association studies than carriers of the latter in northeast Asian populations. *HLA-B*1521* can be a risk

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Table 1. Backgrounds and HLA-B diplotypes of Japanese carbamazepine-related SJS/TEN patients

ID[a]	ADR type	Sex/Age	Severity score in ophthalmic disorders	Highest BT (°C)	Total area of blistering skin (%)	Systemic complications	Result of DLST to CBZ	Period of onset for CBZ (days)	Coadministered drugs		HLA-B diplotype	
									Drug name	DLST result/period of onset	High resolution	Low resolution
1 (1)	TEN	M/73	1	>39	20	Neutropenia	–	14	Potassium citrate/sodium citrate hydrate	–/4 days	**1511**/4801	**B75**/B48
						Liver dysfunction			Allopurinol	–/>5 years		
									Etizolam	–/>5 years		
									Sodium pravastatin	–/>5 years		
2 (5)[b]	SJS	F/6	At least 1[c]	>37.0	<10%	GI tract disturbance	Not tested	9	None		4006/5101	B61/B51
3 (6)[b]	SJS	F/52	At least 1[c]	Unknown	<10%	Neutropenia Liver dysfunction	Not tested	14	Zonisamide	Not tested/346 days	4601/5901	B46/B59
4	SJS	M/52	0	38	1	GI tract disturbance Neutropenia Liver dysfunction Renal dysfunction	Not tested	51	Tegafur/gimeracil/oteracil potassium	Not tested/38 days	0702/5201	B7/B52
5	SJS	M/32	1	39	5	Liver dysfunction	Not tested	42	None		4002/5401	B60/B54
6 (2)	SJS	F/42	3	>39	5	GI tract disturbance	–	Shorter than 34	Sodium diclofenac	–/1 year	4001/5201	B60/B52
									L-carbocysteine	–/1 year		
									Cefteram pivoxil	–/4 days		
									Olopatadine hydrochloride	Not tested/unknown		
7	SJS	F/64	At least 1[c]	>37.0	10	Liver dysfunction	+	13	Mecobalamin	Not tested/13 days	**1511**/4002	**B75**/B60
8 (3)	SJS	M/45	3	>37.0	5	Liver dysfunction	Not tested	49	None		4801/5601	B48/B56
9 (4)	SJS	M/54	0	<37.0	0.5	None	+	34	None		1501/3501	B62/B35
10	TEN	M/38	3	40.3	40	Liver dysfunction	+	15	Troxipide	–/8 days	1302/4403	B13/B44
									Levofloxacin hydrate	–/15 days		
									Mecobalamin	–/9 days		
									Acyclovir	–/9 days		
11 (7)	TEN	M/17	3	39.7	20	Respiratory involvement Neutropenia Liver dysfunction	+	5	Zonisamide	+/33 days	4601/5601	B46/B56
									Amoxicillin hydrate	+/1 day		
									Promethazine methylenedisalicylate	Not tested/1 day		
12[d]	SJS	M/6	1	Unknown	<10%	Liver dysfunction	–	145	Zonisamide	+/24 days	**1511**/4006	**B75**/B61
13	Probable SJS	F/54	Unknown	<37.0	>10%	Liver dysfunction	Not tested	22	Sodium pravastatin	Not tested/unknown	4006/4403	B61/B44
									Nifedipine	Not tested/81 days		
									Etizolam	Not tested/15 days		
									Lansoprazole	Not tested/46 days		
									Sodium risedronate hydrate	Not tested/46 days		
14	Probable SJS	F/36	At least 1[c]	Unknown	5	None	+	15	Timiperone	Not tested/1 day	1301/**1511**	B13/**B75**
15	Atypical SJS	F/65	1	37.4	0.1	None	+	9	None		**1511**/3501	**B75**/B35

BT, body temperature; DLST, drug lymphocyte stimulation test; CBZ, carbamazepine.
[a]Number in parentheses is ID # from our previous study (Kaniwa et al., 2008).
[b]These patients were also included in Ikeda et al. (2010)
[c]Ophthalmic complications were observed, but severity was unknown.
[d]This patient was excluded from statistical analyses due to likely zonisamide-induced SJS.

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Table 2. Population allele frequencies of individual types of HLA-B75 in various ethnic groups

