A**ntiamphiphysin Antibodies Are Associated With Various Paraneoplastic Neurological Syndromes and Tumors**

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**Background:** Antiamphiphysin antibodies react with a 128-kd protein found in synaptic vesicles. They were first described in patients with paraneoplastic stiff-man syndrome and breast cancer, but studies suggest that they can also occur in patients with other tumors and neurological disorders.

**Objective:** To determine if antiamphiphysin antibodies are associated with various paraneoplastic neurological syndromes and tumors.

**Patients and Methods:** Of 2800 serum samples tested by routine immunohistochemical procedures on sections of paraformaldehyde-fixed rat brain for the detection of autoantibodies associated with paraneoplastic neurological syndromes, 5 were selected because of labeling suggestive of antiamphiphysin antibodies and subsequently confirmed by the results of Western blot analysis using recombinant amphiphysin protein. Controls consisted of 40 patients with various nonparaneoplastic neurological diseases; 101 patients with cancer but without paraneoplastic neurological syndrome; 9 patients with small cell lung cancer, anti-Hu antibodies, and paraneoplastic neurological syndrome; 3 patients with M2-type antimitochondrial antibodies but no neurological disorder; and 30 normal subjects.

**Results:** Of the 5 patients with antiamphiphysin antibodies, patient 1 had sensory neuronopathy, encephalomyelitis, and breast cancer; patient 2 had limbic encephalitis, and small cell lung cancer was detected in the mediastinum after 24 months of follow-up; patient 3 had encephalomyelitis and ovarian carcinoma; and patients 4 and 5 had Lambert-Eaton myasthenic syndrome and small cell lung cancer (patient 4 subsequently developed cerebellar degeneration). None of the 5 had stiffness. Two patients (Nos. 2 and 4) had antimitochondrial antibodies. The two patients (Nos. 4 and 5) with Lambert-Eaton myasthenic syndrome had antibodies directed against the voltage-gated calcium channel, and patient 2 subsequently developed anti-Hu antibodies. In the controls, antiamphiphysin antibodies were detected by Western blot analysis in 3 of 8 patients with anti-Hu antibodies, but in none of the other groups.

**Conclusions:** These data indicate that antiamphiphysin antibodies are not specific for one type of tumor or one neurological syndrome and can be associated with other neural and nonneural antibodies. The simultaneous association of several antibodies in some patients suggests multimodal autoantibody production.

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**S**everal types of autoantibodies have been identified in patients with paraneoplastic neurological syndromes; these include antiamphiphysin antibodies that recognize a 128-kd protein in Western blots of rat and human brain subsequently shown to be the nerve terminal protein amphiphysin.1,2 Antiamphiphysin antibodies were first reported in women with breast cancer and stiff-man syndrome, but studies indicate that they also occur in patients with other tumors and neurological disorders.3,4 Using a series of 5 patients, we have now extended the spectrum of neurological syndromes and tumors that are associated with antiamphiphysin antibodies and suggest that these antibodies are not specific for one type of neurological syndrome and one type of cancer. Also, we found that other neural and nonneural antibodies were present in several patients with antiamphiphysin antibodies, thus making it difficult to detect such antibodies by immunohistochemical analysis alone.

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**REPORT OF CASES**

The 5 patients described below were selected from 2800 patients whose serum
MATERIALS AND METHODS

The serum samples from the 5 patients and all control patients were snap frozen and stored at –80°C until tested. The control group consisted of 40 patients with various nonparaneoplastic neurological diseases; 101 patients with cancer but without paraneoplastic neurological syndrome (37 with SCLC, 25 with gynecological cancer, 25 with colon adenocarcinoma, and 14 with non-SCLC lung tumors); 8 patients with SCLC, anti-Hu antibodies, and paraneoplastic neurological syndrome; 3 patients with M1-type anti-mitochondrial antibodies but no neurological disorder; and 30 normal subjects. All serum samples were immunohistochemically tested as described below. For Western blot experiments, the serum samples from the 5 patients and from the controls (2 normal subjects, 1 patient with anti-mitochondrial antibodies, 16 patients with nonparaneoplastic neurological disorders, 7 patients with SCLC and 7 with breast carcinoma and no neurological disorder, and 8 patients with SCLC, paraneoplastic neurological syndrome, and anti-Hu antibodies) were tested using recombinant human amphiphysin and rat cerebellum extracts.

