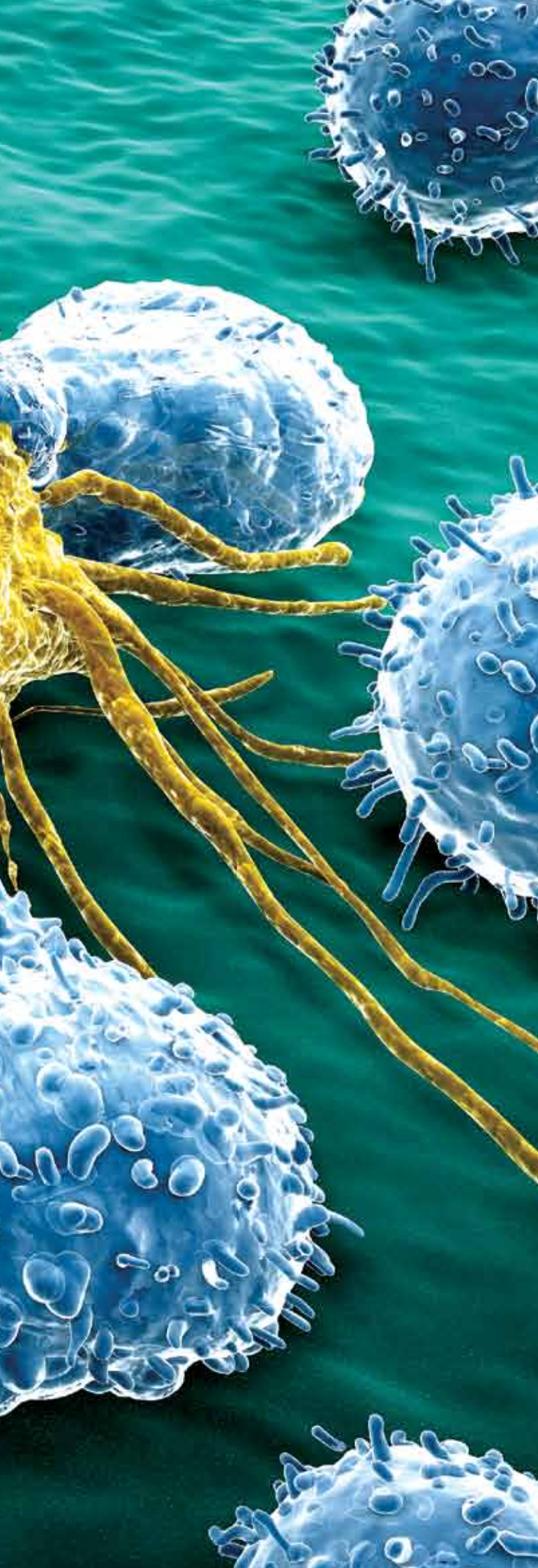


NEW AUTOANTIBODY MARKERS FOR NEUROLOGICAL DISEASES

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ABSTRACT

The spectrum of autoantibodies associated with neurological diseases has expanded rapidly in recent years. Alongside classic anti-neuronal antibodies such as anti-Hu, anti-Yo, and anti-Ri, antibodies against neuronal cell surface antigens, for example glutamate receptors of type NMDA, now play a central role in diagnostics. Early clarification of diseases such as paraneoplastic neurological syndromes, limbic encephalitis and neuromyelitis optica enables timely intervention, which is crucial for a favourable outcome. Autoantibody screening is especially fast and efficient using multiparametric indirect immunofluorescence test (IIFT) systems comprising mosaics of tissue sections together with recombinant-cell substrates. Immunoblots based on extensive panels of purified antigens are ideal for confirming antibody specificities.

PARANEOPLASTIC NEUROLOGICAL SYNDROMES

Paraneoplastic neurological syndromes (PNS) are disorders of the central and peripheral nervous system that occur in direct relation to tumour development. However, they are not caused directly by the tumour, its metastases or by therapeutic side effects. The cells of the tumour express antigens that normally only occur in neurons. These induce the formation of specific antibodies, which then bind to the corresponding antigens in the nervous tissue and damage the neurons. PNS develop in around 15% of malignant diseases, occurring most frequently in small-cell lung carcinoma, neuroblastoma, thymoma, and cancers of the ovary, mamma, uterus and testis.

