Pattern of epitopic reactivity of the anti-Hu antibody on HuD with and without paraneoplastic syndrome

Nobuyuki Sodeyama, Kazuyuki Ishida, Kurt A Jaeckle, Lixin Zhang, Arata Azuma, Masahito Yamada, Hidehiro Mizusawa, Yoshiaki Wada

Abstract

Previous study has shown that the anti-Hu antibody titre of serum samples from patients with paraneoplastic encephalomyelitis/paraneoplastic sensory neuronopathy (PEM/PSN) was significantly higher than that from patients with small cell lung cancer without neurological disturbances (non-PEM/PSN). The aims of this study were (1) to identify the fine epitopes on HuD recognised by the anti-Hu antibody, (2) to determine if the pattern of epitopic reactivity differed between antibodies from patients with and without PEM/PSN, and (3) to determine if the pattern of epitopic reactivity correlated with the clinical features. Recombinant full length HuD and nine deletion fragments were constructed and immunoreacted by western blot analysis with 14 anti-Hu serum samples from eight patients with PEM/PSN and six without PEM/PSN. All anti-Hu serum samples reacted with the deletion fragments containing amino acids (aa) 90–101 or aa 171–206. Some anti-Hu samples reacted with the deletion fragments containing aa 223–234, aa 235–252, or aa 354–373. There was no difference in the pattern of epitopic reactivity between patients with and without PEM/PSN. There was no correlation between the pattern of epitopic reactivity and the clinical features. The anti-Hu antibody titre from patients with PEM/PSN was significantly higher than from patients without PEM/PSN, and the clinical features. The anti-Hu antibody titre from patients with PEM/PSN was significantly higher than from patients without PEM/PSN, suggesting that the presence of the high titre anti-Hu antibody was an important factor in the development of the disease. However, the injection of the anti-Hu antibody into experimental animals has failed to reproduce PEM/PSN. Mice immunised with recombinant HuD produced the high-titre anti-Hu antibody, but did not develop clinical symptoms of PEM/PSN. These findings indicated that the presence of the high-titre anti-Hu antibody was not the critical factor required for the development of PEM/PSN. Therefore, further examination for other possible differences in the anti-Hu antibodies between patients with and those without PEM/PSN might provide important keys to the understanding of the pathogenesis of PEM/PSN.

Three aims of this study were (1) to identify the fine epitopes on HuD recognised by the anti-Hu antibody, (2) to determine if the pattern of epitopic reactivity differed between antibodies from patients with and without PEM/PSN, and (3) to determine if the pattern of epitopic reactivity correlated with the clinical features of neurological disturbances. We have constructed recombinant full length HuD and nine deletion fragments. The immunoreactivities of these expression products with anti-Hu serum samples from patients with PEM/PSN and without PEM/PSN were investigated by western blot analysis.
Materials and methods

SERUM SAMPLES

Fourteen anti-Hu serum samples were obtained from eight patients with PEM/PSN (patient 1-8) and six non-PEM/PSN patients (patients 9–14). Normal control serum samples were obtained from 10 normal healthy persons. Neurological conditions of the patients are as shown in the table.

Isolation of full length HuD cDNA

Total RNA was extracted from normal human frontal lobe and was reverse transcribed. This reaction product was used as a template for polymerase chain reaction (PCR) amplification to produce full length HuD cDNA. The PCR was performed with the following temperature profile: 30 seconds at 94°C, 30 seconds at 57°C, 3 minutes at 72°C (35 cycles), and then 15 minutes at 72°C.

Construction of vectors for expression of full length GST-HuD fusion protein and its deletion fragments

The deletion constructs of HuD cDNA were produced by using restriction enzymes to cleave HuD cDNA, or by PCR with specific primers. HuD cDNA and its deletion constructs were separated by agarose gel, cut out from the gel, and then subcloned in frame into the plasmid expression vector which contained glutathione S-transferase (GST) (GST-HuD constructs). When the insert was digested with restriction enzymes, it was subcloned into the plasmid which had been predigested with the same restriction enzyme. When the insert was a PCR product, it was subcloned into the plasmid which was predigested by Sma I and then added with thimine at the 3’ end.

Expression of full length GST-HuD fusion protein and its deletion fragments in Escherichia coli

The competent Escherichia coli cells were transformed with GST-HuD constructs. Transformed E coli cells were grown to an optical density of 1.0 at 600 nm and isopropyl-b-D-thiogalactopyranoside (Takara) was added. Cells were pelleted by centrifugation and were resuspended in phosphate buffered saline solution and followed by sonication. Glutathione sepharose 4B (Pharmacia) was added to the supernatant and the mixture was centrifuged. After glutathione, elution buffer (Pharmacia) was added and the mixture was centrifuged. The supernatant was used in western blot analysis. Recombinant proteins were obtained as fusion proteins with GST. These fusion proteins expressed by the above techniques are summarised in the figure.