Ethnic group	Population allele frequencies reported in allelefrequencies.net website[a]				
	*HLA-B*1502*	*HLA-B*1515*	*HLA-B*1521*	*HLA-B*1508*	*HLA-B*1511*
Japanese	0.001	Data unavailable	Data unavailable	Data unavailable	**0.004–0.008**[b,c]
Koreans	0.002	0.000	0.000	0.000	0.020
Han Chinese	**0.019–0.124**[b]	0.010	0.000–0.002	0.005–0.015	0.000–0.017[d]
Thai	**0.061–0.085**[b]	Data unavailable	**0.007–0.010**[b]	0.010	**0.010**[b]
Indians	**0.000–0.060**[b]	Data unavailable	Data unavailable	**0.005–0.033**[b]	Data unavailable
Caucasians (West Europe)	0.000	0.000	0.000	0.000–0.004	0.000–0.003
Caucasians (East Europe)	0.000	0.000	0.000	0.000–0.009	0.000
Sub-Saharan Africans	0.000	0.000–0.008	Data unavailable	0.000	0.000
Hispanics	0.000	0.004–0.008	0.000	0.000–0.006	0.000
Arabians	0.000	0.000	0.000	0.000–0.007	0.000
Australian aborigine	0.000–0.007	Data unavailable	0.026–0.135	Data unavailable	Data unavailable

[a]New Allele Frequency Database: http://www.allelefrequencies.net/ (Middleton et al., 2003).
[b]SJS/TEN patients carrying the allele shown in the second row have been reported in the study using an ethnic group shown in the first column.
[c]The frequency of 0.1 was reported by Tanaka et al. (1996).
[d]Higher value than 0.038 in Han Chinese in Beijing was recently reported by Yang et al. (2010).

factor for carbamazepine-induced SJS/TEN for Thai and Australian aborigine. Interestingly, HLA-B75 has not been detected in carbamazepine-induced SJS/TEN Caucasian patients (Lonjou et al., 2006). This may be due to extremely low allele frequencies or no existence of HLA-B75 subfamilies.

*HLA-B*1502* has been reported to have associations with SJS/TEN caused by other aromatic antiepileptic drugs such as phenytoin and lamotrigine in Han Chinese and Thai (Man et al., 2007; Locharernkul et al., 2008). In this study we detected a patient carrying *HLA-B*1511* whose causative drug was probably zonisamide, an aromatic antiepileptic drug. Therefore, *HLA-B*1511* may be also involved in the onset of SJS/TEN induced by other aromatic antiepileptic drugs as well as *HLA-B*1502*, although further investigation is needed.

The odds ratio of *HLA-B*1511* for SJS/TEN obtained in this study was low in comparison with those observed in Thai, Indians, and Han Chinese in Taiwan (25.5, 71.4, and 25.04 respectively) (Chung et al., 2004; Locharernkul et al., 2008; Mehta et al., 2009). One reason for this may be the low allele frequency (<0.01) of *HLA-B*1511* among the Japanese. The administration of multiple drugs to Japanese patients may also contribute to the low odds ratio. Indeed, on average, more than three drugs were administered to the patients in this study. We concluded that patients receiving multiple drugs developed SJS/TEN due to carbamazepine by comparing the periods of latency of the individual drugs prior to SJS/TEN onset. However, we cannot completely exclude the possibility of other causative drugs. Another possibility is that *HLA-B*1502* is more prone than *HLA-B*1511* to cause carbamazepine-induced SJS/TEN. Carbamazepine or its metabolites may covalently (Weltzien et al., 1996) or noncovalently (Wu et al., 2007; Yang et al., 2007) bind more easily to the HLA-B*1502 protein or its binding peptide.

There are no SJS/TEN patients carrying *HLA-B*1511* who had severe ocular complications. This result coincides with the previous report that none of the 71 SJS/TEN patients with ocular surface complications had *HLA-B*1511* (Ueta et al., 2008).

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Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflict of interest to disclose.

References

Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. (1993) Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch Dermatol* 129:92–96. The diagnostic criteria are reflected in the currently used following Japanese guidance; Diagnostic criteria of Stevens–Johnson syndrome (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005) and Diagnostic criteria of Toxic Epidermal Necrolysis (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005).

Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, Chen YT. (2004) Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 428:486.

Ikeda H, Takahashi Y, Yamazaki E, Fujiwara T, Kaniwa N, Saito Y, Aihara M, Kashiwagi M, Muramatsu M. (2010) HLA Class I markers in

Japanese patients with carbamazepine-induced cutaneous adverse reactions. *Eplepsia* 51:297–300.

Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, Sawada J, Furuya H, Takahashi Y, Muramatsu M, Kinoshita S, Abe M, Ikeda H, Kashiwagi M, Song Y, Ueta M, Sotozono C, Ikezawa Z, Hasegawa R; JSAR research group. (2008) HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics* 9:1617–1622.

Locharernkul C, Loplumlert J, Limotai C, Korkij W, Desudchit T, Tongkobpetch S, Kangwanshiratada O, Hirankarn N, Suphapeetiporn K, Shotelersuk V. (2008) Carbamazepine and phenytoin induced Stevens–Johnson syndrome is associated with HLA-B*1502 allele in Thai population. *Epilepsia* 49:2087–2091.

Lonjou C, Thomas L, Borot N, Ledger N, de Toma C, LeLouet H, Graf E, Schumacher M, Hovnanian A, Mockenhaupt M, Roujeau JC; RegiSCAR Group. (2006) A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 18:99–107.

Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, Ng MH. (2007) Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48:1015–1018.

Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, Dave DM, Goyal RK. (2009) Association of HLA-B*1502 allele and carbamazepine-induced Stevens–Johnson syndrome among Indians. *Indian J Dermatol Venereol Leprol* 75:579–582.

Middleton D, Menchaca L, Rood H, Komerofsky R. (2003) Brief communication. New allele frequency database: http://www.allelefrequencies.net. *Tissue Antigens* 61:403–407.

Tanaka H, Akaza T, Juji T. (1996) Report of the Japanese central bone marrow data center. *Clin Transpl* 1996:139–144.

Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Chen P, Lin SY, Chen WH, Konyoung P, Khunarkornsiri U, Auvichayapat N, Pavakul K, Kulkantrakorn K, Choonhakarn C, Phonhiamhan S, Piyatrakul N, Aungaree T, Pongpakdee S, Yodnopaglaw P. (2010) Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. *Epilepsia* 51:926–930.

Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S. (2008) HLA class I and II gene polymorphisms in Stevens–Johnson syndrome with ocular complications in Japanese. *Mol Vis* 14:550–555.

Weltzien HU, Moulton C, Martin S, Padovan E, Hartmann U, Kohler J. (1996) T cell immune responses to haptens. Structural models for allergic and autoimmune reactions. *Toxicology* 107:141–151.

Wu Y, Farrell J, Pirmohamed M, Park BK, Naisbitt DJ. (2007) Generation and characterization of antigen-specific CD4 + , CD8 + , and CD4 + CD8 + T-cell clones from patients with carbamazepine hypersensitivity. *J Allergy Clin Immunol* 119:973–981.

Yang CW, Hung SI, Juo CG, Lin YP, Fang WH, Lu IH, Chen ST, Chen YT. (2007) HLA-B*1502-bound peptides: implications for the pathogenesis of carbamazepine-induced Stevens–Johnson syndrome. *J Allergy Clin Immunol* 120:870–877.

Yang G, Deng YJ, Qin H, Zhu BF, Chen F, Shen CM, Sun ZM, Chen LP, Wu J, Mu HF, Lucas R. (2010) HLA-B*15 subtypes distribution in Han population in Beijing, China, as compared with those of other populations. *Int J Immunogenet* 37:205–212.

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A Young Man with Anti-NMDAR Encephalitis following Guillain-Barré Syndrome

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Key Words
Anti-*N*-methyl-D-aspartate receptor encephalitis · Guillain-Barré syndrome · Parainfectious autoimmune disorder · Male gender

Abstract
A 19-year-old man developed rapidly progressive muscle weakness and dysesthesia in the extremities, and dyspnea after a flu-like episode. Nerve conduction studies showed reduced motor nerve conduction velocities with conduction block, and sensory nerve action potentials could not be evoked. The patient was diagnosed as having Guillain-Barré syndrome (GBS), and was treated with 2 cycles of intravenous immunoglobulin (IVIg) therapy and was assisted by mechanical ventilation. During the recovery course of the illness, he experienced several attacks of psychomotor agitation from the 37th hospital day, and generalized tonic convulsive seizures suddenly developed on the 42nd hospital day. Brain MRI showed high-intensity lesions in the bilateral thalamus and medial temporal lobes. The convulsions were controlled by continuous thiopental infusion (until the 50th hospital day) and mechanical ventilation (until the 84th hospital day). Intravenous methylprednisolone pulse therapy (1,000 mg/day) for 3 days followed by dexamethasone (16 mg/day) was added. After relief of convulsive seizures, prominent orolingual dyskinesia appeared, and on MRI marked atrophy of the bilateral medial temporal lobes was seen. Anti-*N*-methyl-D-aspartate receptor (NMDAR) antibodies in serum and cerebrospinal fluid were positive on the 92nd hospital day. Anti-NMDAR encephalitis usually affects young females but a small number of male cases with this disease have been reported. Our male patient was unique in having GBS, a post-infectious autoimmune disease, as a preceding disease, suggesting that anti-NMDAR encephalitis itself is caused by a parainfectious autoimmune mechanism.

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Introduction

Recently, a unique limbic encephalitis that predominantly affects young females and shows various manifestations, including initial psychosis, and subsequent central hypoventilation, intractable seizures, dysautonomia and prominent orofacial dyskinesia, has been noted [1, 2]. In patients with this disorder a new anti-neural antibody for the NR1/NR2 heteromers of the *N*-methyl-D-aspartate receptor (NMDAR) has been identified as a disease-specific hallmark [3], recently adding that the NR1 is possibly a main epitope [2]. Thus, this disorder is now called anti-NMDAR encephalitis. Although it is now widely accepted that the presence of ovarian teratoma is an important predisposing factor for the development of anti-NMDAR encephalitis [4, 5], the mechanisms that initiate the disease are still incompletely understood.

