Concept of autoimmune neurologic diseases: antibodies attacking neuron (illustration) (Shutterstock.com)

The growing spectrum of neural autoantibodies and associated syndromes

By Dr Jacqueline Gosink

Autoimmune neurology is a rapidly growing field driven by the ongoing discovery of novel neural autoantibodies associated with recognizable clinical syndromes. These diseases can affect the central, peripheral and autonomic nervous systems and may co-occur with cancer. Today's testing landscape encompasses more than 60 neural autoantibodies targeting different intracellular proteins, receptors and ion channels.

Plethora of target antigens

Most initially identified neural autoantibodies are directed against intracellular antigens expressed in the cerebellum, such as Hu, Yo, Ri, CV2, PNMA2, SOX1 and amphiphysin. These antibodies are considered non-pathogenic and are epiphenomena of tumour cells expressing neuronal antigens. It is believed that the misdirected immune response is mediated by cytotoxic T-cells, leading to the loss of neurons. The autoantibodies serve as important biomarkers for paraneoplastic neurological syndromes (PNS) and often provide the first indication of a tumour. The tumours most commonly found in patients with PNS are small-cell lung carcinoma, breast cancer, ovarian cancer, lymphoma, thymoma or seminoma.

The more recently identified autoantibodies target cell-surface antigens such as AQP4, NMDAR, LGI1, CASPR2, AMPAR1/2, GABAR, DPPX and IgLON5. These antibodies are pathogenic and frequently non-paraneoplastic. They cause inflammatory damage to the brain and nerves and can trigger seizures, impairment of visual acuity, psychosis-like symptoms and/or movement disorders. In contrast to PNS, these disorders generally respond well to immunotherapy.

Today's research continues to identify novel autoantibodies at an unprecedented rate, leading to the definition of many

new disease phenotypes. As well as being useful for disease classification and diagnostics, the findings also increase understanding of the autoimmune pathology behind neurological syndromes. This knowledge aids implementation of suitable therapy, which is often complex and multidisciplinary.

Identification of new autoantibodies

Among the methods used to identify new autoantibodies, the indirect immunofluorescence assay (IFA) is one of the most versatile, especially in cases of previously unspecified target antigens. Patient sera that show a characteristic staining pattern on neural tissue, but do not react with any known antigens, are used as the starting point. To identify the antigens, immunocomplexes formed between patient antibodies and brain tissue cryosections are analysed by immunoprecipitation followed by mass spectrometry. Use of cryosections ensures authentic presentation of the antigens in their natural environment and conformation. The new discoveries are confirmed by IFA using human cells recombinantly expressing the antigen, and additionally verified by neutralization analyses. The cell-based assays (CBAs) are subsequently useful for serological diagnostics and studies on the clinical relevance of the autoantibodies.

This approach has been used by the EUROIMMUN-affiliated Institute for Experimental Immunology *(https://www.euroimmun.de/en/contact/external/institute-f-exp-immunology/)* in collaborative studies to identify more than twenty autoantigens in neurological diseases, among them AP3B2, ATP1A3, CLIP1, CNTN1/CASPR1, CPT1C, ERC1, flotillin-1/2, GluRδ2, GRIPAP1, hexokinase-1, Homer-3, ITPR1, KCNA2, NBCe1, neurochondrin, RGS8, ROCK2, RyR2, STX1b and septin-3 [1].

The following sections detail some novel autoantibody markers to emerge in recent years from this and other research and their clinical associations observed in patients.

Autoimmune encephalitis

Established markers for autoimmune encephalitis include autoantibodies against NMDAR, AMPAR1/2, LGI1, CASPR2 and GABABR, DPPX, IgLON5 and mGluR5. Many of these autoantibodies are rare and about 50% of patients meeting the criteria for autoimmune encephalitis are seronegative for known neural autoantibodies. Identification of new autoantibodies helps to extend the testing spectrum. Among the new test parameters are anti-**adenylate kinase 5 (AK5) antibodies**, which occur in severe, non-paraneoplastic, autoimmune limbic encephalitis. Patients experience severe memory deficits which remain even with immunotherapy. Altered or loss of smell sensation is also observed in some patients [2]. Autoantibodies against **gamma-aminobutyric acid receptor type A (GABAAR)** are a further new marker and are associated with rapidly progressing encephalopathy and/or seizures occurring in all age groups [3,4]. There is usually clinical improvement with immunotherapy.

