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Neurobiology of autoimmune encephalitis

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Autoimmune encephalitis presenting with amnesia, seizures, and psychosis is highly topical in basic and clinical neuroscience. Recent studies have identified numerous associated autoantibodies, targeting cell-surface synaptic proteins including neurotransmitter receptors (e.g. NMDA receptors (NMDARs)) and a secreted protein, LGI1. *In vitro* and *in vivo* analyses of the influence of the autoantibodies have begun to clarify their causal roles. Of particular interest is the generation of recombinant monoclonal antibodies from patients' B cells with anti-NMDAR encephalitis. Patient monoclonal antibodies could be useful to reveal their direct, detailed pathogenicity. Such identification and characterization of autoantibodies could create new categories of neurological diseases and promote the understanding of patho-physiologic roles of target proteins in human brain function.

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Introduction

Autoimmune neurological disorders are caused by the production of aberrant, pathogenic autoantibodies to self-antigens. In the peripheral neuromuscular junction, it is well-known that autoantibodies to the postsynaptic nicotinic acetylcholine receptor and the presynaptic voltage-gated calcium channel induce myasthenia gravis and Lambert-Eaton syndrome, respectively [1]. In the central nervous system (CNS), antibodies to GluA3 subunit of AMPA receptors (AMPARs) were serendipitously found in patients with Rasmussen's encephalitis in 1994 [2] although a causative role of the autoantibodies in the development of disease was not clearly shown.

In 2000, taking advantage of the typical immunohistochemical staining pattern of mouse brain sections by patient serum antibodies, autoantibodies to mGluR1 were discovered from two patients who had Hodgkin's disease and paraneoplastic cerebellar ataxia [3]. This study elegantly demonstrated that the patients exclusively have autoantibodies to mGluR1 using the mGluR1 knockout (KO) mouse as a negative control for the immunohistochemical staining and that the passive transfer of autoantibodies into mouse cerebellum causes cerebellar ataxia. In addition, the group showed the detailed actions of autoantibodies on mGluR1 functions by the electrophysiological and mouse behavior analysis [4]. Although cerebellar ataxia with mGluR1 autoantibodies is rare, this investigation has served as a prototypical study of CNS autoimmune diseases.

Subsequently, antibodies to the GluN2B subunit of NMDA receptors (NMDARs) were reported in patients with chronic forms of epilepsia partialis continua, based on the serum antibody binding to the GluN2B subunit detected by Western blotting [5]. In contrast, antibodies to the GluN1 subunit of NMDARs were identified in patients with ovarian tumors, psychiatric symptoms, amnesia, seizures, and impaired consciousness, based on their apparent hippocampal neuropil-staining pattern in rodent brain sections [6]. The GluN1 autoantibodies react with conformation-dependent epitopes as they do not work with Western blotting. The latter cases have been well established as 'anti-NMDAR encephalitis' that is the most common form of autoimmune encephalitis [7].

Since 2009, nonbiased proteomic approaches using serum or cerebrospinal fluid (CSF) samples from patients with autoimmune CNS disorders have continuously expanded autoantibodies against cell-surface synaptic proteins, which target neurotransmitter receptors [AMPA (GluA1/GluA2), $GABA_A$, $GABA_B$, glycine, and dopamine-2 receptors and mGluR5] and other types of proteins, CASPR2 and LGI1. Furthermore, autoantibodies to DNER, DPPX (DPP6), DCC, IgLON5, Neurexin-3α, DPP10, ADAM23, CSMD1, ODZ1, and TMEM132A were reported (Table 1) [1,8,9]. These antibodies could be pathogenic by directly interfering with synaptic functional proteins. However, the linkage between these diverse autoantibodies and clinical symptoms/diagnoses remains incompletely understood.

The recent attempt to obtain and characterize recombinant monoclonal antibodies from the patients' B cells with anti-NMDAR encephalitis [10••] and advanced structural insights into ion channel proteins (e.g.

