Multiparametric serological testing in autoimmune encephalitis using recombinant immunofluorescence cell substrates and EUROTIDE technology

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Introduction

Recently, several new antigenic targets of autoantibodies associated with autoimmune forms of encephalitis were identified and found to be located on the neuronal cell surface: glutamate (Glu) receptors (type NMDA and type AMPA), GABA_B receptors (GABABR), and the VGKC-complex antigens, LGI1 and CASPR2. Since the clinical features associated with these anti-neuronal surface antibodies (ANSA) often overlap, it is appropriate to test for all of the ANSA as well as the classical paraneoplastic antibodies. Using a multiparametric BIOCHIP mosaic, the frequencies of ANSA and classical paraneoplastic antibodies were analyzed by indirect immunofluorescence assay (IIFA).

Methods

cDNAs for glutamate receptors (type NMDA; subunit NR1 and type AMPA; Glur1/Glur2), GABA_A receptors (B1), LGI1 and CASPR2 were inserted into eukaryotic expression vectors and transfected into HEK293 cells. The recombinant cells were grown and acetone-fixed on glass slides, which were fragmented to BIOCHIP mosaics, the frequencies of ANSA and classical paraneoplastic antibodies were analyzed by indirect immunofluorescence assay (IIFA). (IIFA).

Results

Out of 2716 requests for anti-neuronal antibody testing received between October and December 2010, 108 patients were positive for specific antibodies*. ANSA were found in 63% of positive patients: anti-glutamate receptor (type NMDA) 38%, anti-glutamate receptor (type AMPA) 0%, anti-GABAB receptor 3%, anti-LGI1 11%, and anti-CASPR2 11%. Classical paraneoplastic antibodies were detected in 31% of specimens: anti-Hu 6%, anti-Yo 8%, anti-Ri 9%, anti-Ma 2%, anti-PCA-2 1%, anti-CV2 4%, and anti-amphiphysin 1%. Parallel detection of two different anti-neuronal antibodies occurred in 7% of cases: anti-Hu/anti-CV2 3%, anti-glutamate receptor (type NMDA)/anti-Hu 2%, anti-glutamate receptor (type NMDA)/anti-GABA_A receptor 1%, anti-glutamate receptor (type NMDA)/anti-CASPR2 1%. In 31% of cases, the detected antibody was different from that requested.

Conclusion

Application of recombinant cell-based BIOCHIP mosaics and EUROTIDE-based IIFA allows monospecific detection and differentiation of ANSA. The overall prevalence of ANSA was approximately twice the prevalence of the classical paraneoplastic antibodies, with reactivity against glutamate receptor (type NMDA) being most frequently detected in this panel. The findings substantiate the importance of multiparametric serological testing in suspected cases of autoimmune-mediated encephalitis.

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* The sum of percentages is 101% due to rounding up
Detection of autoantibodies in bullous pemphigoid, epidermolysis bullosa acquisita, and pemphigus vulgaris by indirect immunofluorescence with a BIOCHIP Mosaic

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Introduction

Bullous pemphigoid (BP) is characterized by autoantibodies against the NC16A domain of BP180 and the C-terminal globular domain of BP230. In contrast, epidermolysis bullosa acquisita (EBA) is associated with autoantibodies against the non-collagenous domain 1 (NC1) of collagen VII, and pemphigus foliaceus and pemphigus vulgaris (PV) are characterized by autoantibodies against the ectodomains of desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), respectively.

Methods

Transfected HEK293 expressing the C-terminal globular domain of BP230, the NC1 domain of collagen VII (Col7-NC1), the ectodomains of Dsg1 or Dsg3 were used in combination with bacterially expressed BP180-NC16A and primate esophagus cryosections in a BIOCHIP format for indirect immunofluorescence (IIF). IgG autoantibodies were determined in patients with BP (n = 55), EBA (n = 57), PV (n = 24), and in 154 healthy blood donors (HBD).

Results

Anti-BP180-NC16A antibodies were detected in all 55 (100%) BP sera (PV 0%, EBA 2%), and in 24 (44%) of these additional anti-BP230 reactivity was found (PV 0%, EBA 2%). Anti-Col7-NC1 antibodies were present in 50 (88%) of EBA sera (PV 0%, BP 2%). Anti-Dsg3 antibodies were detected in all 24 (100%) PV sera, and in 9 (28%) of these additional anti-Dsg1 reactivity was found. No autoantibodies against Dsg1 and 3 were found in EBA and BP sera. Lower prevalences were obtained with conventional IIF microscopy using esophagus: 21 (88%) PV sera produced a desmosomal pattern, whereas 39 (71%) BP and 43 (75%) EBA sera produced a basement membrane pattern. HBD sera did not show any of the analyzed IgG specificities.

