REVIEW
ARTICLES

Autoimmune Encephalitis: A Disease of the 21st Century at the Crossroads of Neurology and Psychiatry

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Abstract—Autoimmune encephalitis is a group of neurological diseases characterized by brain damage by autoantibodies towards extra- or intracellular structures of the nervous system that act as antigens. The combination of neurological and mental disorders, as well as the ability to identify a specific "antigen and antibody" axis, makes these diseases extremely interesting from the standpoint of "molecular psychiatry" and the creation of new experimental models of cognitive processes, clinical diagnosis and targeted treatment. However, despite active research in this direction and a large number of specific antibodies, the diagnosis of autoimmune encephalitis is often extremely difficult and can be made only by clinical criteria. This study provides an overview of the available data on the history of discovery and study of autoimmune encephalitis, describes the methods for searching of antibodies that exist today and the further prospects for studying this group of diseases.

"The brain is a monstrous, beautiful mess."

Susannah Cahalan, Brain on Fire:
My Month of Madness

INTRODUCTION

One of the most exciting discoveries in neurology of the 21st century is the isolation of antibodies to n-methyl-D-aspartate receptors (NMDAR) and the identification of the cause of acute psychosis and behavioral disorders in young patients with ovarian teratoma [1]. The isolation of these antibodies is one of the small bridges in the big world of "molecular psy-

chiatry," when a discovery of a specific substrate explained the development of mental disorders.

Following the first discovery, many laboratories started to work in this area, and, currently, one or two new antibodies are described every year [2]. The occurrence of each of these antibodies may lead to the occurrence of psychiatric symptoms in patients: acute psychosis, as well as behavioral and cognitive impairments.

In approximately 60% of patients, psychiatric symptoms appear at the beginning of the disease and become leading in clinical picture [3]. Usually these patients are hospitalized or treated by psychiatrists, often with the diagnosis of "schizophrenia," while correct diagnosis and immunosuppressive therapy may completely cure the patient and return them to a normal life. Dramatic effect of therapy and the curability of these states lead to isolation of a separate group of autoimmune encephalitis and the active study of pathogenic antibodies and substrates.

Autoimmune encephalitis (AE) is a group of neurological diseases characterized by brain damage by autoantibodies to the extra- or intracellular structures of the nervous system, which act as antigens [4, 5]. This group of diseases is divided into two large subgroups: paraneoplastic encephalitis, whose development is associated with the presence of a tumor and

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Abbreviations: AIT, autoimmune thyroiditis; AE, autoimmune encephalitis; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl- 4-isoxazole propionic acid; AMPAR, AMPA receptor; CASP2R, contactin-associated protein 2 receptor; DPPX, dipeptidyl peptidase-like protein; D2R, dopamine receptor 2; GABAaR, GABAa receptor; GABAb, GABAb receptor; HEK, human embryonic kidney cells; LGI1, Leucine-rich, glioma inactivated 1 protein; mGlu5R, metabotropic glutamate receptor 5; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; PANDAS, pediatric autoimmune neuropsychiatric diseases due to streptococcal infection.

Table 1. Classical "paraneoplastic" encephalitides with antibodies against intracellular antigens

Antigen	Clinical symptoms (most often)	Tumor	References
Anti-Hu (ANNA-1)	Encephalitis (limbic, cortical, brainstem), polyneuropathy, autonomic impairments	>90% (small-cell lung cancer)	33
MA (MA1, MA2)	Encephalitis (limbic, brainstem), poly-neuropathy	>90% (ovary, breast, colon cancers)	55
Amphiphysin	Limbic encephalitis, encephalopathy, stiff-person syndrome, degeneration of the cerebellum	>90% (small-cell lung cancer, mammary adenocarcinoma)	36
Anti-Ri (ANNA-2)	Limbic encephalitis, encephalopathy, stiff-person syndrome, degeneration of the cerebellum, dystonia	>90% (small-cell lung cancer)	35
CV2 (CRMP5)	Encephalitis, optic neuritis, retinitis, polyneuropathy, myelopathy, Lambert-Eaton syndrome, degeneration of the cerebellum, movement disorders (chorea and other)	>90% (small-cell lung cancer, thymoma)	37
Anti-GAD antibodies	Encephalitis (limbic, cortical, brainstem), stiff-person syndrome, degeneration of the cerebellum	<5% (thymoma, kidney cancer, mammary or colon adenocarcinoma)	73
Other paraneoplastic neurological syndromes			
ZIC-4	Subacute degeneration of the cerebellum	Small-cell lung cancer	74
Yo-1 (PCA-1)	Subacute degeneration of the cerebellum	Often (ovary cancer, breast cancer)	34
Tr-receptor	Subacute degeneration of the cerebellum	Hodgkin's lymphoma	73
SOX-1	Lambert-Eaton syndrome	Small-cell lung cancer	74

subsequent antigen presentation, and idiopathic encephalitis, where autoantibodies are produced without any association with any oncological process [4].

Three groups of antibodies were separated using the same principle:

(1) antibodies to intracellular antigens or, as they are often called, "classical" paraneoplastic antigens (Hu, Yo, Ma2, CV2, and amphiphysin), which in most cases are associated with the presence of a tumor;

(2) antibodies to the surface neuronal membrane that may be detected in both paraneoplastic and idiopathic cases (metabotropic glutamate receptor 5 (mGlu5R), GABAb receptor (GABAbR), NMDAR, α-amino-3-hydroxy-5-methyl- 4-isoxazole propionic acid (AMPAR), and contactin-associated protein 2 receptor (CASP2R);

(3) antibodies that are more often detected idiopathically (glutamate decarboxylase (GAD65), dipeptidyl peptidase-like protein (DPPX), IGloN5, glycine receptor (GlyR)).

