

SHORT REPORT

Anti-amphiphysin I antibodies in patients with paraneoplastic neurological disorders associated with small cell lung carcinoma

A Saiz, J Dalmau, M Husta Butler, Q Chen, J Y Delattre, P De Camilli, F Graus

Abstract

Patients with stiff man syndrome and breast cancer develop anti-amphiphysin I antibodies that primarily recognise the C terminus of the protein. Anti-amphiphysin I antibodies have also been identified in a few patients with paraneoplastic neurological disorders (PND) and small cell lung cancer (SCLC). The frequency of anti-amphiphysin I antibodies in patients with SCLC and PND was analysed and the epitope specificity of these antibodies was characterised.

Anti-amphiphysin I antibodies were evaluated by immunohistochemistry on human and rat cerebellum and immunoblots of rat brain homogenates. Serum samples included 134 patients with PND and anti-Hu antibodies (83% had SCLC), 44 with SCLC and PND without anti-Hu-antibodies, 63 with PND and either Yo, Ri, or Tr antibodies, 146 with SCLC without PND, and 104 with non-PND. Positive serum samples were confirmed with immunoblots of recombinant human amphiphysin I and immunoreacted with five overlapping peptide fragments covering the full length of the molecule.

Serum samples positive for anti-amphiphysin I antibodies included those from seven (2.9%) patients with PND and two (1.4%) with SCLC without PND. Six of the seven anti-amphiphysin I antibody positive patients with PND had SCLC (three with Hu-antibodies), and one had anti-Hu-antibodies but no detectable tumour. The PND included encephalomyelitis/sensory neuropathy (five patients), cerebellar degeneration (one), and opsoclonus (one). All anti-amphiphysin I antibodies reacted with the C terminus of amphiphysin I, but seven also recognised other fragments of the molecule.

In conclusion, anti-amphiphysin I antibodies are present at low frequency in patients with SCLC irrespective of the presence of an associated PND. All anti-amphiphysin I antibody positive serum samples have in common reactivity with the C terminus of the protein.

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Amphiphysin I, a nerve terminal protein with a putative role in endocytosis,¹ is recognised by autoantibodies present in the serum and CSF samples from female patients with paraneoplastic stiff man syndrome (SMS) and breast cancer.²⁻³ The anti-amphiphysin I antibodies of these patients predominantly react with the C terminus of the protein.¹⁻⁴ A recent study⁵ identified anti-amphiphysin I antibodies in the serum of three patients with small cell lung carcinoma (SCLC) and paraneoplastic encephalomyelitis and sensory neuronopathy (PEM/SN), a disorder usually associated with anti-Hu-antibodies.⁶ The serum of one of these patients had anti-Hu antibodies but none of them developed typical SMS. Although this study suggested that PEM/SN in patients with SCLC may associate with several autoantibody specificities and that anti-amphiphysin I antibodies are not restricted to SMS, the frequency and specificity of these associations were not defined. A few additional cases of anti-amphiphysin I antibody positive patients with PND other than SMS associated with either breast or lung cancer were reported.⁷ To clarify these clinical-serological associations is important because these antibodies are considered good markers of specific types of paraneoplastic syndromes and tumours.

In the present study, we analysed the frequency of anti-amphiphysin I antibodies in a large series of patients with PND other than SMS, to determine whether: (1) anti-amphiphysin I antibodies are associated with a particular type of PND or tumour and (2) whether amphiphysin I autoepitopes differ in patients with different clinical syndromes.

