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Adenylate Kinase 5 Autoimmunity in Treatment Refractory Limbic Encephalitis

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Abstract

We report two men with limbic encephalitis (LE) refractory to corticosteroids, IVIg and plasma exchange. Both patients had serum/CSF antibodies that reacted with the cytoplasm of neurons. Probing of a hippocampal cDNA library resulted in the isolation of adenylate kinase 5 (AK5). Patients' antibodies, but not those of 111 controls, recognized AK5-expressing phage plaques. Human AK5-affinity purified antibodies reproduced the neuronal immunolabeling of patients' antibodies, and co-localized with a rabbit AK5 antibody, confirming that the brain autoantigen was AK5. Detection of antibodies to AK5 in LE patients carries a poor prognosis, and suggests the prompt use of aggressive immunosuppression.

Keywords

limbic encephalitis; paraneoplastic; adenylate kinase 5; antibodies

INTRODUCTION

Clinical experience suggests that non-viral immune-mediated limbic encephalitis (LE) occurs more frequently than previously thought. Recognition of this disorder has been facilitated by improved neuroimaging techniques and the discovery of several autoantibodies that are used in diagnostic tests (Bataller and Dalmau, 2006). Yet in about 20% of patients with clinical, MRI, and cerebrospinal fluid (CSF) findings compatible with immune-mediated LE, studies for all known autoantibodies are negative (Bataller et al., 2006). We have investigated two LE patients whose syndrome had a relentless progression to dementia despite treatment with corticosteroids, intravenous immunoglobulin (IVIg), and plasma exchange. Both had antibodies to previously unknown neuronal antigens, which were characterized as adenylate kinase 5 (AK5).

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Patient 1

A 71 year-old man developed rapidly progressive memory deficits, delusional thoughts, confusion, agitation and aggressive behavior. The neurological examination did not reveal focal deficits. The CSF showed no cells, normal protein (46 mg/dl) and glucose (58 mg/dl), increased IgG index (0.7) and positive oligoclonal bands. EEG was normal and the brain MRI demonstrated T2 and FLAIR hyperintensities in the right medial temporal lobe with mild contrast enhancement. CT of the chest, abdomen and pelvis, and body [18F] Fluorodeoxyglucose PET (FDG-PET) were normal. Testing for paraneoplastic and voltage-gated potassium channels (VGKC) was negative. Treatment with corticosteroids, IVIg, and plasma exchange was not effective. The behavioral symptoms mildly improved with risperidone, but the memory deficits and cognitive dysfunction continued to progress. At follow-up 13 months after symptom presentation, he was severely demented and there was no evidence of cancer.

Patient 2

This 72 year-old man presented in January 2006 with rapidly progressive confusion, personality change and difficulty recognizing familiar faces. The neurological examination revealed severe short-term memory loss, without focal deficits. CSF showed pleocytosis (18 WBC/ul, 100% lymphocytes), normal glucose concentration, elevated protein concentration (142 mg/dl) and high IgG index (0.92). EEG demonstrated occasional right central sharp waves with no epileptiform discharges. MRI FLAIR and T2 sequences showed bilateral medial temporal lobe hyperintensities that enhanced after contrast administration. CT of the chest, abdomen and pelvis, and FDG-PET were normal. Testing for anti-double stranded DNA, SSA/Ro, SSB/La, rheumatoid factor, ANCA, and salivary gland biopsy were unrevealing. Analysis for paraneoplastic and VGKC antibodies was negative. Treatment with corticosteroids and IVIg was ineffective. In June 2006 the patient had several generalized seizures; the neurological examination showed severe dementia. The patient died one month later as a result of neurologic deterioration. Postmortem studies were limited to ruling out a prion disease, and the immunoblot of frozen brain tissue did not reveal the presence of protease resistant prion protein (Prp 27-30).