Tables 1 and 2 show current list of antibodies and possible diverse clinical manifestations. In addition, for AEs with antibodies to the surface neuronal membrane, there is a different classification of antigen localization [6]:

(1) antibodies to neurotransmitter receptors (NMDAR, AMPAR, mGluR5, GABAaR, GABAbR, GlyR, and dopamine receptor 2 (D2R));

(2) antibodies to transmembrane proteins (CASPR2, DPPX, IgLON5, Neurexin3a, etc.);

(3) antibodies to secreted proteins (Leucine-rich, glioma inactivated 1 protein, and LGI1).

According to the presence of antibodies and their type, it is possible to predict the rate of progression of the disease, the prognosis, and the response to therapy, which makes a search for specific antibodies extremely important in each case.

In addition, the discovery of antibodies to surface neuronal antigens led to a burst of new ideas in the field of psychiatry: the hope arose that the molecular and cellular bases of psychiatric diseases may be unraveled and the theory of the relationship between immunological disorders and the development of schizophrenia received a new area of development [7]. In addition to the pathogenetic role of antibodies, levels of AE antibodies in acute psychoses began to be actively studied. It was revealed that in some cases patients with acute psychosis have antibodies to NMDAR (from 1.46 to 20 % according to various studies, depending on the criteria for selection of group of patients and methods for antibodies evalua-

Table 2. Autoimmune encephalitides with antibodies to surface neuronal membrane or synaptic proteins

Antigen	Clinical symptoms	Tumor	References
NMDAR	Prodromal symptoms, psychiatric disturbances, epileptic seizures, memory impairments, paroxysmal movement episodes, catatonia, autonomic disorders, consciousness decline, coma, hypoventilation	Ovary teratoma, rarely carcinoma (10−45% of all cases depending on age) in HSV-encephalitits survivors. Idiopathic	1
AMPAR	Limbic encephalitis, psychiatric disturbances	70% (pulmonary cancer, breast cancer, thymoma)	43
GABAbR	Limbic encephalitis, often seizures	50% (pulmonary cancer, neuroendocrine cancer)	44
GABAaR	Refractory seizures, status epilepticus or Kozhevnikov epilepsy, stiff-person syndrome, opsoclonus	Rarely, reported in thymoma	48
LGI1	Limbic encephalitis, 60% hyponatremia, faciobrachial dystonic seizures	<10% (pulmonary cancer, thymoma)	41
CASPR2	Encephalitis, Morvan syndrome, neuromyotonia	<40% (thymoma)	41
GlyR	Stiff-person syndrome, progressing encephalomyelitis with rigidity and myoclonus, limbic encephalitis, degeneration of the cerebellum, optic neuritis	Rarely	42
IgLON5	Motor impairments during sleep, behavioral disturbances, obstructive sleep apnea, respiration disorders, dysarthria, dysphagia, ataxia, chorea	No	51
DPPX	Diarrhea, consciousness decline, psychiatric disorders, tremor, myoclonus, epileptic seizures, nystagmus, hyperekplexia, ataxia, encephalomyelitis with rigidity and myoclonus	No	47
mGluR5	Limbic encephalitis, Ophelia syndrome	Often, Hodgkin's lymphoma	45
D2R	Encephalitis with involvement of basal ganglia, Sydenham's chorea	Rarely	16
Neurexin3	NMDA-like encephalitis, orofacial dyskinesia	Reported idiopathically and after malaria	52

tion). Based on these studies, "red flags" were identified, which can help to diagnose AE in acute psychoses, and the possibility of immunotherapy also started to be discussed more actively [3].

Although most antibodies have been described in the last 15−20 years and AE is a pathology of the 20th and 21st centuries, the first conceptions on the possible organic nature of mental illnesses, and subsequently of their autoimmune etiology, occurred much earlier. The chronological sequence of the AE study is shown in Fig. 1.