Immunoblot of full length GST-HuD fusion protein and its deletion fragments

Fusion proteins were separated by 11% sodium dodecyl sulphate polyacrylamide gel electrophoresis and was electroblotted on to nitrocellulose membranes. After blocking, the membranes were immunolabelled with patients’ serum samples, and followed by reaction with horse radish peroxidase labelled affinity isolated goat anti-human IgG (Sigma).
Epitopes on HuD

Detection of the anti-Hu antibody titre
The anti-Hu antibody titre was determined by a semiquantitative western blot analysis. The titre was defined as the greatest dilution of each serum that stained the band of full length GST-HuD fusion protein on western blot.

Results
Western blot analysis showed that all 14 anti-Hu serum samples and anti-GST antibody reacted with full length GST-HuD fusion protein at 63 kDa. Ten serum samples from normal healthy persons did not react with full length GST-HuD fusion protein. The immunoreactivity of 14 anti-Hu serum samples with each deletion fragment was shown in the figure. The anti-GST antibody reacted with all deletion fragments at the presumed molecular weights. The results indicate that amino acids (aa) 90–101 and aa 171–206 are the major epitopes with which all anti-Hu sera react, and aa 223–234, aa 235–252, and aa 354–373 are the minor epitopes with which only some anti-Hu serum samples react. There was no difference in the pattern of epitopic reactivity between patients with PEM/PSN and patients without PEM/PSN. There was no correlation between the pattern of epitopes and the clinical features of neurological disturbances (table). The titre of each anti-Hu serum is also shown in the table. The anti-Hu antibody titre from patients with PEM/PSN was significantly higher than that from patients without. Their results were also confirmed by our study. Overlap of the titre between those patients with and those without PEM/PSN was also shown by Graus et al. These findings might indicate that titre of the anti-Hu antibody is not a single critical factor for the development of PEM/PSN.

In conclusion, aa 90–101 and aa 171–206 of HuD are the major epitopes with which all anti-Hu samples react, and aa 223–234, aa 235–252, and aa 354–373 are the minor epitopes with which only some anti-Hu serum samples react. Our analyses suggested that the pattern of epitopic reactivity of the anti-Hu antibody on HuD was not associated with the development or clinical features of PEM/PSN.

Discussion
In our study, there were two major epitopes with which all anti-Hu serum samples reacted and three minor epitopes with which only some anti-Hu serum samples reacted on HuD. A major epitope, aa 90–101, was in the first RNA recognition motif (RRM) outside the ribonucleoprotein consensus sequence (RNP), and another major epitope, aa 171–206 was in the second RRM, possibly including a portion of the RNP 1. One minor epitope, aa 354–373, was in the third RRM outside the RNP. These three epitopes were located between the carboxy terminal of the RNP 1 and the carboxy terminal of each RRM. Differing from these three epitopes, two minor epitopes, aa 223–234 and aa 235–252, were within the interval region between the RRM 2 and RRM 3.

Manley et al have already reported the epitope mapping of the anti-Hu antibody on HuD and showed that only deletion fragments of HuD which contained the first or second RRM were recognised by all the anti-Hu antibodies. The presence of the major epitopes is also confirmed by our study. The novel finding in the current study is the discovery of three minor epitopes recognised by the anti-Hu antibody. This finding was considered to be relevant because all bands were detected at the presumed molecular weights and such reactivity was not seen with normal control serum samples under the conditions of western blot analysis used.

There was no correlation between the pattern of epitopic reactivity and the development of PEM/PSN in patients positive for anti-Hu antibody, or the clinical neurological features. We could not identify a specific epitopic reactivity pattern which delineated patients likely to develop PEM/PSN. Our results suggest that the pattern of epitopic reactivity of the anti-Hu antibody on HuD was not a critical factor for the development or clinical features of PEM/PSN.

Dalmau et al showed that the anti-Hu antibody titre from patients with PEM/PSN was significantly higher than that from patients without. Their results were also confirmed by our study. Overlap of the titre between those patients with and those without PEM/PSN was also shown by Graus et al. These findings might indicate that titre of the anti-Hu antibody is not a single critical factor for the development of PEM/PSN.

In conclusion, aa 90–101 and aa 171–206 of HuD are the major epitopes with which all anti-Hu serum samples react, and aa 223–234, aa 235–252, and aa 354–373 are the minor epitopes with which only some anti-Hu serum samples react. Our analyses suggested that the pattern of epitopic reactivity of the anti-Hu antibody on HuD was not associated with the development or clinical features of PEM/PSN.

We thank Des Tomohiko Mizutani, Jun Takagiwa, and Hirotaro Mizu for providing anti-Hu serum samples. This work was supported in part by Health Science Research Grants (Mechanism of Abnormal Deposition in Dementia Brain) of the Ministry of Health and Welfare of Japan to MY.

Pattern of epitopic reactivity of the anti-Hu antibody on HuD with and without paraneoplastic syndrome

Nobuyuki Sodeyama, Kazuyuki Ishida, Kurt A Jaeckle, Lixin Zhang, Arata Azuma, Masahito Yamada, Hidehiro Mizusawa and Yoshiaki Wada

doi: 10.1136/jnnp.66.1.97

Updated information and services can be found at:
http://jnnp.bmj.com/content/66/1/97

These include:

References
This article cites 8 articles, 3 of which you can access for free at:
http://jnnp.bmj.com/content/66/1/97#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Immunology (including allergy) (1943)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/