Autoimmune cerebellar ataxia

Numerous novel autoantibodies have been reported in patients presenting with idiopathic cerebellar/brainstem ataxia. Autoantibodies to **neurochondrin**, a neuronal cytosolic protein which plays a role in cell-surface localization of certain membrane-bound proteins, have been described in patients with non-paraneoplastic rapidly progressive rhombencephalitis with poor neurologic outcomes [5,6]. Autoantibodies to **glutamate receptor δ2 (GluRδ2)** have been observed in association with visual deficits and ocular motility abnormalities and appeared with young age, infectious prodromes and lymphocytic pleocytosis [7]. Autoimmunity targeting the **adaptor protein 3 subunit B2 (AP3B2)**, a synaptic vesicle coat protein, was found in patients with subacute onset and rapidly progressive gait ataxia [8,9]. **RhoGTPase-activating protein 26 (ARHGAP26)** is a further novel target antigen in autoimmune cerebellar ataxia, cognitive impairment and psychosis [10].

Autoantibodies with a more prominent cancer association include those against **inositol 1,4,5-trisphosphate receptor type 1 (ITPR1)**, an intracellular channel that mediates calcium signalling. These are associated with cerebellar ataxia, seizures, myelopathy and neuropathy, with around 45% of cases linked to an underlying tumour [11]. Antibodies against **the regulator of G-protein signaling 8 (RGS8)**, a Purkinje cell protein which belongs to a class of proteins involved in the regulation of central nervous system

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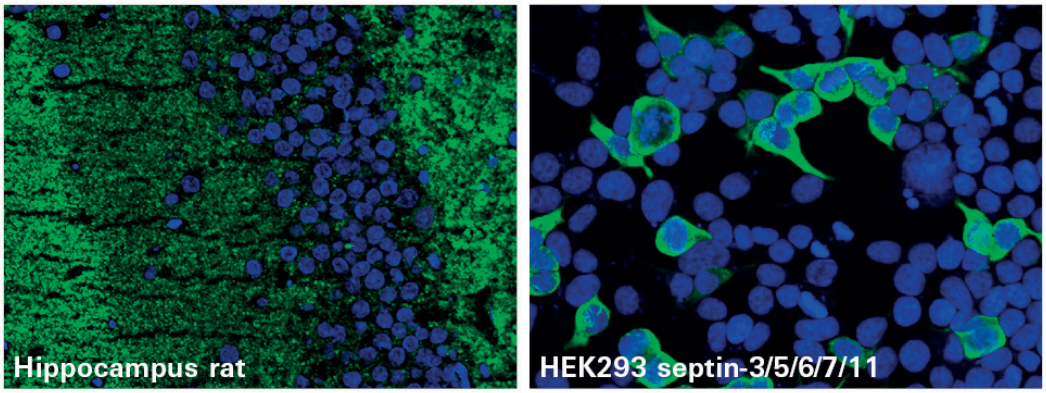

Figure 1. Detection of anti-septin-3 autoantibodies on tissue and transfected-cell substrates (EUROIMMUN)

>> (CNS) actions such as synaptic plasticity, memory and vision, have been described in patients with cerebellar syndrome associated with lymphoma [12]. Anti-**metabotropic glutamate receptor type 1 (mGluR1)** antibodies are a marker of a treatable form of cerebellar ataxia which may also be associated with lymphoma [13].

Septins have also been recently identified as target antigens of autoimmunity (Fig. 1). Septins are cytoskeletal proteins with multiple roles in cell division, cellular polarization, morphogenesis and membrane trafficking. Autoantibodies against septin-3 have been newly described in patients with paraneoplastic cerebellar ataxia [14]. Anti-septin-5 antibodies have been previously characterized in patients with non-paraneoplastic cerebellar ataxia, while anti-septin-7 antibodies were found in patients with encephalopathy with prominent neuropsychiatric features [15]. Thus, the different anti-septin antibodies appear to be associated with different clinical phenotypes.