The method used to detect antiamphiphysin antibodies was slightly modified from that described elsewhere for the detection of paraneoplastic antibodies and conforms to the guidelines for the detection of such antibodies. Immunohistochemical testing was performed on frozen cerebellar and brainstem sections from brains removed from anesthetized rats perfused in vivo with 4% paraformaldehyde and 0.2% picric acid and postfixed for 24 hours in the same solution. The sections were incubated for 2 hours at room temperature with the serum samples and diluted at least 100-fold in phosphate-buffered saline and blocked with 1% bovine serum albumin to saturate nonspecific protein binding sites. The sections were then incubated with biotinylated rabbit anti–human IgG antibodies (Jackson, Baltimore, Md) or with pig anti–rabbit IgG antiserum (Dakopatts, Glostrup, Denmark). The color reaction was developed using diaminobenzidine.

Antimitochondrial antibodies were detected by screening serum samples from the patients and controls immunohistochemically on frozen sections of rat kidney, liver, and stomach for nonneural antibodies using the procedures previously described. M1-type antimitochondrial antibodies demonstrated a typical granular cytoplasmic staining of the distal renal tubules, mainly of the medullary, hepatocytes, and gastric parietal cells. In our laboratory, a sample is considered positive at a titer of greater than 1:10.

CASE 1

In April 1994, a 75-year-old woman developed sensory loss and pain in her lower limbs. On examination in December 1994, the absence of paresthesia was noted in both legs. Pain and thermal sensation were almost normal. There was no motor deficit, and tendon reflexes were absent. Electrophysiological tests showed absent sensory responses in both legs. Motor conduction velocities were normal. The cerebrospinal fluid specimen contained 0.69 g/L protein, with a normal level of IgG and 1 lymphocyte per cubic millimeter. The patient’s neurological status progressively deteriorated, and she developed cerebellar dysarthria, truncal ataxia, slight memory loss, and epilepsy. She had no stiffness. A computed tomographic scan of the brain showed no tumoral lesion or cerebellar atrophy. A breast adenocarcinoma, discovered in May 1996, was treated with surgery and radiotherapy. During the following months, the ataxia progressed and then stabilized. In September 1997, the patient was able to walk using a Zimmer frame.

CASE 2

In August 1996, a 55-year-old male smoker complained of mood changes and became abnormally anxious and depressed. His neurological status deteriorated progressively, and he had olfactory and auditory hallucinations. He developed severe anterograde memory loss, but his retrograde memory was normal. In February 1997, electroencephalographic tests showed epileptic discharges in the temporal lobes. The results of cerebrospinal fluid analysis were normal. T1-weighted magnetic resonance images of the brain showed high signals in both hippocampi, especially the left. There was no stiffness. After 24 months of follow-up, a lymph node metastasis of small cell lung cancer (SCLC) was discovered in the mediastinum.

CASE 3

In December 1994, a 50-year-old woman complained of gait disturbance and paresthesia in her hands. Severe proximal and distal motor deficit with hand amyotrophy, diffuse areflexia, and a bilateral Babinski sign progressively developed. Severe sensory loss, affecting all modalities of sensation, appeared in all 4 limbs. There was...
of the tongue with surgery and radiotherapy. In June 1996, he complained of increasing gait difficulties. Examination in July 1997 showed a proximal and distal motor deficit, with absence of both Achilles tendon reflexes. The patient’s mouth was dry. There was no stiffness. Electrophysiological test results were indicative of LEMS, showing reduced motor action potentials in the lower limbs, with a 500% improvement after brief maximal exercise, a 20% decrease after stimulation at 3 Hz, and a 250% improvement after stimulation at 20 Hz. Screening for antibodies to the P/Q-type VGCC proved positive. There was no recurrence of the tongue carcinoma, and a computed tomographic scan of the chest showed a mediastinal tumor, identified as SCLC on surgical biopsy.

CASE 5

In 1972, a 73-year-old man was treated for carcinoma of the tongue with surgery and radiotherapy. In June 1996, a mediastinal tumor, identified as SCLC on surgical biopsy.