THE HISTORY OF THE DISCOVERY OF AUTOIMMUNE ENCEPHALITIS

The Era of Clinical Observations and Descriptions of Individual Nosologies

Although neurosyphilis is not an autoimmune disease and is a well-known infectious process with a specific pathogen, *Treponema pallidum*, we start our review from it. During the treatment of patients with syphilis doctors caught the idea that "madness and insanity" may have a biological cause: at the end of the 19th century, patients with delirium, schizophreniform disorders, and neurosyphilis were observed by all psychiatrists in Europe [9]. Neurosyphilis and its psychiatric manifestations, viz., dementia, depression, delirium, psychosis, episodes of arousal, hallucinations, depersonalization, and cognitive impairments, were the most frequent reason for hospitalization in psychiatric clinics from the late 19th century to the 1940s until penicillin was discovered [10]. The frequency of psychiatric manifestations in neurosyphilis is extremely high, from 33 to 86% [11]; however, the mechanism of their development in some cases is not completely clear. Previously, it was confirmed by studies of pathological autopsy material of the brain that there are lesions and *Treponema pallidum* is present in these regions. This led to the formation of views on the relationship between persistent infection and the development of manifestations of neurosyphilis [12]. However, recent studies using magnetic resonance
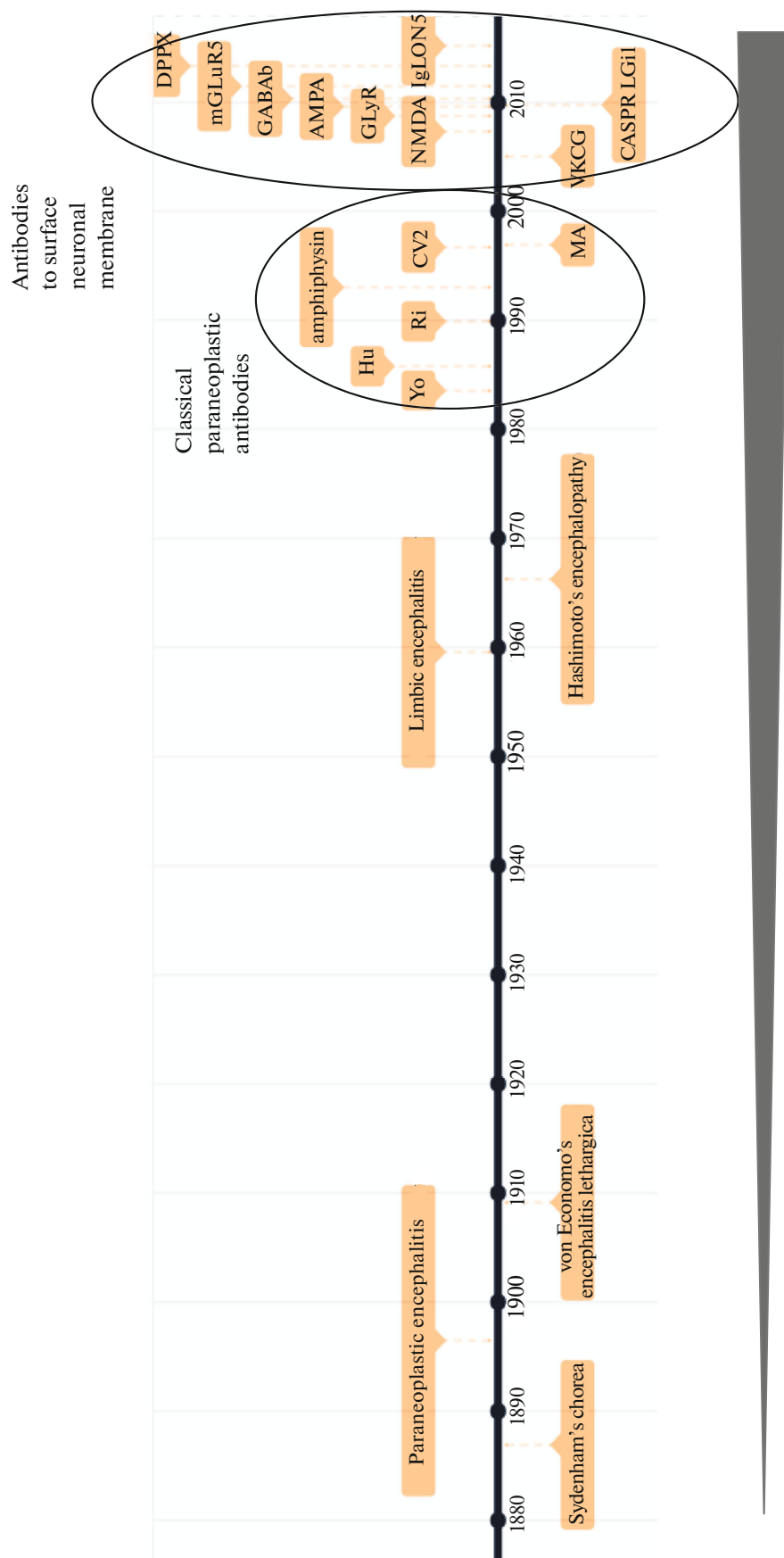
Fig. 1. The chronological sequence of the study of autoimmune encephalitides.

imaging (MRI) and other methods of neuroimaging have shown that neurosyphilis, like AE, is often associated with normal MRI or brain atrophy of varying severity, as well as a pronounced antibody response in the form of immunoglobulins in the cerebrospinal fluid (CSF) [9]. This suggests that psychotic disorders may develop not only in the presence of focal and bacterial damage but also for other reasons:

(1) the immediate toxic effect of Treponema on neurons, which disturbs the normal functioning of neurons and cortex;

(2) production of antibodies due to a persistent chronic infection, which interact with different molecular targets and lead to the development of psychiatric disorders.

Many studies have shown that the permeability of the blood–brain barrier is impaired in neurosyphilis and antibodies to *Treponema pallidum* are synthesized intrathecally [13], while the exact mechanism of the occurrence of mental disorders in patients without focal neurological pathology and lesion areas in MRI is currently not known. In addition, according to a number of studies with antibiotic therapy, the classical picture of neurosyphilis and generalized paralysis, which was previously well studied, changed [14], and syphilis is still a "great imitator."

Similarly, in a number of other infectious pathologies, researchers have discussed the theory of the development of schizophrenic-like mental disorders and their relationship to antibodies (for example, in patients with antibodies to *Toxoplasma gondii* [15]).

Neurosyphilis was one of the first and most common diseases in which psychiatric manifestations was assumed to have a clear structural basis in the form of a brain lesion caused by *Treponema pallidum*. The first described AEs include encephalitis lethargica, Sydenham's chorea, and Hashimoto's encephalopathy. In addition, Rasmussen's encephalitis and FIRES syndrome are historically considered within the framework of AE-like diseases; however, due to almost complete lack of knowledge about the pathogenesis of these diseases and the availability of screening antibodies, these diseases will not be considered in this review [6].

Encephalitis lethargica. Historically, von Economo's encephalitis lethargica, whose pandemia was observed from 1915 to 1925 after the epidemic of influenza, is assumed to have postinfectious autoimmune genesis. Due to the sudden development and the same sudden disappearance of the disease, its causes are virtually unknown; however, clinically, patients developed a picture of weakness, fatigue, sleep disorders such as lethargy, ophthalmoplegia, catatonia, and symptoms of parkinsonism and psychosis.

Assumptions on the autoimmune genesis of encephalitis lethargica were made on the basis of several clinical and epidemiological facts:

(1) the pandemic of encephalitis after the "Spanish flu" pandemic (influenza that affected approximately 30% of the world's population at the beginning of the 20th century);

(2) the relationship between pharyngitis in 55% of patients with the presence of elevated titers to anti-streptolysin;

(3) the pathomorphological data of the brain studies of the deceased from encephalitis lethargica, which showed the presence of inflammatory lymphocytic infiltrates in the midbrain and basal nuclei, as well as the presence of oligoclonal antibodies in the CSF, the presence of antibodies to the basal nucleus antigens, and a positive response to steroid therapy [16].