Demyelinating diseases

Autoantibodies against **aquaporin-4 (AQP4)** are a highly specific, pathogenic marker for neuromyelitis optica spectrum disorders (NMOSD), a group of inflammatory demyelinating disorders of the CNS affecting the optic nerve, spinal column and brainstem. CBA is the gold standard for anti-AQP4-IgG testing and is now included in the diagnostic algorithm for NMOSD [16].

Autoantibodies against **myelin oligodendrocyte glycoprotein (MOG)** are a marker for MOG antibody-associated encephalomyelitis (MOG-EM), which is clinically similar to NMOSD but is now recognized as a distinct disease [17]. Recent evidence suggests that MOG-EM may be more common than NMOSD. Determination of AQP4 and MOG antibodies helps to delimit the diseases from each other and also from multiple sclerosis (MS), which can resemble NMOSD clinically in the initial stages.

Autoantibodies against the **flotillin-1/2** heterocomplex, a peripheral membrane protein that is involved in axon outgrowth and regeneration of the optic nerve, have been observed in a subset of about 1–2% of patients with bona fide MS [18], but not in patients with other neural autoantibody-associated diseases or in healthy blood donors. This suggests that anti-flotillin antibodies may be specific for MS, although their clinical and pathological relevance has not yet been clarified.

Autoimmune nodopathies

Autoantibodies against nodal/paranodal proteins are emerging biomarkers for a novel class of neuropathies known as autoimmune nodopathies [19]. These diseases have clinical similarity to Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy (CIDP) but are pathologically distinct. The antibodies target membrane proteins located at or around the nodes of Ranvier – gaps in the myelin sheath that facilitate fast conduction of nerve signals. The target antigens include **neurofascin 186 (NF186)**, **neurofascin 155 (NF155)**, **contactin 1 (CNTN1)** and **contactin-associated protein 1 (CASPR1)**.

