Plasmablasts and neuroimmunological disorders

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Abstract

Neuroimmunological disorders are diseases of the nervous system, wherein the immune system contributes to tissue injury and repair. Autoantibodies are useful biomarkers for the diagnosis of neuroimmunological disorders and evaluating disease activity. Emerging evidence indicates that several autoantibodies are associated with neuroimmunological diseases. While the differential diagnostic process based on the positivity of autoantibodies has been established, the mechanisms underlying the production of these autoantibodies still need to be investigated. Autoantibodies are not necessarily pathogenic, and some are involved in immune regulation. Autoantibody-producing plasmablasts are involved in both pathogenicity and immune regulation of diseases. Thus, comparisons between these pathogenic and regulatory plasmablasts may give us clues understanding the machinery of autoantibody-related neuroimmunological diseases. Moreover, elucidating these mechanisms may allow the development of new immune-modulatory therapies to facilitate regulatory B cell function in neuroimmunological diseases. To this end, herein the roles of plasmablasts in neuroimmunological disorders are discussed.

Introduction

Autoantibodies are the key in pathogenicity or as biomarkers in the diagnosis of autoimmune diseases. A report has suggested that 2.5% of the population suffers from pathologies caused by autoantibodies [1]. In this review, an overview of the autoantibody-related neuroimmunological disorders and the pathophysiological mechanism of autoantibody-producing plasmablasts (PB) are discussed.

Pathogenicity of autoantibodies

Autoimmune reaction is a phenomenon in which the immune system responds to host antigens, and it is important to distinguish it from autoimmune diseases induced by autoantibodies. In general, high-affinity autoantibody-producing B cells are eliminated by negative selection in the bone marrow; however, low to moderate affinity autoantibody-producing B cell can differentiate and appear in peripheral blood. Indeed, a part of them are known to be involved in immune regulation [2], thus autoantibodies, intrinsically, are not necessarily pathogenic. It is also known that some autoantibodies are useful in the diagnosis of autoimmune diseases even if they are not pathogenic. For instance, differences in the intracellular recognition sites of the antinuclear antibodies have been classified and used for the differential diagnosis of connective tissue diseases. For example, recently, the antibodies against aminoacyl-tRNA synthetase (anti-ARS antibody), observed in a part of myositis, have been used as the biomarkers for predicting association with interstitial pneumonia or tumor [3].

After the peripheral appearance, auto-reactive B cells undergo a maturation process (affinity maturation) in lymphoid organs that increases the binding affinity of the autoantibody during B-cell differentiation. The B-cell interactions with the lymphoid tissue factors and auto-reactive T cells result in the formation of a lymphoid structure together and produce pathogenic autoantibodies. Recently, Ludwig et al. classified pathogenic autoantibodies into 7 categories based on their functions [4]. Among those, either receptor inhibition of neurotransmitter or induction of the local tissue inflammation is observed in neuroimmunological disorders (Table 1).

As an example of inhibition of neurotransmitter, Myasthenia gravis (MG) is a neuromuscular junction disease, where the autoantibodies against the acetylcholine receptor (AChR) inhibit post-synaptic neurotransmission, which results in muscle...
weakness and fatigue. Anti-AChR antibodies mainly consist of IgG1 and IgG3 subclass, thus their function is complement-dependent cytotoxicity as well as antibody-dependent cellular cytotoxicity. The antibodies also make AChR cross-link together and physically inhibit acetylcholine (Ach) binding to the receptors, and lesser extent functionally block Ach-binding site [5]. On the other hand, there is another autoimmune disease that causes MG, anti-muscle-specific kinase (MuSK) antibody consists of IgG4 subclass that rarely causes complement-dependent cytotoxicity but decreases AChR clustering required for prompt neurotransmission. Recently, it is reported that IgG4 autoantibodies targeting Ranvier’s node protein NF155 or contactin-1 in the peripheral nerves causes loss of paranodal transverse bands and demyelinating polyneuropathy which is found in less than 10% of chronic inflammatory demyelinating polyneuropathy [6,7].

Another group of the disease is autoimmune limbic encephalitis. Autoantibodies against N-methyl-D-aspartate receptors (NMDARs) are reported to induce synaptic structural changes, which cause reversible loss of NMDARs, impair glutamate receptor signaling, and result in learning dysfunction, memory and other behavior observed in patients with anti-NMDAR encephalitis [8]. Recently, several other autoantibodies inhibitive to neurotransmitter receptors and related proteins in the central nervous system (CNS) have been reported to be associated with autoimmune limbic encephalitis, such as antibody against α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), γ-aminobutyric acid (GABA), and leucine-rich glioma-inactivated 1 (LG11) [9–11]. Within them, anti-GABA_{α}R antibodies cause extra-limbic cortical and subcortical lesions [9]. Of note, brain lesions show only mild nonspecific perivascular lymphocytes infiltration and reactive astrocytes [12]. The pathogenesis of these newly identified autoantibodies related to limbic encephalitis still needs to be investigated.