In addition, immunohistochemical analysis of sporadic cases of encephalitis lethargica in later periods showed the presence of antibodies mainly against neurons, as well as T- and B-cell infiltration in the basal nucleus region. However, sometimes it cannot be ruled out that antibodies against the basal nuclei occurred after their damage by some other pathological process [17].

Sydenham's chorea. Sydenham's chorea was described by Paracelsus as "the dance of St. Witt," whereas in the 17th century Sydenham gave it an accurate clinical description, and he also described acute rheumatic fever. However, the relationship between the two diseases was suggested later, in the 18th century, by Richard Bright and further supported by a number of researchers. Researchers at that time wrote: "Of two children with rheumatic fever, one is necessarily sick with chorea." The classical description of Sydenham's chorea is a combination of chorea, sometimes with the development of paresis, and behavioral disorders (mainly, the obsessive-compulsive spectrum) after an infection caused by group A hemolytic streptococcus.

At the end of the 20th century, the expanded term PANDAS (pediatric autoimmune neuropsychiatric diseases due to streptococcal infection) was introduced. The proposed mechanism for the development of these diseases is the cross-reactivity of antibodies to streptococcus and basal ganglia, which leads to extrapyramidal movement disorders and psychiatric manifestations [16]. Recently, a number of studies considered Sydenham's chorea as encephalitis with antibodies to D2R [18].

Hashimoto's Encephalopathy. In 1966, Brain et al. described a case of episodic encephalopathy associated with autoimmune thyroiditis (AIT). A few months after the detection of AIT, the patient (a 48-year old man) periodically had pareses of different localizations, a decreased level of consciousness, and cognitive impairments that persisted despite therapy with hormones, anticoagulants, and thyroxine; however, 1 year later it had regressed completely. Doctors suggested the relationship between AIT and brain damage on the background of thyroid pathology, as the

described picture of the disease did not fit into any of the known pathologies at that time [19].

Further, many attempts have repeatedly been made to classify these conditions, develop criteria, and understand the etiology of Hashimoto's encephalopathy. However, due to the rarity of disease and unknown mechanism of brain damage, these investigations were not successful.

The main clinical manifestations include epileptic seizures, resistant to antiepileptic therapy, headache, hallucinations, stroke-like episodes and other focal neurological symptoms, impairments of cognitive functions and consciousness up to coma, behavioral and mood disorders, ataxia, and dementia. The frequency of occurrence of this disease is 2.1/100 000 (the National Institute of Health of the United States classifies this disease as rare); it occurs more frequently in women, has a fluctuating nature of pathology, and responds to immunosuppressive therapy. The autoimmune genesis of encephalopathy is also confirmed by a number of laboratory tests. The patients have a high titer of antibodies to thyroperoxidase or thyroglobulin, whereas in most cases euthyroidism or a slight change in thyroid function was observed [20]. A number of studies showed the presence of antibodies in the CSF which specifically bound to cerebellar astrocytes. The CSF often had an elevated level of IgG and/or oligoclonal bands [21]. Several studies have shown the presence of immunoreactive receptors of thyroid-stimulating hormone in the cerebral cortex [22]. In 2002, antibodies to alpha-enolase were found [23], which were detected in 68 to 83.3% patients with Hashimoto's encephalopathy according to various studies [24].

However, the antineuronal autoimmune response is not fully confirmed: the data of single-photon emission computer tomography on brain hypoperfusion, high expression of alpha-enolase in the vascular endothelium and the presence of antibodies to it in other vasculitis (systemic lupus erythematosus, rheumatoid arthritis, etc.) served as basis for a hypothesis on the vascular genesis of Hashimoto's encephalopathy [21].

Accordingly, currently, there are three views on the mechanism of development of Hashimoto's encephalopathy [21]:

(1) autoimmune vasculitis of the central nervous system;

(2) autoimmune encephalitis with antibodies against thyroid and central nervous system antigens;

(3) metabolic pathology due to the toxic effect of thyrotropic hormone on the central nervous system.

However, nowadays, a number of researchers doubt not only the genesis but also the existence of this pathology.

Oncology Development and Description of the Group of Paraneoplastic Encephalitis

The next era in the study of AE is a description of the group of paraneoplastic encephalitides (Table 1). Classification and studies of this group of diseases began in the same era as the molecular description of various types of tumors in the 1950s. Prominent researchers in autoimmune neurology believe that the first case of autoimmune paraneoplastic encephalitis was described by Oppenheim in 1888 [25] in the article "Neurological symptoms associated with carcinomatosis without detected changes in the brain." A 54-year-old woman was first admitted to the Charite Clinic in Berlin in June 1887. Her neurological status included agnosia, mood changes, and severe aphasia; communication was possible only with the simplest gestures. A few days later, the patient died, autopsy revealed gastric cancer; however, there were no visible changes in the brain, including meningeal membranes and vessels. Histological examination revealed no significant changes such as metastases and vascular or nerve pathologies. Given the strange combination of neurological symptoms without any pathological changes, Oppenheim began to look for similar cases in the archives of the clinic and found a similar description 1 year before where a woman with epileptic seizures and breast cancer had no changes in a pathological morphological study of brain tissue. Oppenheim suggested the presence of a "toxic effect" of the tumor on the brain, even in the absence of metastases. In 1929, subacute cerebellar degeneration was described in patients with oncological pathology and only half a century later, in 1948, Denny-Brown [26] described a rapidly progressing sensory neuronopathy in patients with lung cancer and suggested the need for "metabolic studies in these cases to identify the cause of lesions of the nervous system." In 1949, the term "paraneoplastic" was introduced by Guichard and Vignon to describe the neuropathy that arose in a patient with uterine cancer [27].