Classic onconeural antibodies in PNS are directed against intracellular neuronal antigens such as Hu, Yo, Ri, amphiphysin, CV2, Ma1, Ma2/Ta, PCA-2, Tr and SOX1. If a positive result is obtained for these antibodies, the probability of a tumour is over 95%. Thus, if corresponding clinical symptoms are present and differential causes have been excluded, the detection of well-characterised anti-neuronal autoantibodies is considered sufficient to make a definitive diagnosis of PNS. Moreover, the antibodies may be detected up to five years before the tumour appears. Hence, in positive cases, PET scans should be performed on a regular basis to search for the tumour, which can then be excised at an early stage.

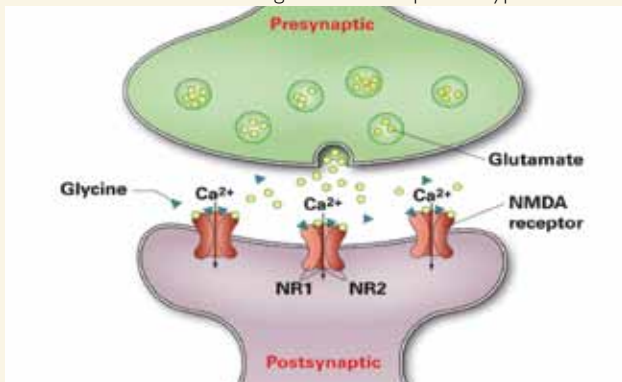
STIFF-PERSON SYNDROME

Stiff-person syndrome is a rare neurological disease, which can be paraneoplastic or non-paraneoplastic in origin. The disease manifests with severe progressive muscle stiffness, typically in the spine and lower extremities. Paraneoplastic cases are associated with antibodies against amphiphysin. Non-paraneoplastic cases are characterised by autoantibodies against glutamate acid decarboxylase (GAD), which are found in 60-90% of patients. However, anti-GAD antibodies are not specific markers for stiff-person syndrome as they also occur in other neuronal diseases and, in particular, diabetes mellitus type 1.

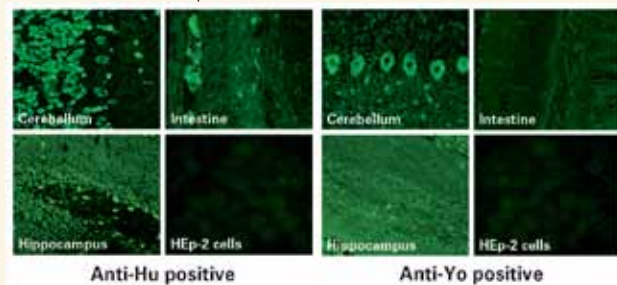
AUTOIMMUNE LIMBIC ENCEPHALITIS

Autoimmune limbic encephalitis encompasses a range of disorders manifesting with memory deficits, neuropsychiatric symptoms and epileptic seizures. A few years ago this condition was primarily attributed to classic paraneoplastic antibodies. However, in recent years several novel autoantibodies have been described as being associated with this condition. They differ from classic antibodies in that they are directed against target antigens on the cell surface of neurons, typically canal or receptor proteins. Examples include antibodies against glutamate receptors of types NMDA (see figure 1) and AMPA, against GABAB receptors and against the voltage-gated potassium channel-associated proteins LGII and CASPR2. In some cases the autoantibodies are associated with malignancies, but in many cases no tumour is detected. Thus, the associated encephalitides are classified as facultative PNS. →

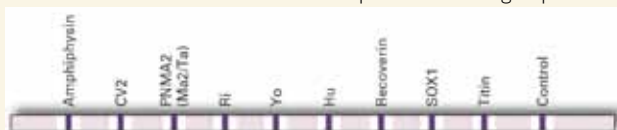
▼ **FIGURE 1:** Structure of glutamate receptor of type NMDA



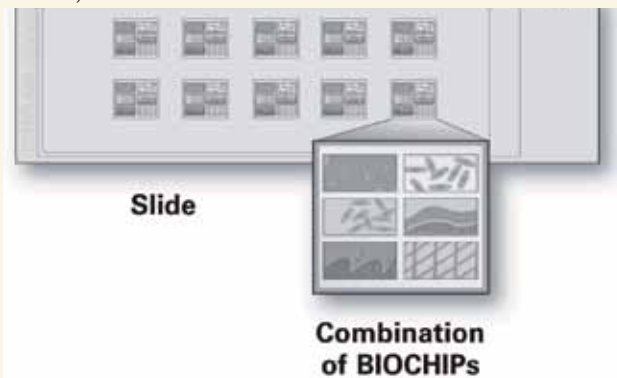
▼ **FIGURE 2:** IIFT patterns for anti-Hu and anti-Yo antibodies