MATERIALS AND METHODS

Patients' sera and CSF were kept frozen at -80°C . Sera and CSF from 111 patients were used as controls, including 37 patients with LE, 7 cerebellar degeneration, 6 encephalomyelitis, 3 optic neuritis, 2 polyneuropathy, 2 stiff-person syndrome and 54 patients with small cell lung cancer (SCLC) without neurological symptoms. Overall, in 45 of these patients the neurological disorder was a paraneoplastic manifestation of cancer. Nineteen of these patients had breast cancer and 20 had SCLC.

Immunohistochemistry

Immunohistochemistry was performed with frozen 7 μm -thick sections of rat and human brain, rat liver, kidney, and bowel tissues fixed in paraformaldehyde, using patients' CSF (1:5), sera (1:200) and human AK5-affinity purified antibodies (1:1), and the avidin-biotin-peroxidase method, as reported (Dalmau et al., 1999). Double immunolabeling using human AK5-affinity purified antibodies and polyclonal rabbit anti-AK5 antibody (1:50) (Abgent, San Diego, CA) was performed using the appropriate Alexa Fluor secondary antibodies (1:2000) (Molecular Probes, OR).

Screening of a hippocampal cDNA expression library and immunoblot

A uni-ZAP-XR rat hippocampal library (Stratagene, La Jolla, CA) was probed with sera from the patients, as reported (Bataller et al., 2003). Positive clones were purified with several rounds of screening and subcloned into pBluescript using the *in vivo* phage rescue protocol (Stratagene) and sequenced (Bataller et al., 2003). Subsequently, nitrocellulose filters with purified phage plaques expressing AK5 and irrelevant plaques were obtained. Sera (1:500) and CSF (1:10) from patients and controls were examined for antibodies using the avidin–biotin peroxidase method on filters containing AK5 positive plaques, as reported (Bataller et al., 2003).

Affinity purification of antibodies

Filters with purified phage plaques expressing AK5 or irrelevant *E. coli* proteins were incubated with patient's serum (1:1000) overnight at 4°C. After washing, bound antibodies were eluted with sodium citrate pH 2.7 and neutralized with Tris pH 8.8. Purified antibodies were concentrated with a column of protein A-Sepharose and used in immunohistochemical studies as indicated above.

RESULTS

Immunohistochemistry and western blot with patients' sera and CSF

Sera and CSF from both patients, but not from controls, showed an identical pattern of reactivity with the cytoplasm of neurons of rat (Fig 1A) and human brain. Sera and CSF antibodies did not react with systemic tissues, confirming the neuronal specificity of patients' IgG reactivity. Immunoblot studies using proteins extracted from rat brain did not reveal specific reactivity (data not shown).

Identification of AK5 as the target autoantigen

Probing of a rat hippocampal cDNA library with patients' sera resulted in the isolation of six overlapping cDNA clones containing the entire open reading frame of the rat AK5 gene (GenBank accession #XM-001080050). Filters containing AK5 plaques were recognized by the two patients' sera and CSF and the polyclonal rabbit anti-AK5 antibody, but not by control sera (Fig 1B). Immunoblots of proteins from the same representative clones did not react with patients' sera or CSF, suggesting that the AK5 epitopes are conformational.

The human AK5-affinity purified antibodies reproduce the brain reactivity of patients' sera and CSF

Immunohistochemistry with the polyclonal rabbit AK5 antibody revealed an identical pattern of neuronal cytoplasmic reactivity to that obtained with the patients' sera and CSF (Fig 1C). To further confirm that the cytoplasmic reactivity of patients' antibodies was with neuronal AK5, filters with purified AK5-expressing phage plaques (or irrelevant plaques) were incubated with patients' and control sera, and the bound IgG eluted as indicated. These studies demonstrated that the eluted patients' IgG from AK5 filters (but not from filters with irrelevant plaques) reproduced the serum and CSF reactivity with brain (Fig 1 D, E), and co-localized with the immunolabeling of the rabbit polyclonal AK5 antibodies (inset, Fig 1D). These studies confirmed that the brain reactivity of patients' antibodies was specific for AK5.