After Oppenheim the second cases with damage to the central but not peripheral nervous system was described by Brierley and his colleagues in 1960: three cases of the disease with an inflammatory response in the cerebral hemispheres, limbic system and concomitant bronchial carcinoma, kidney leiomyoma, and an unknown lung tumor, respectively [28]. The same case is considered as the first description of the clinical picture of limbic encephalitis. Later, several similar cases were described; in 1968 Corsellis [29] introduced the term "limbic encephalitis" and wrote a review of the literature on this topic.

At the same time, three hypotheses for the development of paraneoplastic encephalitis have been proposed:

(1) neurodegeneration of cells for an unknown reason, which leads to secondary inflammatory infiltration;

(2) a possible viral infection;

(3) main hypothesis of autoimmune lesion of the nervous system by antibodies [30].

At this stage, we note only the description of clinical cases and the study of the morphological pattern of pathology in the brains of deceased people. Anti-Hu antibodies were discovered only 100 years after the clinical description of Oppenheim.

In 1985, the first work was published on the detection of antibodies to various neuronal structures in biological fluids: antibodies to Purkinje cells in paraneoplastic degeneration of the cerebellum were described [31] and neuronal antinuclear antibodies associated with sensory neuropathy and lung cancer, subsequently described as anti-Hu antibodies [32, 33].

Several anti-neuronal antibodies have been described:

in 1983, anti-Yo antibodies [34]; in 1989, anti-Ri antibodies [35] in patients with breast cancer; it was also shown that isolated IgG bound to tumor cells.

In 1993, antibodies to amphiphysin (128 kDa brain protein) were isolated in three patients with Stiff-person syndrome and breast cancer [36], and in 1996, anti-CV2 antibodies (antibodies to the 66 kDa protein) [37] and antibodies to Ma2 in patients with testicular tumor.

At this stage, a standard protocol for antibodies searching has already been developed (see the Methods section).

However, the description of these antibodies and the precise diagnosis of the associated neurological syndrome did not lead to effective treatment of these patients. In most cases, therapy was ineffective and the prognosis was disappointing. This situation was changed only after description of first AE with antibodies to the surface neuronal antigens and good recovery after treatment.

Description of Encephalitides with Antibodies against Neuronal Surface

The next wave of encephalitis research began in 2005, when the Dalmau et al. for the first time clinically described paraneoplastic syndrome in young women with ovarian teratomas and then isolated antibodies to NMDAR [38, 39] (Table 2).

The idea of a relationship between NMDAR and schizophrenic manifestations had been already known; it was found in the 1980s [40], when patients could experience episodes of arousal, psychotic and cognitive impairments after the use of NMDAR antagonists (such as ketamine). Observation of the effect of these drugs led to the creation of the theory of hypofunctioning of NMDAR in schizophrenia, and one of the animal models of schizophrenia was created by the "switching off" of the gene that encodes D-serine racemase, which is required for the production of the NMDAR agonist in the central nervous system, D-serine [7]. However, the antibody–antigen–dependent response that led to these symptoms in patients was described by Dalmau.

First, a group of 12 women, who developed psychiatric behavioral disorders, amnesia, dyskinesias, epileptic seizures, autonomic disorders, as well as a decrease in the level of consciousness or even coma, and the need for ventilation support, were clinically described. After the laboratory examination, it was found that the serum and CSF of patients were immunoreactive against neuropil of the hippocampus and the forebrain; luminescence was the strongest at the level of membrane and the molecular layer of the hippocampus. Antibodies were isolated and found to react with NR1/NR2 NMDAR heteromers. After further examination, 11 patients had ovarian teratomas and 1 patient had mediastinal teratoma, all the examined tumors expressed the NR2B subunit of NMDAR. Tumor removal and immunotherapy resulted in complete recovery in eight of nine patients; three patients without tumor removal died from neurological aggravations. During autopsies, glial changes in brain tissue, rare T-cell infiltrates, and neuronal degeneration, which was more pronounced in the hippocampus, were revealed.

In addition to the description of NMDA encephalitis, in 2005, six patients with new antibodies to potassium channels were described, which in further studies divided into two subtypes: with antibodies against CASP2R and LGI1 [41], who had a good response to therapy and removal of the tumor.

Several years after the description of the clinical picture and antibodies to NMDAR, it became clear that there are many cases of AE. Many of these cases remain unrecognized due to the onset of the disease with psychiatric symptoms, a normal MRI picture or minor changes in MRI. Antibodies searching and discovery are very important due to treatable state of these conditions. NMDAR, CASP2R, LGI1 and other new antibodies were isolated into a separate group of antibodies to the surface neuronal membrane.

In 2008, progressive encephalomyelitis with rigidity and myoclonus was associated with antibodies to GlyR [42]. It is interesting that in this case the clinical picture of encephalitis with hyperekplexia, which was described as a genetic disease caused by the presence of a mutation in the glycine receptor gene, led to the idea of the presence of antibodies to GlyR in this disease, which was confirmed.

In 2009, Lai et al. described antibodies to AMPAR in limbic encephalitis [43], which were detected by screening of CSF and serum of patients in neuronal culture by immunoprecipitation and immunoblotting. In 1994, antibodies to the third subunit of the glutamate receptor subunit, which were detected in Rasmussen's encephalitis and epilepsy with frequent sei-

zures, were described; however, subsequently the pathogenic role of these antibodies was not confirmed.