We here report on a young male patient with anti-NMDAR encephalitis which developed during the recovery course of severe Guillain-Barré syndrome (GBS), and propose that a post-infectious autoimmune mechanism may play an important role in the pathogenesis of anti-NMDAR encephalitis.

Case Report

A 19-year-old man with no history of systemic disease was hospitalized due to muscle weakness and dysesthesia in the extremities following cough and nasal discharge 2 weeks earlier. Neurological examination showed muscle weakness and sensory disturbance in the distal parts of the extremities, and all deep tendon reflexes were absent. Cerebrospinal fluid (CSF) at 5 days after onset of weakness contained a normal cell count, but the concentration of total protein was elevated (52.8 mg/dl). The patient was diagnosed as having GBS and treated with intravenous immunoglobulin (IVIg) therapy (0.4 g/kg/day) for 5 days. However, the symptoms worsened gradually, he was not able to raise his upper limbs and to walk from the 5th hospital day. Furthermore, dysarthria, dysphagia, and dyspnea appeared gradually from the 9th hospital day. He was transferred to our hospital on the 12th hospital day. On the day of transfer, nerve conduction studies showed reduced motor nerve conduction velocities (right median nerve: 11.3 m/s, normal value >55 m/s, right tibial nerve: 36.6 m/s, normal value >45 m/s) with conduction block (fig. 1), and sensory nerve action potentials could not be evoked in both nerves. The F-wave of the right tibial nerve was not evoked. There was neither anti-GM1 IgG antibody nor anti-GQ1b IgG antibody in the serum. Although mechanical ventilation was necessary from the day of transfer, muscle weakness in the extremities and respiratory function gradually improved after the second course of IVIg therapy from the 22nd hospital day for 3 days, and the patient was trained for weaning from mechanical ventilation. However, he started to experience occasional psychomotor agitation from the 37th hospital day, and generalized tonic convulsive seizures suddenly developed following paralytic ileus and tachycardia on the 42nd hospital day. Since the convulsive seizures did not respond to intravenous administration of diazepam, phenytoin, or midazolam, continuous thiopental infusion was used and mechanical ventilation was continued. CSF showed a slightly increased cell count (8/μl, mononuclear cells 7), a highly elevated level of total protein (446 mg/dl), and normal glucose concentration. Brain MRI showed high-intensity lesions in the bilateral thalamus and medial temporal lobes on diffusion and FLAIR images (fig. 2a), and the electroencephalogram showed diffuse spike and wave complexes. He was diagnosed as having some type of autoimmune encephalitis, and intravenous methylprednisolone pulse therapy (1,000 mg/day) for 3 days followed by dexamethasone (16 mg/day) was administered, and used until the 70th hospital day with a gradual dose reduction. Although the generalized convulsive seizures disappeared and the infusion of thiopental was ceased on the 52nd hospital day, orolingual dyskinesia gradually developed. On the brain MRI on the 64th hospital day, the abnormal high-intensity lesions in the bilateral thalamus were reduced, while marked atrophy of the bilateral medial temporal lobes was seen (fig. 2b). Respiratory failure and autonomic dysfunction that included disturbed bowel movement and abnormal cardiovascular responses gradually improved, and he was released from mechanical ventilation on the 84th hospital day. However, the patient remained unresponsive to verbal commands and severe orolingual dyskinesia persisted until he was transferred to a local hospital on the 193rd hospital day.

During investigation of the causes of the encephalitis, there were no laboratory data suggesting viral encephalitis, including herpes simplex, influenza, varicella zoster, cytomegalovirus, measles, and human herpes virus-6. Urological examination was normal and no detectable neoplasm was seen on either chest or abdominal CT. Two years after the onset of seizures, his condition was very similar to when he was discharged from our hospital, and repeated examinations showed no appearance of a tumor.

Immunochemical Analysis of Anti-NMDAR Antibody in Serum and CSF

Detection of anti-NMDAR antibody on the same samples was carried out as follows [6]: cDNA encoding NR1 and NR2B was 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 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 20 min. After non-specific binding was blocked with 10% goat serum in PBS, these cells were incubated with patient sera (1:40) or CSF (1:2) overnight at 4°C 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.

Both serum and CSF obtained from the 92nd hospital day specifically reacted with HEK-293 cells expressing heteromers of NR1/NR2B (fig. 3).

Discussion

In our case, encephalitis was shown by abnormal intensity of the bilateral thalamus and medial temporal lobes on brain MRI at an early stage. However, the former thalamic lesions seemed to be edema due to status epilepticus, and the main lesion of this encephalitis was assumed to be in the bilateral medial temporal lobes, because the thalamic lesions rapidly resolved after treatment, whereas the bilateral medial temporal lobes showed progressive atrophy. Moreover, his clinical manifestations consisted of psychiatric symptoms, intractable seizures, dysautonomia and involuntary movements, and anti-NMDAR antibody was demonstrated in both serum and CSF. He was finally diagnosed as having anti-NMDAR encephalitis.