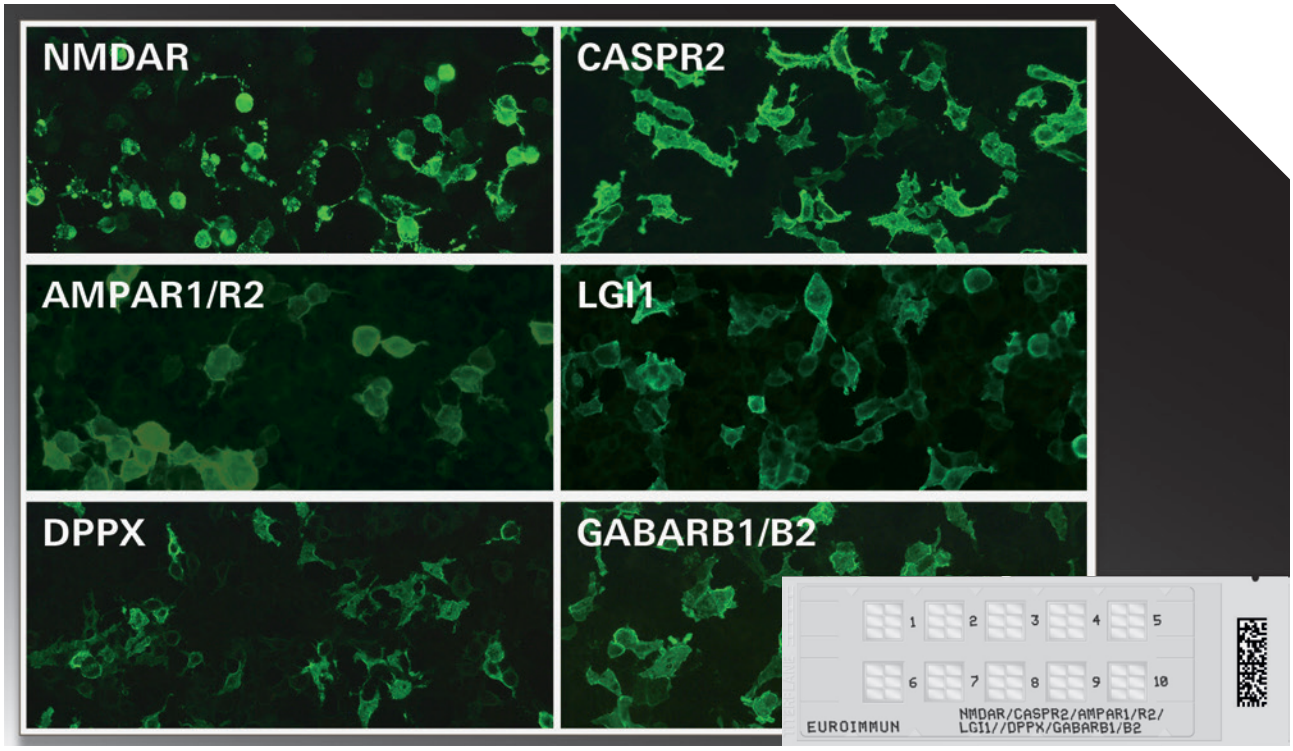

Figure 2. Autoimmune Encephalitis Mosaic 6 for multiparameter autoantibody detection using cell-based substrates (EUROIMMUN)

The autoantibodies are considered pathogenic, and the resulting immune reactions result in slowed conduction or even complete failure of impulse transmission. Autoimmune nodopathies manifest as acute, subacute or chronic onset sensory-motor neuropathies with distinct clinical phenotypes.

Diagnostic test systems

The testing landscape for autoimmune neurological syndromes is continually evolving [20]. Autoantibody detection in conjunction with clinical evaluation and radiographic findings can facilitate diagnosis and prognosis of these diseases. IFA is an indispensable method for autoantibody determination. Tissue sections of nerves, cerebellum, hippocampus and intestine enable comprehensive screening of neural autoantibodies, whereas transfected-cell substrates provide easy, monospecific detection of defined autoantibodies. CBA technology is particularly suitable for neuronal cell-surface antigens, which are often conformation-dependent and fragile and thus unsuitable for the expression and purification procedures required for solid-phase methods such as ELISA or immunoblot. Further, as the antigens do not need to be obtained in purified form, the assays can be developed rapidly, enabling novel parameters to be incorporated promptly into the test repertoire. CBAs are now a core component of serological differential diagnostics for certain neurological diseases, for example anti-NMDAR encephalitis and NMOSD.

CBAs with CE mark are currently available from EUROIMMUN for the detection of autoantibodies against NMDAR, AMPAR 1/2, GABABR, LGI1, CASPR2, DPPX, IgLON5, GAD65, Zic4, DNER/Tr, AQP4, MOG, AChR and MuSK. Further CBAs are commercially available for research use, for example for the detection of antibodies against NF155, NF186, CASPR1, CNTN1, GABAAR, mGluR1, mGluR5, AK5 and flotillin-1/2. Multiple antibodies can be investigated in parallel using BIOCHIP Mosaics composed of different tissue and cell substrates which are incubated simultaneously. BIOCHIP Mosaics with CE mark are available tailored to different diagnostic applications, for example autoimmune encephalitis (Fig. 2), myasthenia gravis and NMOSD.

Immunoblots are suitable for detection of antibodies against more stable antigens, including many intracellular antigens. With multiplex line blots, many different antibodies can be analysed in parallel. In blots of the EUROLINE range, the antigens are contained on individual membrane chips, allowing antigens with widely different properties to be combined in application-oriented profiles. Multiplex EUROLINE profiles are available for detection of up to twelve PNS-associated antibodies (Fig. 3), encompassing the antigens amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, Zic4, GAD65 and DNER/Tr, as well as for detection of different anti-ganglioside antibodies.