In 2010, in [44], during screening of 410 patients, 15 had an antibody response to the hippocampus; however, no other known antibodies were detected. Serum and CSF of these patients were screened on rat hippocampal cultures and immunoreactivity was also detected in relation to neuronal culture. Electrophoresis with immunoprecipitation revealed 90 and 105 kDa bands corresponding to GABAb1R and GABAb2R. Subsequently, GABAbR was determined by mass spectroscopic analysis of immunoprecipitates. The technique described in this work in 2010 has become quite a standard scheme for searching for antibodies in AE with small modifications.

In 2011, antibodies to mGluR5 were detected in Ophelia syndrome [45] and Hodgkin's disease, although the syndrome itself was clinically described in 1982 by the psychiatrist Carr in his teenage daughter [46]. In this case, a technique analogous to the previous work on the detection of antibodies to GABAb2R was used.

In 2013, Boronat et al. described antibodies to DPPX [47].

In 2014, Mar Petit-Pedrol et al. described antibodies to GABAaR also during screening work; in 6 patients out of 140 patients with suspected encephalitis, seizures or epileptic status and an autoimmune response to brain sections of unknown genesis, antibodies to GABAaR were detected during immunoprecipitation [48].

However, prior to this, a similar picture of the disease was suggested in two patients in 2012 on the basis of the clinical picture; four mutations in GABAaR were previously described, which led to generalized epilepsy [49]; this clinically also suggested the presence of antibodies against different variants of GABAR.

In 2015, after the clinical analysis of sleep disorders and PSG patterns, antibodies to IgLON5 were detected by the same method [50], the spectrum of clinical manifestations for which was subsequently expanded [51].

In 2016, the same group of researchers [52] described antibodies to neurexin 3; during a 10-year study, five patients with a similar clinical picture were identified in the form of prodromal symptoms, headache, and symptoms of the gastrointestinal pathology, and then development of epileptic seizures and lowering of consciousness up to coma and the need for ventilation support. The picture of this encephalitis was described as NMDA-like, taking the prodromal symptoms and pronounced impairment of consciousness into account.

Currently, a further search for antibodies is continuing. The concepts of neurobiology of AE have been created and may be used to study pathology at various levels [6]. However, diagnostic criteria are very wide and based more on clinical picture, while the therapy is chosen empirically and often based on the existing expert opinion [53].

Identification and Characterization of Autoantibodies, Confirmation of their Phatogenicity

From the description of Sydenham's chorea and until the first discovery of anti-Hu antibodies, AE has been described clinically. A macro and microscopic picture of the brain pathology has also been studied, and in some cases no significant changes have been found.

It's interesting to note that names of the first antibodies were simply combined from the first letters of patient's names and surnames (Hu, Ri, etc.). It highlights once again that clinical picture played the main role in a process of searching for new antibodies and describing syndromes.

The entire group of paraneoplastic antibodies was isolated and studied using the standard scheme [35]:

(1) Screening of biological fluids (CSF and blood serum) on frozen sections of the brain of deceased people without any neurological diseases, usually the zones of the neuropil of the hippocampus and the cortex [33], or on brain tissue of mice or rats [54]. Sometimes, the antigen could be predicted even at this stage, based on the characteristic localization of antibodies on the membrane or binding of antibodies to a particular brain region. This was the case, for example, in the detection of antibodies to D2R after detection of immunoreactivity against the basal nuclei.

(2) When detecting the immunoreactivity of individual sera or CSF, these samples, in parallel with the control samples, were analyzed by immunoblotting. At the same time, the reactivity of different dilutions of CSF and patients' serum against protein extracts obtained from homogenized brain tissue or from pre-isolated cortical neurons was evaluated. Thus, anti-Hu antibodies have been described to antigen 35−38 kDa.

At that time, a mass spectrometric determination of such amounts of antigen was technically difficult; thus, the antigen was determined by immunoaffinity chromatography. The isolated IgGs to the antigen were applied to the column and a protein extract from neurons of the cerebral cortex was passed through it, which made it possible to obtain concentrated antigen and confirm its mass on a western blot and conduct a mass spectrometric analysis of the concentrated protein [35].

In a number of cases (for example, the description of antibodies to Ma2), modifications of the method were applied: the antigen was determined using the "phage display" method [55].

Since 2010, when the determination of an antigen has become available via mass spectrometry and the work of Lancaster et al. [44] was published, all further studies were performed according to a standard proce-

dure and mainly as screening. For only two antibodies, an antigen was predicted on the basis of clinical manifestation (hyperekplexia in the case of encephalitis with antibodies to the glycine receptor) and a possible common pathogenic antibody IgLON5 for a group of patients with a pattern of sleep disorders according to polysomnography.

The search for antibodies is usually performed according to the standard scheme (Fig. 2).

The first stage is determination of immunoreactivity of patients' serum or CSF using a neuronal culture and/or immunohistochemical staining of rodent brain sections. Immunocytochemical analysis is usually performed as follows: a rat hippocampal neuronal culture is incubated with antibodies to the NR1 subunit of NMDAR (control) or with CSF from patients. Then it's possible to check if fluorescent signals are localized in the same areas [39]. The complex containing the autoantibody is isolated from the cell culture and the protein is identified by high performance liquid chromatography with tandem mass spectrometry. It's important to remember that detected proteins may itself be complexes of antibodies with other proteins. Therefore, direct binding of antibodies is evaluated on transfected cell cultures expressing candidate antigens.

Unfortunately, it is not always possible to isolate antigens from neuronal cultures, despite the rapid development of immunoproteomics. The biochemical properties of a number of proteins, such as insolubility in detergents (NMDAR), or post-transcriptional modifications (pronounced lipidation or phosphorylation) make it difficult to purify and analyze by mass spectrometry. In addition, some patient antibodies bind weakly to rodent antigens-orthologues, which leads to false-negative results [6].