Anti-NMDAR encephalitis usually affects young females, and an early series of patients with this disease was found to have a high association of ovarian teratoma [3, 4]. Thus, the pathogenetic significance of ovarian teratoma in this encephalitis was investigated and it was proposed that, since the ovarian teratomas obtained from diseased patients showed mature- and immature-appearing neurons with expression of NR2B and/or NR2A [3, 4], ectopically expressed NMDARs in ovarian teratoma contribute to the production of antibodies to NMDARs [3]. Thus, early removal of ovarian teratoma has been recommended for patients with this disease [7]. However, a recent study on a large number of patients with anti-NMDAR encephalitis has shown that this ovarian tumor could not be found in about 40 to 80% of adult patients with the disease [1, 2, 8], although careful follow-up examinations on the detection of tumors are always required in these patients. Increased recognition of this unique encephalitis has also disclosed that children and adolescents also encounter it [9, 10], although the frequency of associated ovarian teratoma was much lower in children than adults. Additionally, a small number of male cases with anti-NMDAR encephalitis [11] were reported, indicating that other causes besides ovarian teratoma can produce encephalitis.

In the pathogenesis of anti-NMDAR encephalitis, the antibody immune response has been shown to be more relevant than cytotoxic T-cell mechanisms [12] and the vast majority of patients with this type of disease have a history of prodromal flu-like symptoms. It was, therefore, suggested that the preceding flu-like illness leads to the triggering of abnormal antibody production targeting NMDARs [12]. In this situation the presence of ovarian teratoma with a high expression of NMDAR epitopes may predispose or exaggerate the production of anti-NMDAR antibodies; these NMDAR antibodies then cause a specific, titer-dependent, and reversible decrease in NMDAR surface density and synaptic localization, especially in the hippocampus [13], resulting in learning, memory, and other behavioral deficits seen in patients with anti-NMDAR-encephalitis. Thus, this encephalitis seems to be causally related to a parainfectious autoimmune mechanism. Immunosuppressive therapy, including corticosteroid, plasma exchange and IVIg, has been used for the treatment of this disease [1]. Recently, rituximab, an anti-CD20 monoclonal antibody, is expected to accelerate the recovery of patients with this type of disease [14].

Our young male patient with anti-NMDAR encephalitis lacked testicular or mediastinal teratoma, which was previously reported to be of paraneoplastic origin [11]. On the other hand, his clinical course was characterized by a preceding attack of GBS: this disease is a representative post-infectious peripheral demyelinating neuropathy with underlying autoimmune abnormalities [15]. Although a causative relationship between GBS and anti-NMDAR encephalitis has not been studied, it is likely that GBS-related abnormal immune reactions secondarily caused another autoimmune state with anti-NMDAR encephalitis. Although serial examinations of anti-NMDAR antibodies in both serum and CSF might be useful in clarifying the underlying immune condition of this patient, there were no available samples. This case report has provided the clinical evidence that a parainfectious autoimmune reaction is an important pathogenetic mechanism in the development of anti-NMDAR encephalitis.

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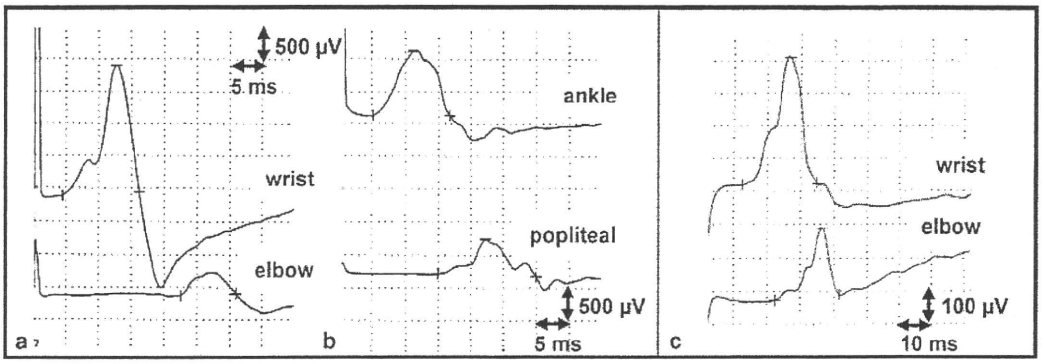

Fig. 1. Motor nerve conduction study of the right median nerve and the right tibial nerve. **a** Right median nerve on the 12th hospital day. **b** Right tibial nerve on the 12th hospital day. These studies showed reduced motor nerve conduction velocities (MCV) with conduction block (median nerve 11.3 m/s, tibial nerve 36.6 m/s). The sizes of the compound muscle action potential of the right median nerve were 2.0 mV at the wrist and 0.33 mV at the elbow, and those of the right tibial nerve were 1.0 mV at the ankle and 0.57 mV at the popliteal fossa, respectively. **c** Motor nerve conduction study of the right median nerve on the 94th hospital day, showing improvement of the MCV (23.2 m/s) and severity of the conduction block (the sizes of the compound muscle action potential were 0.404 mV at the wrist and 0.225 mV at the elbow). MCV of right tibial nerve on the 94th day was not evoked.