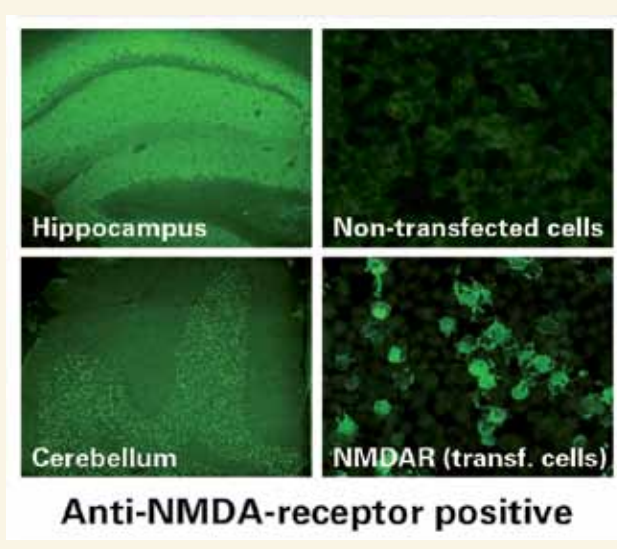
▼ **FIGURE 3:** EUROLINE with comprehensive antigen panel



▼ **FIGURE 4:** Positive reaction for anti-glutamate receptor (type NMDA) antibodies in IIFT



▼ **FIGURE 5:** BIOCHIP Mosaic



The autoantibodies are ascribed a direct pathogenic role. Binding of the autoantibodies to the corresponding membrane proteins interferes with synaptic signal transduction, resulting in neuropsychiatric deficits.

A favourable prognosis for autoimmune limbic encephalitis is highly dependent on early diagnosis and intervention. The immediate initiation of immunotherapy and, in paraneoplastic cases, tumour removal helps to stabilise the patients and improve their overall outlook.

ANTI-NMDA RECEPTOR ENCEPHALITIS

Anti-NMDA receptor encephalitis is an inflammatory encephalopathic autoimmune disease, which is characterised by highly specific autoantibodies against glutamate receptors of type NMDA (N-methyl-D-aspartate). The disease was first described in 2007 and is currently still widely underdiagnosed. It frequently affects young women with ovarian teratoma, but is also observed in older female patients, in women without tumours, in men and in children. Paraneoplastic cases represent 35-40% of the total. Patients usually present with symptoms such as memory loss, disorientation, confusion, paranoid thoughts, visual or auditory hallucinations, dyskinesias, decrease in consciousness, lethargy, seizures and autonomic instability.

Detection of anti-glutamate receptor (type NMDA) autoantibodies constitutes a key criterion for diagnosis of anti-NMDA receptor encephalitis. Their analysis is particularly important for differential diagnostics in patients with encephalitis of unknown origin, i.e. non-infectious etiology, and in young women with de novo epilepsy. When a positive serological result is obtained, a comprehensive teratoma investigation should be undertaken.

ANTI-AMPA, GABAB AND LGII/CASPR2 ENCEPHALITIDES

Limbic encephalitis triggered by autoantibodies against glutamate receptors (type AMPA), anti-GABAB receptors, LGII or CASPR2 occur less frequently than anti-NMDA receptor encephalitis. The different disease subtypes manifest with varying symptom complexes, encompassing memory deficits, seizures, confusion, disorientation, neuromyotonia, agitation, behavioural problems, hallucinations, paranoia, hyponatremia, myoclonus, dysautonomia and sleep or consciousness disturbances. Tumours are found with differing frequencies, more commonly in patients exhibiting anti-AMPA (70-75%) or anti-GABAB receptor (50-80%) antibodies than in individuals with anti-LGII (0-10%) or anti-CASPR2 (0-35%) positivity. Limbic encephalitis associated with anti-glutamate receptor (type AMPA) antibodies occurs predominantly in women, while the anti-GABAB receptor and LGII/CASPR2 subtypes are found more frequently in men.

NEUROMYELITIS OPTICA

The inflammatory autoimmune disease neuromyelitis optica (NMO) is a rare form of the group of acquired demyelinating diseases of the central nervous system. It is characterised by degradation of the insulating sheath of at least one optical nerve and at the same time or a few months later the spinal cord. Symptoms encompass acute visual disorders including blindness, impaired mobility and loss of bladder and bowel control. Without adequate therapy, half of patients become blind in one or both eyes or cannot walk without supports within five years.

Highly specific autoantibodies are found frequently in NMO. The antibodies were first described as NMO-IgG. The protein aquaporin-4 (AQP-4) was later identified as the target antigen. The determination of anti-AQP-4 antibodies is particularly useful for serologically differentiating NMO from classic multiple sclerosis.