The second stage—immunohistochemical staining of brain sections or cultures of rodent neurons with antibodies isolated from patients. Unlike the first method mentioned above, in this case two lines of animals (or two types of cultures) are used. The first is the control one, the second is the knockout for the gene of interest (similarly for cell cultures: for example, the staining of the control line and the line with the knockdown of the gene of interest is compared). If a knockout or knockdown leads to the disappearance of fluorescence, this confirms that the patient has antibodies specific to the particular protein.

However, the presence of autoantibodies in the serum/CSF of the patient does not itself indicate their pathogenicity. Therefore, the third mandatory stage of antibodies characterization is confirmation of their pathogenicity. The simplest indirect confirmation of pathogenicity is the correlation between the antibody titer and the severity of the symptoms. More reliable evidences may be obtained in vitro and/or in vivo. A common tool for in vitro analysis is cell cultures, which are used already at the stage of immunocytochemistry; these are usually rat or mouse neuronal
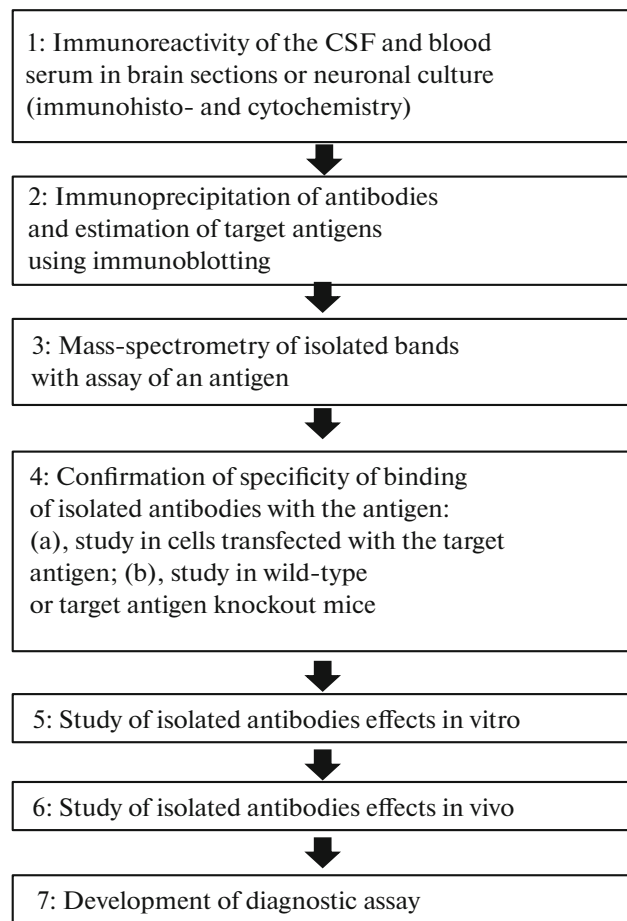

Fig. 2. The standard protocol for the search, isolation, and confirmation of pathogenicity of antibodies in autoimmune encephalitides.

cultures, the methods for obtaining them have been described in detail in the literature [56], or human embryonic kidney cells line 293 (Human Embryonic Kidney, HEK293). Cell lines transfected with plasmids with the genes of the studied antigens, are useful not only for evaluation of the direct binding of antibodies, but also for characterization of their reactivity against individual receptor subunits. Thus, Dalmau et al., 2007 [38] used the HEK293 cell line to study the reactivity of autoantibodies against NMDAR subunits. The cells were transfected with plasmids containing the genes NR1, NR2A, or NR2B (alone or in combination), as well as a control plasmid without inserts. As a result, the cells expressed individual subunits of NMDAR (NR1, NR2A, or NR2B), as well as their functionally active combinations (NR1/NR2B and NR1/NR2A). The resulting cells were incubated with serum or CSF of patients, followed by immunocytochemistry. All 12 samples of patients' serum and CSF patients were reactive against NR1/NR2 heteromers that contained NR2B. Serum and CSF samples from eight patients were also reactive against NR1/NR2A heteromers. Serum samples were not reactive to cells

expressing individual receptor subunits. There was also no reactivity against immunoblots of proteins from cells expressing functional receptors; that is, intact functional heteromers were required for antibody binding. All these data suggested that the main epitopes are probably located on the extracellular domains of the subunits NR2B and NR2A and are conformational.

It is interesting that later (especially with an increase in sample size) this hypothesis became inconsistent with clinical data [39]. The NR2B subunit of NMDAR is expressed predominantly in the hippocampus and other areas of the forebrain. Therefore, the disruption of the function of NR1/NR2B receptors will be relatively local, and is unlikely to cause a clinical picture of the extensive neurological deficit. The discrepancy between the putative molecular mechanism and the clinical picture prompted a new hypothesis: the researchers suggested that the main epitopes for binding autoantibodies are located in the much more common NR1 subunit. This hypothesis was confirmed in a number of experiments: HEK293 cells were transfected with plasmids carrying the NR1 and/or NR2 genes. It was shown that patients' serum and CSF are reactive not only against all NR1/NR2 heteromers, but also against NR1/NR1 homodimers, i. e. autoantibodies recognize exactly the NR1 subunit. To determine the localization of the epitope, the cells were transfected with a plasmid carrying the gene of the modified subunit NR1 (NR1d4): it lacked amino-acid residues 25−380; however, it formed a complex with NR2B. Patients' CSF and serum samples were almost non-reactive against NR1d4/NR2B complex. Thus, it was proved that the main epitope is located in the extracellular region of the NR1 subunit [39].

Cell cultures also revealed that autoantibodies to the GABAa receptor reduce the density of synaptic and extra-synaptic GABAaR [57]. The biochemical experiment deepened the results: the researchers suggested that autoantibodies to GABAa enhance the internalization of receptors, which leads to a decrease in their density. The experiment was performed as follows: rat hippocampal neurons were incubated with the serum of patients or participants from the control group, after which the surface proteins were biotinylated and isolated using granules conjugated with avidin. Neurons that were incubated with patients' serum showed reduced level of surface GABAaR β3 subunits, whereas the total number of β3 subunits remained the same. To determine whether this effect is specific for GABAaR, the content of CluA1 and N-cadherin was evaluated in parallel and no changes were detected. This experiment lead to the hypothesis of receptor internalization caused by autoantibodies.