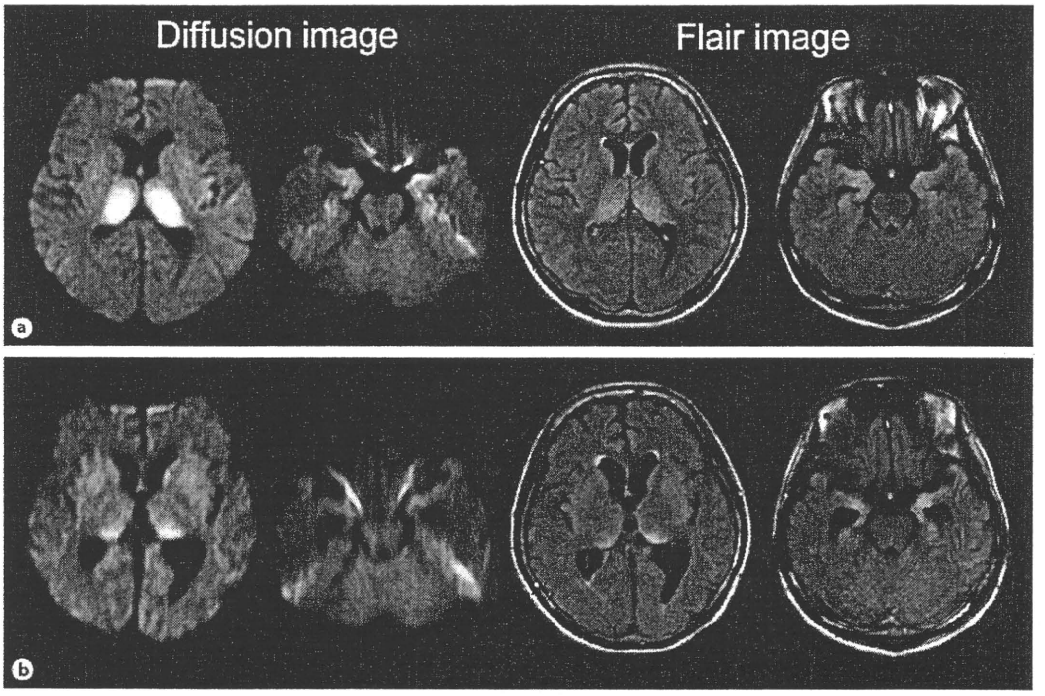

Fig. 2. Brain MRI findings. **a** Images on the 42nd hospital day showed high-intensity lesions in the bilateral thalamus and bilateral medial temporal lobes. **b** Images on the 64th hospital day revealed improved abnormal high-intensity lesions in the bilateral thalamus but progressive atrophy of the bilateral medial temporal lobes.

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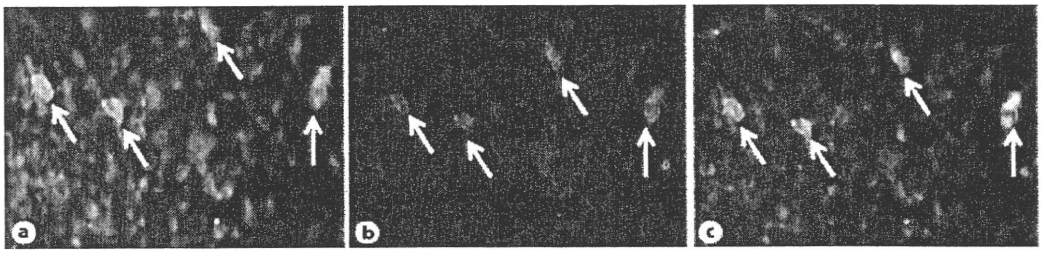

Fig. 3. Immunohistochemical demonstration of antibodies against NMDAR. **a** CSF of the patient showing positive immunoreactivity against heteromers of NR1 and NR2B subunits of NMDAR. **b** Anti-rabbit IgG showing positive immunoreactivity against NR1 subunit of NMDAR. **c** Merge image. Arrows indicate positively stained HEK cells. Immunofluorescence staining (×200).

References

1 Dalmau J, Gleichman A, Hughes EG, Rossi JE, Peng X, Lai M, Dessain S, Rosenfeld MR, Balice-Gordon R, Lynch DR: Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091–1098.

2 Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi MS, Friese MA, Galea I, Kullmann DM, Beeson D, Lang B, Bien CG, Vincent A: *N*-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorders of both sexes. Brain 2010;133:1655–1667.

3 Dalmau J, Tüzün E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR: Paraneoplastic *N*-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25–36.