On the contrary, in cases of Neuromyelitis optica (NMO), IgG1 autoantibodies against aquaporin 4 (AQP4), a water channel expressed on the astrocytes, cause destruction of binding sites in a complement-dependent manner following immune cell infiltration into the CNS, resulting in severe optic neuritis and transverse myelitis [13]. It is important to notice that pathogenic autoantibodies are characterized based on its target receptors expressed on the surface of the cells.

### PB and autoantibodies

Plasma cells (long-lived plasma cells (PC)) are known as cells that produce antibodies, whereas the B-cell differentiation stage before engraftment in the bone marrow is classified as short-lived PB [14]. Although most of IgG antibodies in the peripheral blood is thought to be produced from PC, it has been reported in a murine autoimmune disease model, PB produces pathogenic autoantibodies in peripheral lymphoid tissues [15] that may be called as ‘long-lived’ PB (Figure 1). Among the neuroimmunological diseases, thymus-derived cells that produce pathogenic anti-AChR antibody in MG has been pointed out [16,17]. Since thymus is the place for T-cell maturation, it is possible that the auto-antibody-producing PB can survive by constitutive stimulation by the auto-reactive T cells that recognize AChR as the autoantigen. In anti-MuSK antibody-positive cases, Stathopoulos et al. have reported patients who showed relapse instead of B cell ablation therapy (anti-CD20 antibody therapy, PB is CD20 negative) that PB emerged in peripheral

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Table 1. List of pathogenic autoantibodies.

| Functions | Autoantibodies and their functions (diseases) |
|-----------|-----------------------------------------------|
| 1. Mimicry of receptor activation by hormone | Antibodies against the thyrotropin receptor mimic hormone stimulation (Graves’ disease) |
| 2. Blocking and Alteration of neural transmission | Antibodies against acetylcholine receptor (AChR) or muscle-specific kinase (MuSK) block neural transmission in neuromuscular junction (myasthenia gravis) |
| | Antibodies against voltage-gated calcium channels (VGCC) block neural transmission in neuromuscular junction (Lambert–Eaton myasthenic syndrome) |
| | Antibodies against NMDA, GABA, AMPA receptor, or leucine-rich glioma-inactivated 1 (LG11) alter synaptic sibodies in the CNS (autoimmune limbic encephalitis) |
| 3. Altered cell signaling/ Acantholysis | Antibodies against desmoglein-3 induce an altered signaling in keratinocytes (pemphigus) |
| 4. Blocking of enzymes/ coagulation | Antibodies against ADAMTS13 triggering uncontrolled microthrombosis (acquired thrombotic thrombocypenic purpura) |
| 5. Direct cell lysis | Antibodies against platelets induce cell lysis (autoimmune idiopathic thrombocytopenia) |
| 6. Neutrophil activation | Antibodies against neutrophil cytoplasmatic triggering uncontrolled neutrophil activation (granulomatosis with polyangitis) |
| 7. Induction of Inflammation | Antibodies against aquaporin-4 on astrocytes induce inflammation at the site of autoantibody binding (neuromyelitis optica) |
| | Antibodies against targeting Ranvier’s node (chronic inflammatory demyelinating polyneuropathy) |
| | Antibodies against structural proteins of the skin (pemphigoid disease) |
| | Antibodies against myosin (myocarditis) |
| | Antibodies against citrullinated proteins (rheumatoid arthritis) |

ADAMS13: a disintegrin-like and metalloprotease with thrombospondin type 1 motifs 13; NMDA: N-methyl-D-aspartate; GABA: gamma-aminobutyric acid; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. Modified from Ludwig RJ et al. [4].
blood produced pathogenic anti-MuSK antibodies [18]. Previously, Stathopoulos et al. isolated peripheral blood mononuclear cells (PBMC) from NMO patients and subdivided B cell subpopulations. While NMO patients have anti-AQP4 antibodies, other autoantibodies such as antinuclear antibodies and anti-SS-A/SS-B antibodies often coexist, suggesting an overlap with the connective tissue disease. Based on the report that CD180 negative B cells are autoantibody-producing cells in systemic lupus erythematosus [19], CD180 is employed with common B-cell lineage markers, such as CD19, CD27, and CD38. PB was identified as a subpopulation with CD19⁺CD27highCD38highCD180−/−. NMO patients showed an increase in the number of PB compared with healthy subjects and patients with multiple sclerosis (MS), a major neuroimmunological demyelinating disease of the CNS. More importantly, PB was predominantly produced by anti-AQP4 antibody within B cells [20]. After further analysis of CD138-positive PB in NMO patients who expressed tissue-oriented CXCR3 and had common variable region mutations in antibody genes in both cerebrospinal fluid (CSF) and peripheral blood in the same time at relapse, suggesting the possibility of PB migration from peripheral blood to central nervous tissue [21]. Bennett et al. also have shown that CNS pathologies can be passively induced in animal models using manufactured anti-AQP4 antibodies from CD138-positive PB obtained from CSF [22]. These studies suggest that the pathogenic PB infiltrate into CNS in NMO (Figure 1). Unlike in the cases of anti-AChR antibody-positive MG, there is no place in the CNS for PB to survive after interaction with auto-reactive T cells. In NMO, both the anti-AQP4 antibody produced from PB engrafted in the bone marrow and the PB appearing at the time of relapse may cause CNS pathology in NMO.