Neuronal cultures are also used to perform electrophysiological experiments, during which it was shown that antibodies change potentials and currents in nerve cells: for example, antibodies to GABAaR selectively reduce the amplitude and frequency of miniature inhibitory postsynaptic potentials [57] and also reduce synaptic currents mediated by GABAR [6]. In another experiment on cerebellar slices of mice, it was shown that the inward current induced by the selective agonist of the metabotropic glutamate receptors was significantly reduced in the presence of IgG that were isolated from the patients serum. After washing away antibodies, the current was restored.

No less interesting are the methods used to confirm pathogenicity in vivo. Pioneers in this area are Coesmans et al. [58]. First, in accordance with the standard procedure [59], eye movements in laboratory mice were analyzed. After the baseline measurements (control data), a pump was implanted that injected purified and concentrated IgG solution from either a patient or a healthy control into mice cerebellum (flocculus area) at a predetermined rate. All mice injected with patients' IgG showed a decrease in the amplitude of compensatory eye movements.

In 2004, Pellkofer et al. [60] administered recombinant type 1 T-helpers (Th1), which are specific for the autologous onco-neuronal Pnma1 antigen, to female rats. Six days after the administration of Pnma1-specific CD4+ Th1, an inflammatory response in the central nervous system was observed in rats. In animals, encephalomyelitis was localized in the same areas as in patients with paraneoplastic syndrome associated with these antibodies. Thus, it has been confirmed that the reactivity of CD4+ T cells with respect to onco-neuronal antigens is pathogenic and the autoimmune component involving T cells is one of the mechanisms of CNS damage in paraneoplastic syndromes.

The same approach was used to show pathogenicity of individual antibodies: IgG from a patient with a high titer of antibodies to amphiphysin induced muscle spasms in rats (their severity correlated with the dose of antibodies administered). They resemble spasms typical for stiff-person syndrome [61]. In 2010, in this experiment, the pathogenicity of antibodies to NMDAR was confirmed [62].

In 2012, in vivo studies moved to a new level: the models of Sydenham's chorea and PANDAS-associated disorders in rats were created by immunization with group A streptococcal antigen. Results from studies [63] allows to consider this group of pathologies as encephalitis with antibodies to D2R.

The experiments described above are laborious and technically difficult. However, the most important results are obtained in vivo, and animal models are imperative to reliably confirm the pathogenicity of individual autoantibodies.

CONCLUSIONS: THE MODERN STATE OF THE PROBLEM

There are several important reasons for searching for new antibodies in AEs:

(1) Clarification of the pathogenesis of the disease, since most of the antibodies are pathogenic, which has been proven in animal experiments [64];

(2) Finding antibody allows the use of immunotherapy and, in some cases, predicts the response to therapy and the prognosis of the disease;

(3) Antibody positivity may become the basis for prescribing more effective but dangerous second line treatment if the refractoriness to the first-line drugs occurred.

In addition, there are several problems of antibodies determination in AE:

(1) The detection and determination of all known antibodies is available only to a small number of laboratories;

(2) It is possible to detect low unspecific antibody titers in other neurological diseases. GlyR antibodies were found in Creutzfeldt—Jakob disease [65], NMDAR in Creutzfeldt—Jakob disease [66] and MELAS syndrome [67], GABAaR in genetically confirmed Goettington's disease [68], and sometimes antineuronal antibodies are detected in healthy volunteers [69];

(3) Most of the available studies focus on IgG detection, whereas the role of IgA and IgM antibodies is unknown, although cell cultures demonstrate changes in the presentation of the NMDA receptor under the action of these antibodies [70];

(4) There is no correlation between the antibody titer and the severity of clinical manifestations, there are no clear protocols for treatment (choice of cytostatics, etc.), treatment is mostly empirical.

Despite the short-term existence of this disease as a clinical nosology, nowadays AE may be considered as a unique disease.

First of all, antibodies detected in AE with different psychiatric symptoms may be one of the possible "molecular substrata" of psychiatric diseases and may lead to new concepts of pathogenesis of psychiatric disorders. Using AE antibodies scientists can study cognitive processes, for example, short-term memory.

Secondly, it is quite interesting to consider AE from the point of view of switching off the receptor function: now AE is considered together with its "genetic twins," diseases with a genetic mutation in the gene of the receptor or another protein that is an antigen.

As an example, the identified mutations in GRIN1, as well as GRIN2A (NR2A) and GRIN2B (NR2B) lead to the development of epileptic encephalopathies. The clinical phenotype for GRIN1-encephalopathy is described as a combination of hyperkinesis, epileptic seizures, and sleep-wake disorders, which is very similar to the pattern of AE with antibodies to NMDAR [71].

Nowadays encephalitis with antibodies to glycine receptors and hyperekplexia, and a mutation in GlyR gene leading to hyperekplexia, a mutation in the neurexin gene in autism and behavioral disorders similar to pathology in antineurexin antibodies encephalitis [6], involvement of the temporal lobe in AE with antibodies to LGI1 and hereditary temporal epilepsy with mutation in this gene [72] were already described. All these facts make AE an unique disease where it is possible to evaluate the disturbances of "antibody—receptor—gene" axis functioning not only in animal models, but also in different patients.

Thus, in addition to the routine clinical task of diagnosing and determination of the optimal treatment tactics, AE is a very interesting disease for further study of neurobiology of cognitive and behavioral processes, as well as immunogenetic interactions.

COMPLIANCE WITH ETHICAL STANDARDS

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