4 Vitaliani R, Mason W, Ances B, Zwerdling T, Jiang Z, Dalmau J: Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. Ann Neurol 2005;58:594–604.

5 Iizuka T, Sakai F, Ide T, Monzen T, Yoshii S, Iigaya M, Suzuki K, Lynch DR, Suzuki N, Hara T, Dalmau J: Anti-NMDA receptor encephalitis in Japan. Long-term outcome without tumor removal. Neurology 2008;70:504–511.

6 Tachibana N, Shirakawa T, Ishii K, Takahashi Y, Tanaka K, Arima K, Yoshida T, Ikeda S: 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. Intern Med 2010;49:2167–2173.

7 Seki M, Suzuki S, Iizuka T, Shimizu T, Nihei Y, Suzuki N, Dalmau J: Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. J Neurol Neurosurg Psychiatry 2010;79:324–326.

8 Kamei S, Kuzuhara S, Ishihara M, Morita A, Taira N, Togo M, Matsui M, Ogawa M, Hisanaga K, Mizutani T, Kuno S: Nationwide survey of acute juvenile female non-herpetic encephalitis in Japan: relationship to anti-N-methyl-D-aspartate receptor encephalitis. Intern Med 2009;48:673–679.

9 Florance NR, Davis RL, Lam C, Szperka C, Zhou L, Ahmad S, Campen CJ, Moss H, Peter N, Gleichman AJ, Glaser CA, Lynch DR, Rosenfeld MR, Dalmau J: Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. Ann Neurol 2009;66:11–18.

10 Niehusmann P, Dalmau J, Rudlowski C, Vincent A, Elger CE, Rossi JE, Bien CG: Diagnostic value of *N*-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. Arch Neurol 2009;66:458–464.

11 Eker A, Saka E, Dalmau J, Kurne A, Bilen C, Ozen H, Ertoy D, Ogurz KK, Elibol B: Testicular teratoma and anti- *N*-methyl-D-aspartate receptor-associated encephalitis. J Neurol Neurosurg Psychiatry 2008;79:1082–1083.

12 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 2009;118:737–743.

13 Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, Parsons D, Lynch DR, Dalmau J, Balice-Gordon RJ: Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci 2010;30:5866–5875.

14 Ishiura H, Matsuda S, Higashihara M, Hasegawa M, Hida A, Hanajima R, Yamamoto T, Shimizu J, Dalmau J, Tsuji S: Response of anti-NMDA receptor encephalitis without tumor to immunotherapy including rituximab. Neurology 2008;71:1921–1922.

15 Griffin JW, Sheikh K: The Guillain-Barré syndrome; in Peripheral Neuropathy 4th ed, Dyck PJ, Thomas PK (eds): Elssevier and Saunders, Philadelphia, 2005, pp 2197–2220.

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BRIEF COMMUNICATION

HLA Class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions

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Summary

Carbamazepine (CBZ) is frequently used for treating epilepsy, but this drug causes cutaneous adverse drug reactions (cADRs) that may range from mild to severe. It is reported recently that the human leukocyte antigen HLA-B*1502 is associated with Stevens-Johnson syndrome (SJS) induced by CBZ in Han Chinese. We examined HLA class I in 15 Japanese patients who fulfilled the diagnostic criteria for CBZ-induced cADRs (mild in 10 and severe = SJS in 5). HLA-B*1518, HLA-B*5901 and HLA-C*0704 alleles showed higher relative risks (above 10.0) for severe cADRs. The haplotype (HLA-A*2402-B*5901-C*0102) had high relative risk (16.09) for severe cADRs. In patients with severe cADRs, frequencies of HLA-A*1101, HLA-A*3303, HLA-B*1501, HLA-B*4403, HLA-B*5101, HLA-B*5201, HLA-C*0702, and HLA-C*1202 alleles are relatively lower than in the Japanese population. These data may suggest that HLA-B*5901 is one of the candidate markers for CBZ-induced SJS in Japanese.

KEY WORDS: Carbamazepine, Stevens-Johnson syndrome, HLA class I, HLA-B*5901, Cutaneous adverse drug reactions.

Background

Skin rash is a well-known complication of antiepileptic drug (AED) treatment. The risk of cutaneous adverse drug reactions (cADRs) of AED treatment is reported to be higher compared to drugs other than AEDs (Roujeau et al., 1995). In particular, carbamazepine (CBZ), which is commonly used to treat partial epilepsy, frequently causes a wide spectrum of cADRs including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug-induced hypersensitivity syndrome (DIHS), macropapular eruption, and mild skin rash. CBZ induces cADRs in 2.9% of Japanese patients (http://www.info.pmda.go.jp/). Recently, a strong association has been reported between a genetic marker, the human leukocyte antigen HLA-B*1502 and SJS induced by CBZ in Han Chinese (Chung et al., 2004; Hung et al., 2006). However, HLA-B*1502 is rare in the Japanese population and is not found in patients with SJS induced by CBZ (Kashiwagi et al., 2008). The genetic markers for SJS seem to be heterogeneous in each race (Ueta et al., 2008). In the present study, we try to identify the HLA class I genetic markers in the Japanese population that may predict patients at high risk of cADRs induced by CBZ.