In January 1993, a 67-year-old man complained of progressive weakness in his legs. In February 1994, a moderate proximal motor deficit and depressed tendon reflexes were noted in both legs. Electrophysiological tests showed reduced motor action potentials in the lower limbs, with a 210% improvement after brief maximal exercise, a 40% decrease after stimulation at 3 Hz, and a 158% improvement after stimulation at 20 Hz, indicating Lambert-Eaton myasthenic syndrome (LEMS). The results of screening for antibodies to the P/Q-type VGCC were positive. An SCLC was discovered in March 1994, and the patient received chemotherapy and radiotherapy, without improvement. He was discharged in May 1994 and was unavailable for follow-up until May 1995, at which time he became bedridden. There was severe truncal and appendicular weakness, with absence of both Achilles tendon reflexes. The tendon reflexes were depressed. A computed tomographic scan of the brain showed severe cerebellar atrophy. There was no stiffness. The tumor was still localized to the mediastinum.

CASE 4

In January 1993, a 67-year-old man complained of progressive weakness in his legs. In February 1994, a moderate proximal motor deficit and depressed tendon reflexes were noted in both legs. Electrophysiological tests showed reduced motor action potentials in the lower limbs, with a 210% improvement after brief maximal exercise, a 40% decrease after stimulation at 3 Hz, and a 158% improvement after stimulation at 20 Hz, indicating Lambert-Eaton myasthenic syndrome (LEMS). The results of screening for antibodies to the P/Q-type VGCC were positive. An SCLC was discovered in March 1994, and the patient received chemotherapy and radiotherapy, without improvement. He was discharged in May 1994 and was unavailable for follow-up until May 1995, at which time he became bedridden. There was severe truncal and appendicular cerebellar ataxia with dysarthria, overshadowing the symptoms of LEMS. The tendon reflexes were depressed. A computed tomographic scan of the brain showed severe cerebellar atrophy. There was no stiffness. The tumor was still localized to the mediastinum.

Figure 1. Western blots using recombinant amphiphysin. Lane 1, rabbit anti-recombinant amphiphysin antiserum; lane 2, patient 1; lane 3, patient 2; lane 4, patient 5; lane 5, patient 3; lane 6, patient 4; and lane 7, antimitochondrial antibodies from a control patient. Molecular weights are shown on the right. The serum samples from all the patients and the rabbit react with a doublet of bands corresponding to 125- to 138-kd molecular weight, resulting from spontaneous degradation of the recombinant protein.

Of the 2800 serum samples routinely tested for paraneoplastic autoantibodies, 5 demonstrated labeling of the rat cerebellum and brainstem suggestive of antiampiphysin antibodies. On Western blots using recombinant human amphiphysin, all 5 labeled a doublet of bands with an apparent molecular weight of 125 to 128 kd, identical to those recognized by the rabbit antiampiphysin antiserum (Figure 1). When tested on Western blots of brain cerebellum, all 5 serum samples labeled a 128-kd band probably corresponding to amphiphysin. As with the control serum samples, additional bands were sometimes seen. The immunological data for these patients are summarized in the Table.

When tested immunohistochemically on rat cerebellum and brainstem, serum samples from patients 1, 3, and 5 demonstrated a pattern typical of antiampiphysin antibody. In the cerebellum, there was diffuse staining of the neuropil in the molecular layer, the cytoplasm of the Purkinje cells being less intensively labeled, and intense granular staining was seen in the periphery of the perikarya of the Purkinje cells and the granule cell layer, suggesting staining of the synaptic terminals (Figure 2, A). In the brainstem, fiber tracts were labeled and large neurons showed the same pattern of staining as Purkinje cells, whereas glial cells were never stained. Serum samples from patients 2 and 5 demonstrated a somewhat different pattern. In addition to the labeling typical of antiampiphysin antibody, granular cytoplasmic staining of neurons and glial cells was seen.