Table 1

Cell-surface targeting autoantibodies in encephalitis

Cell-surface target antigens	Associated diseases (characteristic symptoms)	Refs.
(1) Neurotransmitter receptors		
NMDAR (GluN1)	Anti-NMDAR encephalitis (psychosis, seizure, autonomic instability; teratoma-associated)	[6,7]
AMPAR	LE (seizure, psychosis)	[39]
mGluR1	Cerebellar ataxia (Hodgkin's disease associated)	[3,4]
mGluR5	LE, Ophelia syndrome	[58]
$GABA_AR$	Encephalitis (seizure, thymoma-associated)	[40,41,59]
$GABA_BR$	LE	[60]
Glycine R	PERM, stiff-person syndrome	[61,62]
Dopamine-2 R	Basal ganglia encephalitis (movement and psychiatric features)	[19,20•]
(2) Transmembrane proteins		
CASPR2	Encephalitis, peripheral nerve hyperexcitability (NMT)	[31,63]
DNER	Paraneoplastic cerebellar degeneration	[64]
DPPX (DPP6)	Encephalitis (diarrhea)	[42•,65]
DCC	NMT associated with thymoma	[9,66]
IgLON5	Abnormal sleep movements, tauopathy	[67,68]
Neurexin-3α	Encephalitis	[25]
(3) Secreted protein		
LGI1	Anti-LGI1 encephalitis (LE, facio-brachial dystonic seizures, hyponatremia)	[9,31,32]

LE, limbic encephalitis, PERM, progressive encephalomyelitis with rigidity and myoclonus; NMT, neuromyotonia.
Other neuronal autoantibody targets reported include DPP10, ADAM23, CSMD1, ODZ1, and TMEM132A although their pathogenic roles remain unclear due to the limited cases [9].

NMDARs [11•,12,13], AMPARs [14–16], and GABA receptors [17,18]) will in concert open a new avenue for understanding precise molecular mechanisms of action of autoantibodies. Here, we review recent progress in cell-surface autoantibodies associated with CNS disorders, particularly encephalitis, and discuss the potential of autoantibodies as research tools in basic neurobiology.

Identification of autoantibodies

Identifying autoantibodies is the first step to understand the pathogenic mechanism of autoimmune encephalitis. Previously, the discovery of autoantibodies depended on the serendipity or the deep insights and hypothesis of investigators (e.g. autoantibodies to mGluR1 [3] and NMDARs [6]). The recent advance in immunoproteomics has accelerated the identification of autoantibodies against cell-surface neuronal proteins [1,8,9]. Representative screening procedures include: first, cell-surface staining of rodent cultured neurons and/or immunohistochemical neuropil staining of rodent brain sections by patient serum or CSF antibodies (Figure 1a), second, isolation of the autoantibody-mediated immune complex from its bound cultured neurons and protein identification by mass spectrometry (Figure 1b), and third, cell-based binding assay to determine whether the patient antibodies directly bind to the cell-surface expressed candidate protein using transfected heterologous cells. Further assessment, disappeared staining with patient antibodies of brain sections in which the target protein is knocked-out or of cultured neurons in which the target is knocked-down, shows that the patient contains the autoantibodies exclusively to the single target protein, providing strong evidence linking the autoantibodies to the disease (Figure 1c).

Immunohistochemical analysis of brain sections by patient antibodies often provides the valuable information, as some candidate target proteins may show cell-specific or region-specific expression in the brain (e.g. dopamine-2 receptor is dominantly expressed in the striatum [19]). We should take account into staining patterns the patient antibodies give when we choose the cultured neurons from which target proteins are immunopurified. Although very powerful, the immunoproteomics analysis using cultured neurons is not always effective and sometimes misses the target proteins. Their biochemical properties, such as detergent insolubility (e.g. NMDARs) and post-translational modifications (e.g. highly lipidated or phosphorylated), may hinder their purification and mass spectrometry detection. In addition, some patient antibodies may have reduced binding to the rodent orthologs, leading to false-negative results (e.g. anti-dopamine-2 receptor antibodies [20•]).

Because so far, many neurotransmitter receptors have been reported as targets of autoantibodies (Table 1) [1,8,9], it may be worthwhile to explore whether autoantibodies against other neurotransmitter receptors (e.g. serotonin receptor) and auxiliary subunits of neurotransmitter receptors (e.g. TARP for AMPARs [21], Neto for kainate receptors [22], and GARLH for $GABA_A$ receptors [23]) are involved. Because previously identified target molecules are often mutated in congenital CNS disorders, target-based screening is well worth doing based on the