Patients and Methods

Patients

We classified cADRs into two categories: group A (10 patients) with mild cADRs such as exanthema and rash with or without fever, and group B (five patients) with severe cADRs such as SJS, TEN, and DIHS. The diagnosis of each cADR was based on the clinical criteria provided by Pharmaceuticals and Medical

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Devices Agency (PMDA) (http://www.info.pmda.go.jp/juutoku/juutoku_index.html). TEN and SJS are both defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema, and high fever. However, SJS is defined as skin detachment of 10% or less of the body surface area, whereas TEN is defined as skin detachment of more than 10%, excluding staphylococcal scalded skin syndrome (Roujeau et al., 1995). DIHS is characterized by multiorgan involvement such as hepatitis and nephritis accompanied by systemic manifestations, including fever, eosinophilia, and lymphadenopathy, in addition to skin rashes. The five patients with severe cADR were diagnosed as SJS by the above-mentioned clinical criteria, patients B-2 and B-4 were reported in a previous report (Kashiwagi et al., 2008), and patient B-5 was included in a previous paper (Kaniwa et al., 2008). The characteristics of the patients are shown in Table 1. We conducted HLA genotyping after obtaining informed consent from each patient by the methods approved by the ethical committee of our hospital.

HLA genotype

High-resolution typing of HLA class I loci was performed by the sequence-based method using the SeCore sequencing kits (Invitrogen Corp., Brown Deer, WI, U.S.A.) and the ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Using the kits for HLA-A, -B, and -Cw, exons 2–4 of each gene were amplified and sequenced to identify the genetic polymorphisms. HLA-A, -B, and -Cw alleles were estimated using the ASSIGN SBT software version 3.2.7b (Conexio Genomics, Freemantle, Western Australia, Australia).

Statistical analysis

The HLA-A and -B allele frequencies obtained from 493 Japanese healthy subjects were used as the frequencies for Japanese general population (Table 2). The HLA-C allele frequencies obtained from 114 Japanese healthy subjects were used as the frequencies for Japanese general population (Table 2). Relative risks were calculated according to the reference (Marsh et al., 2000). Relative risk in this study is defined as hK/Hk, where h is the allele frequency in patients with the antigen, k is the allele frequency in the patients without the antigen, H is the allele frequency in healthy controls with the antigen, and K is the allele frequency in controls without the antigen.

Results

HLA-A

Ten patients in group A and five patients in group B were analyzed (Table 2). In group A, relative risk was the highest for HLA-A*2603 allele (5.37), and zero for HLA-A*0201 and HLA-A*2601 alleles. In group B, the highest relative risk was 2.03, and zero risk was observed for HLA-A*1101 and HLA-A*3303 alleles among alleles with relatively high frequencies in the Japanese population.

Table 1. Clinical characteristics of patients in groups A and B

Type and Pt number	Age	Sex	Epilepsy	Associated disease	Dose of CBZ (mg)	Latency to cADRs (days)	Concurrent AEDs	History of cADRs
A-1	12	M	PE	Brain tumor	?	11	–	–
A-2	7	F	PE	Hyperthyroidism	?	30	–	CLB, ZNS
A-3	11	M	RS	–	?	4	–	
A-4	1	F	PE	Tuberous sclerosis	60	12	–	
A-5	8	M	PE	Sequelae of encephalitis	100	35	VPA	PB
A-6	7	F	PE	Mental retardation	150	17	VPA+ZNS	CBZ, PHT
A-7	6	F	PE	Sequelae of encephalitis by influenza vaccine	110	9	–	
A-8	9	M	PE	Autism	180	12	VPA	
A-9	14	F	PE	Mental retardation	200	30	–	
A-10	7	F	PE	–	100	14	VPA	ZNS, PHT
B-1	60	F	PE	–	100	1	–	CBZ, PHT
B-2	33	M	PE	Operated AVM	400	7	–	
B-3	38	F	PE	SLE	?	?	–	PHT
B-4	24	F	PE	Sequelae of Influenza encephalitis	?	7	–	
B-5	52	F	PE	SLE	100	11	ZNS	ABPC

M, male; F, female; PE, localization-related epilepsy; RS, Rasmussen syndrome; AVM, arteriovenous malformation; SLE, systemic lupus erythematosus; cADRs, cutaneous adverse drug reaction; Concurrent AEDs, concurrently used AEDs on cADRs; CLB, clobazam; ZNS, zonisamide; PB, phenobarbital; CBZ, carbamazepine; PHT, phenytoin; ABPC, aminobenzylpenicillin.

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