Materials and methods

Serum samples were obtained from 241 patients with PND, 146 with SCLC without PND or anti-Hu-antibodies, and 104 with non-paraneoplastic neurological disorders usually considered in the differential diagnosis of PND (cerebellar disorders 56, sensory neuropathy 41; opsoclonus seven). Among the 241 patients with PND, 134 had PEM/SN (83%

Service of Neurology and Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain
A Saiz
F Graus

Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, USA
J Dalmau

Department of Cell Biology and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Conn, USA
M Husta Butler
P De Camilli

Service of Neurology, Hôpital de la Pitié-Salpêtrière, INSERM U134, Paris, France
Q Chen
J Y Delattre

Correspondence to:
Dr Francesc Graus, Servei de Neurologia, Hospital Clínic, Villarroel 170, Barcelona 08036, Spain. Telephone 0034 3 2272213; fax 0034 3 2275414; email graus@medicina.ub.es

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had SCLC), 44 had SCLC and PND without anti-Hu antibodies (paraneoplastic cerebellar degeneration 30; PEM/SN seven; opsoclonus seven), and 63 had PND and either Yo, Ri, or Tr antibodies. Serum samples were collected in three of the participant laboratories (Barcelona, Paris, and New York) and kept frozen at -70°C . Serum samples from two patients with paraneoplastic SMS and breast cancer were used as positive controls.

Immunohistochemistry on human and rat cerebellum (serum screening dilution 1:500) and immunoblot of rat brain homogenate (serum dilution 1:10 000) were done as previously described in detail.^{8,9} To make the anti-amphiphysin I antibody detection more uniform, all immunoblots were prepared in the same laboratory (Hospital Clinic, Barcelona) and nitrocellulose strips containing the same amount of immobilised antigen were sent to the other two participating laboratories (Paris and New York) that processed the strips with the same protocol.

CRITERIA FOR THE PRESENCE OF ANTI-AMPHIPHYSIN I ANTIBODIES

A serum was considered positive for anti-amphiphysin I antibodies when immunoblots of rat brain homogenate showed a reactive band of identical electrophoretic mobility (around 128 kDa) than that obtained with the positive control. All positive and seven randomly taken negative serum samples were subsequently evaluated by indirect immunofluorescence on formaldehyde fixed rat cerebellar tissue² and immunoblots of recombinant human amphiphysin I⁴ at Yale University (MHB, PDC). Serum samples were tested at dilutions of 1:4 and 1:500 for the immunofluorescence and the immunoblot experiments respectively. Clinical information was blinded from investigators performing these immunoblots.

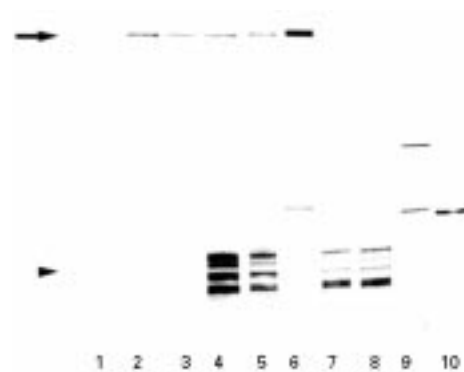
EPITOPE ANALYSIS

Glutathione S-transferase (GST) fusion proteins consisting of five overlapping fragments of amphiphysin I were prepared as described previously.⁴ The five GST fusion proteins were subjected to SDS-PAGE (10% acrylamide) and western blotting as previously described.¹⁰ Monoclonal antibodies directed to each fragment of amphiphysin I were used to identify the correct band. Positive and negative human serum samples were also used as controls.

Results

IMMUNOBLOT STUDIES

Nine serum samples immunoreacted with a band identical with that obtained with the positive control in immunoblots of rat brain homogenate (figure). In all of them, the presence of anti-amphiphysin I antibodies was confirmed in immunoblots of recombinant human amphiphysin I, whereas seven negative serum samples by the screening criteria, blindly examined with the positive ones, did not react with the recombinant protein. All nine positive samples reacted with fragment V of amphiphysin I that includes the C terminus



Immunoblots of rat brain homogenate probed with a normal human serum (lane 1), serum from paraneoplastic SMS and breast cancer with anti-amphiphysin I antibodies, as positive controls (lanes 2,3), PND and SCLC (lanes 4–8), and SCLC without PND (lanes 9–10). Three serum samples from patients with PND and SCLC (lanes 4–6) immunoreacted with a band (around 128 kDa) of the same electrophoretic mobility as that recognised by the positive anti-amphiphysin I antibody serum samples (arrow). Four serum samples also presented anti-Hu antibodies (arrowhead).

of the molecule,⁴ but the reactivity with the other four fragments of amphiphysin I was not uniform with seven samples recognising multiple fragments.