Table

<table>
<thead>
<tr>
<th>Patient No./Age, y/Sex</th>
<th>Neurological Syndrome</th>
<th>Tumor</th>
<th>Amphiphysin Antibody Titer</th>
<th>Other Antibodies</th>
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</thead>
<tbody>
<tr>
<td>1/75/F 14</td>
<td>SSN/PEM</td>
<td>Breast</td>
<td>1:1000</td>
<td>None</td>
</tr>
<tr>
<td>2/55/M 21</td>
<td>Limbic encephalitis</td>
<td>SCLC</td>
<td>1:5000</td>
<td>Anti-M</td>
</tr>
<tr>
<td>3/50/F 31</td>
<td>EM</td>
<td>Ovary</td>
<td>1:5000</td>
<td>None</td>
</tr>
<tr>
<td>4/67/M 45</td>
<td>LEMS and PCD</td>
<td>SCLC</td>
<td>1:2000</td>
<td>Anti-VGCC</td>
</tr>
<tr>
<td>5/73/M 66</td>
<td>LEMS</td>
<td>SCLC</td>
<td>1:1000</td>
<td>Anti-M</td>
</tr>
</tbody>
</table>

*SN indicates sensory neuropathy; EM, encephalomyelitis; LEMS, Lambert-Eaton myasthenic syndrome; PCD, paraneoplastic cerebellar degeneration; SCLC, small cell lung cancer; M, mitochondrial; and VGCC, voltage-gated calcium channel.
with diffuse labeling of the neuropil. When tested immunohistochemically on rat kidney, liver, and stomach, these 2 serum samples demonstrated labeling characteristic of M2-type antimitochondrial antibody (titer, 1:160 and 1:2500), while the serum samples from the 3 patients with M2-type antimitochondrial antibody, but no neurological disease, demonstrated granular labeling in the neuropil and cytoplasm of neurons and glial cells (Figure 2, B). Thus, some of the granular cytoplasmic staining seen in the serum samples from patients 2 and 5 may be due to antimitochondrial antibodies. Also, a second serum sample from patient 2, obtained 11 months after the first antibody determination, showed nuclear labeling of neurons, with sparing of the nucleoli, suggestive of anti-Hu antibodies; this was confirmed by Western blotting using recombinant HuD protein and whole cerebellar extracts (Figure 3).

On immunohistochemical testing, none of the 182 control serum samples demonstrated labeling suggestive of antiamphiphysin antibodies. On Western blot analysis using recombinant human amphiphysin, none of the 57 control serum samples proved positive, except samples from 3 of 8 patients with anti-Hu antibodies.

**COMMENT**

Although antiamphiphysin antibodies have been reported in several patients, to our knowledge detailed clinical data are available in only 6 cases. In 1993, Folli et al described 3 women with breast carcinoma who had contracture and stiffness of the limbs and continuous motor unit activity at rest on electromyographic testing, consistent with stiff-man syndrome. The trunk was usually spared, and oligoclonal bands of IgG were present in the cerebrospinal fluid specimens from 2 of the patients. Their serum samples reacted with a protein doublet of 128 kd on Western blots of human cortex. In 1993 and 1994, 2 more patients were described by the same group as having stiff-man syndrome and serum samples that were reactive with the 128-kd protein, now identified as amphiphysin. In 1993, Grimaldi et al mentioned 2 patients with stiff-man syndrome and antibodies that reacted with a 125- to 130-kd protein on Western blots of total rat brain homogenate; one had carcinoma of the colon and the other Hodgkin disease. In 1996, Dropcho reported the detailed clinical description of 3 patients with SCLC.
and antibodies that reacted with human amphiphysin: 2 had subacute sensory neuronopathy (SSN) and paraneoplastic encephalomyelitis (PEM), and 1 had stiffness and myoclonus associated with symptoms of encephalomyelitis. Finally, in 1997, Lennon et al. described, in abstract form, 11 patients with antibodies that reacted with recombinant human amphiphysin; these patients did not have stiff-man syndrome, but they did have PEM with various features, including limbic encephalitis, choreoathetosis, myoclonus, stiffness, and rigidity. The tumors involved were breast or lung carcinomas; 2 patients had no known cancer.