On the other hand, about half of anti-NMDA receptor encephalitis is paraneoplastic neurologic syndrome related to ovarian teratoma, and the recurrence is about 15%, and in those cases, ovarian teratoma can recur [23]. It is also well known that autoantibodies against voltage-gated calcium channels (VGCC) cause Lambert–Eaton myasthenic syndrome, typically as a paraneoplastic neurologic syndrome due to small-lung cell carcinomas, inhibiting pre-synaptic calcium channels which are required for acetylcholine supply in the neuromuscular junction [24]. There are also cases of thymoma-related MG as well as tumors associated with NMO [25]. In these cases, autoantibody production is seen as a normal immune response in a cross-reaction against tumor antigens (non-self antigens), where immune background may be different from autoantibodies positive cases without tumor. In the future, it is expected that the characteristics of PB that produce autoantibodies in neuroimmunological disease patients will be clarified that can lead to the development of therapeutics based on its pathological background.

**Pathogenic PB and regulatory PB**

Although it is understood that the interaction with lymphoid tissue factors and auto-reactive T cells is
important in the differentiation of autoantibodies-producing PB, B cell intrinsic factor before differentiation into PB is not completely understood. Recently, Rubtsova et al. reported that abnormal lymphoid follicular formation was increased when T-bet, one of the transcription factors, was expressed in B cells of a murine autoimmune disease model. When T-bet was specifically knocked out in the B cells, the mice showed reduction of autoantibody production and delayed progression of the disease [26]. While pathogenic autoantibodies recognize cell surface proteins, in some cases with autoimmune diseases have nonpathogenic autoantibodies that recognize intracellular proteins. We still do not know whether it is due to differences in antigens or differences in the B cell intrinsic factor responsible for antibody production.

Additionally, there are cases in which a neuroimmunological disease is suspected clinically, however, the autoantibodies are negative, and show progressive course of the disease without treatment. Takewaki et al. reported patients with relapses of neurological symptoms similar to MS but without any abnormal findings on the image as normal-appearing imaging-associated, neuroimmunologically justified, autoimmune encephalomyelitis (NINJA) [27]. Of note, in the peripheral blood of those patients, the frequency of PB was increased. At present, autoantibodies specific to this disease group have not been identified, however in neuroimmunological diseases that are difficult to diagnose using other clinical tests, the same immune background can be predicted by analyzing the dynamics of PB. There is also potential clinical usefulness of PB that in the patients presenting cognitive decline and intractable epilepsy, there might be immune-mediated causes and usually they are followed up without receiving enough treatment.

To this end, we should take into consideration that there is a two-faceted role of PB. For the PB that produces anti-AQP4 antibody in NMO, the signal of interleukin 6 (IL-6), a pro-inflammatory cytokine, is important for its survival and antibody production [20], then NMO treatment using anti-IL-6 receptor antibody underwent clinical trial showed the preferable effect [28]. On the other hand, some reports have highlighted regulatory roles of PB that produce the immunosuppressive cytokine interleukin 10 (IL-10) [29,30]. Matsumoto et al. found that PB produces IL-10 in the draining lymph nodes of mice that developed experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and both Blimp1 and IRF4 are important for differentiation of those PB [30]. When B cells were specifically knocked out from these transcription factors, EAE model worsened. Moreover, in an autoimmune disease model, regulatory role of PB that produces interleukin 35 (IL-35), a suppressive cytokine, is also reported [31]. Researches that focus on differences in gene programs in the background of differentiation of PB, especially when they produce pathogenic or regulatory autoantibody, are awaited.

**Conclusion**

While the involvement of pathogenic autoantibodies in neurolimmunological diseases and their clinical characteristics have been clarified, the upstream of their autoantibody production still needs further investigations. PB is an autoantibody-producing cell, which can be a biomarker for categorizing diseases with a similar immune background. Since it has become clear in recent research that those with various functions are also found in the PB, analyzing the details of PB may make it possible to diagnose and evaluate the disease state of autoantibody-related neuroimmunological diseases.

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