IMMUNOHISTOCHEMISTRY STUDIES

None of the nine positive anti-amphiphysin I antibody serum samples could be identified by immunohistochemistry on human cerebellum. In rat cerebellar sections evaluated by the avidin-biotin immunoperoxidase technique, the presence of anti-amphiphysin I antibodies was shown in only one serum sample, which corresponded to that with the strongest immunoreactivity in immunoblots. However, when the nine serum samples were evaluated by indirect immunofluorescence using a higher concentration,² all but one gave an immunoreactive pattern compatible with anti-amphiphysin I antibodies.

CLINICAL-IMMUNOLOGICAL CORRELATION

Seven anti-amphiphysin I antibody positive patients had PND(2.9%) and two had SCLC without neurological disorders (1.4%). Six of the seven patients with PND had SCLC that was diagnosed after onset of the neurological symptoms. In one patient, who also was anti-Hu-antibody positive, the tumour could not be found at necropsy. We include this patient in the PND group because the patient presented typical clinical and neuropathological features of PEM/SN with neuronal loss and inflammatory infiltrates in the dorsal root ganglia and spinal cord. None of the patients without PND or with PND associated with anti-Yo, Ri, or Tr antibodies had anti-amphiphysin I antibodies.

The clinical features of the seven patients with PND and anti-amphiphysin I antibodies are summarised in the table. Five of the seven patients developed symptoms of PEM/SN and four of them also had high titres of anti-Hu-antibodies. The other two patients had paraneoplastic cerebellar degeneration and

Clinical features of anti-amphiphysin positive patients with paraneoplastic neurological disorders

Patient	Age/sex	Clinical syndrome	Hu-Ab	Time (months) PND/tumour	Cancer	Outcome
1	60/F	Sensorimotor neuropathy	Yes	-23	SCLC	Necropsy: PEM/SN†
2	58/M	PEM/SN	Yes	-7	SCLC	Necropsy: PEM/SN†
3	71/M	PEM/SN and LEMS	Yes	-17	SCLC	Necropsy: PEM/SN†
4	64/M	Sensory neuropathy	No*	0	SCLC	Death: pulmonary embolus
5	64/M	Cerebellar syndrome	No	-18	SCLC	Alive for 7 years
6	74/M	Opsoclonus/myoclonus	No*	-1	SCLC	Necropsy: perivascular/meningeal inflammatory infiltrates. Moderate neuronal loss in dentate nucleus and Purkinje cells
7	80/F	Sensory neuropathy	Yes	n.a.	None	Necropsy: PEM/SN**

PEM/SN=Paraneoplastic encephalomyelitis/sensory neuronopathy; LEMS=Lambert-Eaton myasthenic syndrome; SCLC=small cell lung carcinoma; NA=not applicable.

*Patients had low titre of Hu-Ab similar to that found in 16% of SCLC without paraneoplastic neurological disorders.¹¹

†Necropsy findings typical of PEM/SN with multifocal inflammatory infiltrates, reactive gliosis, and neuronal loss.

opsoclonus/myoclonus syndrome. The frequency of anti-amphiphysin I antibodies in patients with PND and SCLC (3.9%) with (2.7%) or without (6.8%) anti-Hu-antibodies was not statistically different from that in patients with SCLC without PND.