Our patients extend the spectrum of neurological syndromes associated with antiamphiphysin antibodies. Two patients (Nos. 4 and 5) had SCLC and LEMS with a typical electrophysiological pattern and anti-VGCC antibodies; in patient 4, paraneoplastic cerebellar degeneration appeared in the following months. The association of LEMS and paraneoplastic cerebellar degeneration has been reported in several other patients and is probably not fortuitous, suggesting a pathophysiological link between the 2 disorders. Patient 1 had breast cancer, SSN with the electrophysiological abnormalities of SSN, and central nervous system manifestations of PEM. Patient 3 had ovarian carcinoma and developed severe sensory-motor neuropathy, with electrophysiological abnormalities indicative of a neuronopathy. The presence of pyramidal syndrome showed that she had encephalomyelitis. Although rare, the combination of SSN and PEM has already been reported in patients with gynecological cancer and is a classic paraneoplastic disorder. In the last patient (No. 2), the clinical and radiological data indicated limbic encephalitis that was associated with SCLC. Taken as a whole, the 26 patients described with antiamphiphysin antibodies (including our 5) show that these antibodies occur in association with several types of tumors, with gynecological cancer occurring in only 9 of the 26 patients. The clinical features are also heterogeneous. However, stiffness was present in 12 of the 26 patients, but it should be clarified whether a true stiff-man syndrome or a PEM with rigidity and spasm, or both, can be associated with antiamphiphysin antibodies.

**Antiamphiphysin Antibodies** contrast with the well-known anti-Hu and anti-Yo antibodies, which show a strong correlation with the type of neurological disorder and the type of cancer. Most patients with anti-Hu antibodies have SCLC and SSN/PEM, while anti-Yo antibodies are strongly associated with paraneoplastic cerebellar degeneration and gynecological tumors. This observation, together with the intracellular localization of amphiphysin, is evidence against a direct role of antiamphiphysin antibodies in neurological disorders. Such a conclusion is further supported by the fact that 2 of our patients had LEMS, a disorder of the neuromuscular junction thought to depend on anti-VGCC antibodies. It is possible that the humoral response directed against amphiphysin may simply be an epiphenomenon of the autoimmune process that directly mediates the neurological disease.

The current hypothesis concerning paraneoplastic neurological disorders is that the autoimmune response is directed primarily against tumor antigens that are also present in the nervous system. Dropcho reported that SCLC and breast carcinomas in patients without neurological disorders can express amphiphysin messenger RNA. Thus, patients with tumors may be prone to develop antiamphiphysin antibodies. However, Folli et al. found no antiamphiphysin antibodies in 30 control patients with cancer, and we found no labeling suggestive of antiamphiphysin antibodies in 101 patients with SCLC, gynecological cancer, or colon carcinoma. Thus, the incidence of antiamphiphysin antibodies in patients with cancer, but without paraneoplastic disorders, is probably low. So far, these antibodies have only been seen in patients with neurological disorders consistent with paraneoplastic syndromes; therefore, whatever their pathogenic role, they are useful tools for the diagnosis of tumors in patients with suspected paraneoplastic disorders.

Many laboratories use routine immunohistochemical testing as a screening method for the detection of paraneoplastic antibodies. In 2 of our patients, the typical labeling of antiamphiphysin antibodies was significantly modified by the presence of M1-type antimitochondrial antibodies or anti-Hu antibodies, emphasizing the need to test serum samples with an unusual staining pattern on noncerebral tissues and by Western blotting using recombinant protein or brain homogenates. Three of our 5 patients had several autoantibodies in their serum samples, and of these 3 patients, 1 had antiamphiphysin, anti-VGCC, and antimitochondrial antibodies, and 1 had anti-Hu, anti-mitochondrial, and antiamphiphysin antibodies. Antiamphiphysin antibodies have also been reported in association with anti-Hu and anti-glutamic acid decarboxylase antibodies, and we found that the serum samples from 3 of 8 patients with PEM/SSN and anti-Hu antibodies reacted with recombinant amphiphysin. These data are in agreement with those of reports indicating that patients with paraneoplastic neurological disorders can sometimes produce several autoantibodies that react with the nervous system or that non–organ-specific antibodies are seen in patients with cancer or paraneoplastic neurological syndromes. Patients with cancer, especially those with paraneoplastic neurological disorders, are probably prone to develop a complex state of autoimmunity leading to the simultaneous production of several antibodies, both specific and nonspecific for the nervous system.

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