Conclusion

The IIF BIOCHIP Mosaic based on a combination of transfected HEK293 and bacterially expressed BP180-NC16A is easy to interpret and represents a highly sensitive and specific tool for the parallel determination of autoantibodies against BP180-NC16A, BP230, Col7-NC1, Dsg1, and Dsg3. It is a simple and time-saving alternative to conventional IIF employing cryosections of esophagus or salt-split human skin, which often produce equivocal patterns.


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Detection of PLA2R specific autoantibodies in patients with idiopathic membranous nephropathy using PLA2R producing HEK293 cells

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Introduction

Idiopathic membranous nephropathy (IMN) is one leading cause of nephrotic syndrome in Caucasian adults. Up to 70% of patients with IMN exhibit autoantibodies of the IgG4 subclass directed against M-type phospholipase A2 receptor (PLA2R). The aim of the current study was to evaluate a cell-based immunofluorescence assay for the determination of anti-PLA2R.

Methods

A cDNA encoding full-length PLA2R isoform 1 was used for transient transfection of HEK293 cells. 48 hours after transfection, cells were fixed and used as substrates for indirect immunofluorescence. Antibody titers of follow-up samples from 11 IMN patients under therapy were monitored and results were compared with reactions of sera from healthy blood donors (n=150).

Results

With the recombinant cell-based assay PLA2R-specific antibodies (IgG1-4) were detected in 6 of 11 IMN patients (specificity 100%). During a monitoring period of up to 9 months there was a decrease in antibody titers in five patients.

Conclusion

Detection of autoantibodies in patients with IMN may delineate those patients who need immunosuppressive therapy in order to reduce proteinuria and prevent loss of renal function. The new substrate is suited for broad screening in the detection of anti-PLA2R antibodies in nephrology.

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Computer-Aided Immunofluorescence Microscopy (CAIFM) in the diagnosis of autoimmune and infectious diseases

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**Introduction**

Indirect immunofluorescence (IIF) has not been able to keep pace with most other analytical techniques used in diagnostic laboratories. Whereas there are some automated technical solutions for IIF incubation about to appear on the market, the performance of result evaluation is still in its infancy. The patterns are predominantly recorded and interpreted by visual microscopic examination and the results documented in paper or electronic form.

The user is forced to permanently alternate between the microscopic image and the records – and to shift his point of focus is tiring and often leads to incorrect allocation of results, especially since the evaluation is generally performed in a dark room. CAIFM was developed to support the laboratory experts in diagnosing antibodies.

**Microscope configuration**

A motorised camera-microscope with special IIF relevant functions was designed (EUROPATTERN by EUROIMMUN), containing a magazine with a capacity for 50 slides, each with 10 reaction fields, or 10 slides, each with 50 reaction fields. A matrix code scanner enables slide identification and an incremental encoder identifies the field position.

**Interactive microscopy**

Starting with any of the 500 reaction fields by entering an ID or by mouse click, the substrates are selectively or consecutively visualized without eye-pieces at the computer screen. The slides are moved and focussed using a 3D actuator. Results are interpreted by the expert, they are entered via mouse and keyboard, images are recorded at the push of a button and automatically allocated and archived together with the results. A dark room is not required because the images on the screen are very bright. Owing to the casing around magazine and microscope stage, sunlight is kept out and the fluorescence in the substrates is protected from fading.

The 500 reaction fields are examined and interpreted automatically. The system autofocuses and takes an adjustable number of images by means of a camera, followed by visual or software-based diagnosis. The EUROPATTERN software allows automated assessment of IIF patterns, at present for anti-nuclear antibodies on HEp-2/HEp-2010 cells. The software performs a positive/negative differentiation and identifies deposited patterns, including many pattern combinations. If a sample has been incubated at different dilutions, the software merges the results of the individual analyses into one report form, which shows the recognised pattern and the antibody titer. Fluorescence image and computer interpretation are displayed together on the computer screen to be confirmed with one mouse click or modified if deemed necessary. In an optional two-step screening approach, all images defined as negative are sorted out, and the expert individually confirms or reclassifies only the remaining positive results.

**Automatic pattern recognition**

The system automatically merges all reaction field images for each substrate, the patterns are predominantly recorded and interpreted by visual microscopic examination and the results documented in paper or electronic form. The patterns are displayed together on the computer screen to be confirmed with one mouse click or modified if deemed necessary. In an optional two-step screening approach, all images defined as negative are sorted out, and the expert individually confirms or reclassifies only the remaining positive results.

**Laboratory management software**

The EUROPATTERN Microscope is integrated into EUROLabOffice (EUROIMMUN) which supports IIF processing by automatic protocol generation, interconnection with further laboratory devices (e.g. dilution/incubation systems) or analytical techniques (ELISA, Immunoblot, RIA), data exchange with the pattern recognition software, merging of test results and archiving IIF images in electronic report forms.

**Conclusion**

CAIFM reinforces the practical suitability of IIF. Results are interpreted at the office PC, the hands stay clean. The clinical pathologist can view all available IIF images by a simple mouse click and does not need to consult the microscope again in the event that a result must be checked for confirmation.

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