DISCUSSION

Recent studies indicate that LE associated with antibodies to intracellular antigens, including most paraneoplastic antigens (i.e., Hu, CV2/CRMP5, Ma2), are more likely to associate with cytotoxic T-cell mechanisms and decreased response to treatment than those associated with

antibodies to cell surface antigens, including VGKC and N-methylD-aspartate receptor (NMDAR) (Bataller et al., 2006). Furthermore, while some antibodies almost always associate with cancer, others like VGKC antibodies, do so less frequently (Pozo-Rosich et al., 2003).

In keeping with the concept that the encephalitides associated with antibodies to intracellular proteins are usually poorly responsive to treatment, both of our patients had relentless neurological deterioration that led to death in one case. Furthermore, similar to most paraneoplastic antigens, AK5 has critical neuronal-specific metabolic functions, including transfer of energy between cellular compartments and synthesis of RNA and DNA (Ren et al., 2005). Among the 6 members of the AK family (AK1-6), AK5 is specifically expressed in brain, and is primarily located in the neuronal cytosolic fraction (Van Rompay et al., 1999). Aberrant methylation of the AK5 gene has been reported in breast cancer (Miyamoto et al., 2005).

Antibodies to AK5 have not been previously identified in association with human disease. However, increased CSF concentration of AK may occur in patients with extensive brain damage caused by stroke and brain tumors (Buttner et al., 1986; Ronquist et al., 1977). To examine whether antibodies to AK5 could have been triggered after neuronal damage by encephalitis of unknown etiology or as a remote effect of an occult breast cancer, we included 50 control patients with paraneoplastic encephalitis or cerebellar degeneration and 19 with breast cancer. None of the patients had antibodies to AK5 in serum or CSF, suggesting that the antibodies of our patients were likely related to the immune disorder.

Several protein kinases have recently been found to be target antigens of autoimmune encephalitis, paraneoplastic or not (Sabater et al., 2005; Sabater et al., 2006). These disorders are usually poorly responsive to therapy except for Sydenham's chorea that may associate with antibodies that activate Ca²⁺/calmodulin-dependent protein kinase II (Kirvan et al., 2003). When antibodies to brain kinases occur in association with paraneoplastic syndromes, the tumor most frequently involved is lung cancer. Therefore, to determine if the immune response to AK5 could have been triggered by an occult lung cancer, we looked for anti-AK5 antibodies in sera of 74 patients with SCLC. All sera were negative for anti-AK5 antibody, suggesting that these antibodies are not present in lung cancer patients. Overall, these findings suggest that AK5 should be added to the list of protein kinase antigens related to autoimmune encephalitis.

In the last 5 years the profiling of autoantigens associated with non-viral LE has expanded, allowing the identification of target autoantigens in approximately 80% of patients (Bataller et al., 2006; Vincent et al., 2004; Dalmau et al., 2007). The two patients presented here narrow the remaining group of cases for whom no autoantigens are known. Our findings suggest that detection of AK5 antibodies in patients with LE carries a poor prognosis, and should prompt consideration of aggressive immunosuppression.

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REFERENCES

- Bataller L, Dalmau J. The clinical and immunological diversity of limbic encephalitis: a model for paraneoplastic disorders. *Hematol. Oncol. Clin. North. Am* 2006;20:1319–1335. [PubMed: 17113466]
- Bataller L, Kleopa KA, Wu GF, Rossi JE, Rosenfeld MR, Dalmau J. Autoimmune Limbic Encephalitis in 39 Patients: Immunophenotypes and Outcomes. *J. Neurol. Neurosurg. Psychiatry*. 2006 Published Online First: 15 September 2006. doi:10.1136/jnnp.2006.100644