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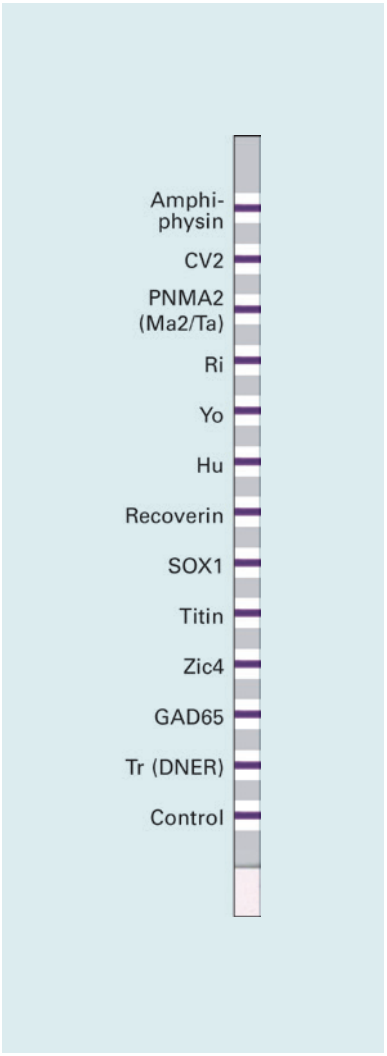

Figure 3. EUROLINE Paraneoplastic Neurological Syndromes 12 Ag test (EUROIMMUN)

Table 1. Definitions of antigen/protein short-form names

Short-form name	Definition (bold, defined in text)
AChR	acetylcholine receptors
AK5	**adenylate kinase 5**
AMPAR1/2	glutamate receptors (type AMPA1/2)
AP3B2	**adaptor protein 3 subunit B2**
AQP4	**aquaporin-4**
ARHGAP26	**RhoGTPase-activating protein 26**
ATP1A3	sodium/potassium-transporting ATPase subunit alpha-3
CASPR1	contactin-associated protein 1
CASPR2	contactin-associated protein 2
CLIP1	CAP-Gly domain-containing linker protein 1
CNTN1	**contactin 1**
CPT1C	carnitine O-palmitoyltransferase 1, brain isoform
CV2	CV2/collapsin response mediator protein 5 (CRMP5)
DNER/Tr	delta/notch-like epidermal growth factor-related receptor
DPPX	dipeptidyl aminopeptidase-like protein 6
ERC1	ELKS/RAB6-interacting/CAST family member 1
GABAAR	**gamma-aminobutyric acid receptor type A**
GABABR	**gamma-aminobutyric acid receptor type B**
GABAR	gamma-aminobutyric acid receptor
GAD65	glutamic acid decarboxylase 65 kDA isoform
GluRδ2	**glutamate receptor δ2**
GRIPAP1	GRIP1-associated protein 1
Hu	Index patient
IgLON5	immunoglobulin-like cell adhesion molecule 5
ITPR1	**inositol 1,4,5-trisphosphate receptor type 1**
KCNA2	potassium voltage-gated channel subfamily A member 2
LGI1	leucine-rich glioma-inactivated protein 1
mGluR1	**metabotropic glutamate receptor type 1**
mGluR5	metabotropic glutamate receptor type 5
MOG	**myelin oligodendrocyte glycoprotein**
MuSK	muscle-specific kinase
NBCe1	electrogenic sodium-bicarbonate cotransporter 1
NF155	**neurofascin 155**
NF186	**neurofascin 186**
NMDAR	glutamate receptor (type NMDA)
PNMA2 (Ma2/Ta)	paraneoplastic antigen Ma2
RGS8	**regulator of G-protein signaling 8**
Ri	Index patient
ROCK2	Rho-associated protein kinase 2
RyR2	ryanodine receptor 2
SOX1	SRY-box transcription factor 1
STX1b	syntaxin-1B
Yo	Index patient
Zic4	Zic family member 4

Outlook

The growing interest in autoantibody-associated neurological disease has fuelled the recent discovery of many novel neural autoantibodies and the characterization of their associated clinical syndromes. Autoantibody testing is now a vital tool in the diagnosis, prognosis and management of various diseases that were previously often misdiagnosed as psychiatric, degenerative or infectious conditions. It is especially useful for differentiating potentially immuno-therapy-responsive syndromes from those that are unlikely to benefit from this line of treatment. Autoantibody detection can also guide cancer screening to detect tumours at an early and highly treatable stage. Continued identification of novel autoantibodies will help to expand the testing repertoire further and close diagnostic gaps. Additional research will probe the underlying immune mechanisms and patho-physiology with the aim of developing new therapeutic strategies.

Note

For readability, predominantly the short form names are used for the antigens/proteins. However, for completeness, the full names have been provided in Table 1.

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