Discussion

The main finding of the present study is that the frequency of anti-amphiphysin I antibodies was low in patients with SCLC and PND. The group of patients with the highest frequency of anti-amphiphysin I antibodies (6.8%) corresponded with those with SCLC, PND, and negative anti-Hu-antibodies. However, even for this group the frequency of anti-amphiphysin I antibodies was not significantly different from that in patients with SCLC without PND.

The clinical range of anti-amphiphysin I antibody positive patients was not uniform. None developed clinical or neurophysiological features of SMS, five patients had PEM/SN and two developed other PND (see below). Four out of the five PEM/SN patients had anti-Hu-antibodies, a not surprising finding considering that the great majority of patients with PEM/SN, 95% in this series, had anti-Hu-antibodies.⁶ Necropsy findings of the four patients with PEM/SN harbouring both anti-Hu-antibodies and anti-amphiphysin I antibodies were similar to those reported in anti-Hu-antibody positive patients with PEM/SN but without anti-amphiphysin I antibodies.⁷ Although postmortem of one of our anti-amphiphysin I antibody positive patients did not show a tumour, this patient had anti-Hu-antibodies and typical clinical and postmortem features of PEM/SN raising the possibility that a small, tumour was overlooked.

The other two patients presented paraneoplastic cerebellar degeneration and the opsoclonus/myoclonus syndrome respectively. Patients with paraneoplastic cerebellar degeneration and SCLC, who do not develop other features of PEM/SN, usually lack anti-Hu-antibodies but 36% of them harbour P/Q type voltage gated calcium channel antibodies probably related to a higher than expected association with the Lambert-Eaton myasthenic syndrome.¹² Similarly, anti-Hu-antibodies are rarely identified in paraneoplastic opsoclonus/myoclonus associated with SCLC although a few patients have been reported.^{13, 14} Excluding these exceptions, when paraneoplastic cerebellar degeneration and

opsoclonus/myoclonus develop as isolated syndromes in patients with SCLC, they are not generally associated with any specific antineuronal antibody. Our series supports this notion, because anti-amphiphysin I antibodies were identified in only 3% of paraneoplastic cerebellar degeneration and 14% of patients with opsoclonus-myoclonus and SCLC.

The low frequency of anti-amphiphysin I antibodies in patients with SCLC with PND, the lack of association with a particular neurological syndrome, and the similar frequency of anti-amphiphysin I antibodies in patients with SCLC without PND is by contrast with that described with anti-Hu-antibodies that are particularly associated with PEM/SN syndrome and SCLC.⁶ This is also by contrast with the close link between anti-amphiphysin I antibodies and SMS associated with breast cancer.²

Our study suggests that the optimal immunohistochemical method to detect antineuronal antibodies depends on the type of antibody. In the current study, the use of recommended techniques and serum dilutions to detect antineuronal antibodies¹⁵ failed to identify anti-amphiphysin I antibodies which, however, were readily demonstrated using higher serum concentrations and a different protocol.² In any case, immunoblot seems more sensitive than immunohistochemistry to detect anti-amphiphysin I antibodies.

The anti-amphiphysin I antibodies from all our patients recognised fragment V (C terminus) of human amphiphysin I, although many of them also recognised additional fragments. This finding is in agreement with that previously reported in patients with SCLC with PEM/SN and patients with SMS and breast cancer. Interestingly, in a series of 30 mouse monoclonal antibodies which were raised against human amphiphysin I, only three were directed against fragment V.¹⁰ This indicates that the epitopes recognised by the human serum samples do not simply coincide with the most immunogenic portion of the molecule.

Anti-amphiphysin I antibodies may be useful in predicting the paraneoplastic origin of a neurological disorder in a given patient without known cancer because, in agreement with a previous report,⁵ we did not find anti-amphiphysin I antibodies in a large series of patients without PND but whose symptoms were initially suspected to be paraneoplastic. However, the low incidence of anti-

amphiphysin I antibodies in patients with PND and SCLC represents a limitation for its use as a diagnostic marker of PND other than SMS.

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