- Bataller L, Rosenfeld MR, Graus F, Vilchez JJ, Cheung NK, Dalmau J. Autoantigen diversity in the opsoclonus-myoclonus syndrome. *Ann. Neurol* 2003;53:347–353. [PubMed: 12601702]
- Buttner T, Hornig CR, Busse O, Dorndorf W. CSF cyclic AMP and CSF adenylate kinase in cerebral ischaemic infarction. *J. Neurol* 1986;233:297–303. [PubMed: 3021918]
- Dalmau J, Gultekin SH, Voltz R, Hoard R, DesChamps T, Balmaceda C, Batchelor T, Gerstner E, Eichen J, Frennier J, Posner JB, Rosenfeld MR. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. *Brain* 1999;122:27–39. [PubMed: 10050892]
- Dalmau J, Tuzun 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 anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann. Neurol* 2007;61:25–36. [PubMed: 17262855]
- Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nat. Med* 2003;9:914–920. [PubMed: 12819778]
- Miyamoto K, Fukutomi T, Akashi-Tanaka S, Hasegawa T, Asahara T, Sugimura T, Ushijima T. Identification of 20 genes aberrantly methylated in human breast cancers. *Int. J. Cancer* 2005;116:407–414. [PubMed: 15818620]
- Pozo-Rosich P, Clover L, Saiz A, Vincent A, Graus F. Voltage-gated potassium channel antibodies in limbic encephalitis. *Ann. Neurol* 2003;54:530–533. [PubMed: 14520669]
- Ren H, Wang L, Bennett M, Liang Y, Zheng X, Lu F, Li L, Nan J, Luo M, Eriksson S, Zhang C, Su XD. The crystal structure of human adenylate kinase 6: An adenylate kinase localized to the cell nucleus. *Proc. Natl. Acad. Sci. U.S.A* 2005;102:303–308. [PubMed: 15630091]
- Ronquist G, Ericsson P, Frithz G, Hugosson R. Malignant brain tumours associated with adenylate kinase in cerebrospinal fluid. *Lancet* 1977;1:1284–1286. [PubMed: 68384]
- Sabater L, Bataller L, Carpentier AF, Aguirre-Cruz ML, Saiz A, Benyahia B, Dalmau J, Graus F. Protein kinase Cgamma autoimmunity in paraneoplastic cerebellar degeneration and non-small-cell lung cancer. *J. Neurol. Neurosurg. Psychiatry* 2006;77:1359–1362. [PubMed: 16801349]
- Sabater L, Gomez-Choco M, Saiz A, Graus F. BR serine/threonine kinase 2: a new autoantigen in paraneoplastic limbic encephalitis. *J. Neuroimmunol* 2005;170:186–190. [PubMed: 16165222]
- Van Rompay AR, Johansson M, Karlsson A. Identification of a novel human adenylate kinase. cDNA cloning, expression analysis, chromosome localization and characterization of the recombinant protein. *Eur. J. Biochem* 1999;261:509–517. [PubMed: 10215863]
- Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004;127:701–712. [PubMed: 14960497]

Figure 1.

Panel A: Section of rat temporal cortex immunolabeled with serum of Patient #1. Serum IgG shows intense neuronal cytoplasmic reactivity.

Panel B: Nitrocellulose filter with mixed (~50%) AK5-expressing bacteriophage plaques and irrelevant plaques, incubated with normal serum (N), Patients' sera (C1, C2), and a rabbit polyclonal AK5 antibody (AK5). Normal serum shows no reactivity with AK5 plaques, whereas the patients' sera and anti-AK5 antibody show strong reactivity with AK5-expressing plaques. Irrelevant plaques are barely visible in C1 and C2, but are seen in AK5 producing mild non-specific background signal.

Panel C: Section of rat temporal cortex immunolabeled with a polyclonal rabbit anti-AK5 antibody. Note that the cytoplasmic reactivity is similar to that seen with the patient's serum (panel A).

Panel D: Section of rat temporal cortex immunolabeled with patient IgG eluted from filters containing 100% AK5-expressing plaques. Note that the cytoplasmic reactivity is similar to that produced by patient's serum (panel A), and co-localizes with the polyclonal AK5 rabbit antibody using a double immunolabeling assay (inset with yellow cytoplasmic immunolabeling).

Panel E: Section of rat temporal cortex incubated with patient's IgG eluted from irrelevant plaques produces no reactivity. Similar negative results were obtained with the IgG from normal individuals eluted from AK5-expressing plaques.

Panels A, B, D, E $\times 400$, avidin-biotin-peroxidase technique with mild hematoxylin counterstaining. Inset in D, immunofluorescence, double immunolabeling, $\times 400$.