AUTOANTIBODY DETECTION METHODS

According to current guidelines, autoantibodies in PNS should always be determined using two unrelated laboratory methods, for example IIFT

and immunoblot. Anti-neuronal antibodies can be determined effectively by IIFT using tissue substrates such as cerebellum, hippocampus, nerve and intestine. Characteristic immunofluorescence patterns indicate the presence of particular autoantibodies (see *figure 2*). For example, anti-Hu antibodies show granular fluorescence of the neuronal nuclei on cerebellum and hippocampus. With anti-Yo antibodies the Purkinje cells of the cerebellum and to some extent the hippocampus fluoresce.

Westernblots containing whole extracts of cerebellum offer the complete spectrum of neuronal antigens. They can be supplemented with additionally printed lines of recombinant antigens, for example Hu, Yo and Ri, for additional convenience in interpretation. Line blots contain panels of purified, characterised recombinant antigens. Since the antigens are located at defined positions on the membrane, these blots are extremely easy to evaluate. Line blots containing broad combinations of antigens, for example, the EUROLINE Profile with amphiphysin, CV2, Ma2/Ta, Ri, Yo, Hu, recoverin, SOX1 and titin, provide comprehensive antibody characterisation (see *figure 3*).

Autoantibodies against neuronal surface antigens in limbic encephalitis can be detected monospecifically using recombinant-cell IIFT substrates. These substrates consist of transfected human cells expressing defined, well-characterised whole target antigens or the immunoreactive subunits thereof. They offer high sensitivity and are easy to evaluate. A further advantage of recombinant-cell IIFT systems is that they can be developed in a short time, often in only a few months. Thus, newly identified autoantibody parameters can progress rapidly from the research laboratory into routine diagnostics. Recombinant-cell substrates are available for the detection of autoantibodies against, for example, glutamate receptors of types NMDA (see *figure 4*) and AMPA, GABAB receptors, LGII, CASPR2 and AQP-4. The efficacy of recombinant-cell assays has been confirmed in various clinical studies. For example, the anti-glutamate receptor (type NMDA) IIFT demonstrated 100% clinical sensitivity and specificity for anti-NMDA receptor encephalitis, while the anti-AQP-4 IIFT yielded a sensitivity of 80% for NMO with a specificity of 100%.

MULTIPARAMETRIC SCREENING

Since autoimmune neurological diseases often present with overlapping symptom complexes making them difficult to diagnose, the antibody screening should be as wide ranging as possible. With IIFT BIOCHIP

Mosaics (see *figure 5*), different tissue and recombinant-cell substrates can be analysed simultaneously. Miniature sections of the substrates are positioned next to each other in each test field of a microscope slide and incubated in parallel with one patient sample. The BIOCHIP slides are incubated using the established TITERPLANE technique, which provides standardised, parallel incubation of multiple samples under identical conditions. The procedure is thus fast and reliable and is suitable for use in any laboratory familiar with immunofluorescence. Automation options are available to further boost the efficiency and convenience of the analyses.

The advantages of multiparametric screening are highlighted by a study of findings in 2,716 samples sent to a routine clinical immunology laboratory for analysis of anti-neuronal autoantibodies. The analyses were carried out using BIOCHIP Mosaics and line blot assay. Of the positive cases, 67% exhibited positivity for autoantibodies against neuronal surface antigens, compared to 35% for classic intracellular onconeural autoantibodies. Thus, neuronal surface antibodies appear to be more common than classic paraneoplastic antibodies. Notably, in 31% of the findings, the positive reactions were caused by an autoantibody other than expected according to the analysis order. This large proportion of relevant secondary results underscores the importance of comprehensive antibody profiling in neurological diseases.

PERSPECTIVES

Autoantibody testing is becoming increasingly relevant for neurologists due to the rapidly growing list of novel autoantibodies against neurological targets. Of particular importance is the use of autoantibody parameters as a harbinger of tumour disease. Positivity in neuronal autoantibody tests helps to direct diagnostic efforts towards searching for a neoplasm, enabling early therapeutic intervention. In patients with previous cancer, the presence of autoantibodies may signal a return of the malignancy. Patients with non-paraneoplastic autoimmune disorders also benefit from early diagnosis with prompt immunotherapy. Multiparametric assays provide the greatest efficacy in diagnostic screening, as they identify not just the expected parameters, but also secondary reactivities that would otherwise be missed with a more narrow analysis. Nevertheless, 5-10% of patients with autoimmune limbic encephalitis do not display any currently known autoantibodies. Ongoing research efforts will undoubtedly unearth new neuronal autoantibody targets, enabling this small percentage to be reduced still further. 