Antibodies as mediators of brain pathology

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Abstract

The brain is normally sequestered from antibody exposure by the blood brain barrier. However, antibodies can access the brain during fetal development before the barrier achieves full integrity, and in disease states when barrier integrity is compromised. Recent studies suggest that antibodies contribute to brain pathology associated with autoimmune diseases such as systemic lupus erythematosus and neuromyelitis optica, and can lead to transient or permanent behavioral or cognitive abnormalities. We review these findings here, and examine the circumstances associated with antibody entry into the brain, the routes of access and the mechanisms that then effect pathology. Understanding these processes and the nature and specificity of neuronal autoantibodies may reveal therapeutic strategies towards alleviating or preventing the neurological pathologies and behavioral abnormalities associated with autoimmune disease.

Introduction

There has been much recent study of genetic variants that contribute to abnormal cognition or behavior. While this has been a fruitful area of investigation, there is growing evidence that environmental triggers, perhaps in conjunction with genetic risk factors, are also important in both early and late onset disturbances in brain function ¹. In particular, perturbations in both the innate and the adaptive immune system can alter brain development in the fetus as well as brain function in the adult ^{2,3}. Two major classes of immune effector molecules – cytokines and antibodies - have been demonstrated to affect brain development and brain function ³⁻⁵. In particular, our appreciation of the spectrum of antibodies with this potential keeps growing. It is now appreciated that brain-reactive antibodies can arise as a result of autoimmune disease or as an untoward consequence of an anti-microbial response. Autoimmune diseases such as systemic lupus erythematosus (SLE) and neuromyelitis optica (NMO) are characterized by brain-reactive serology, and Sydenham's chorea, which develops after exposure to group A streptococcus, is characterized by so called signaling autoantibodies - anti-streptococcal antibodies that are cross-reactive with dopamine receptors ^{6,7}. Brain-reactive antibodies may also be a feature of paraneoplastic syndromes, arising through cross-reactivity with tumor antigens.

Here, we review the current understanding of the impact of antibodies on brain development and function. We examine the settings in which antibodies are able to access the 'immune privileged' environment of the central nervous system (CNS), and the routes used for this access. In the context of autoimmune disease, we discuss the known neuronal targets of antibodies, and the antibody-mediated effector mechanisms that mediate brain pathology. We center this discussion on two autoimmune diseases - SLE and NMO - presenting these as paradigms for the study of the potential contribution of antibodies to congenital and acquired brain disease. A deeper understanding of the nature and specificity of neuronal autoantibodies,

and the circumstances and ways in which these antibodies access the CNS should enable new therapeutic strategies towards alleviating or preventing the neurological pathologies and behavioral abnormalities associated with autoimmune disease.

The Blood Brain Barrier

The blood-brain barrier (BBB) is the major interface between molecules in the circulation and the brain. Its architecture has recently been described as a two-walled moat surrounding the brain ^{8,9} which separates blood from interstitial fluid. It is composed of endothelial cells tightly linked by specialized proteins that form the tight junction. Astrocytes lay down a basement membrane (glia limitans) in which pericytes reside. The endothelial barrier and the glia limitans help control the composition of the interstitial fluid in the brain and help shield the brain from the surrounding interstitial fluid. Barrier properties are most restrictive in the capillaries, and are less so in the venules. Perivascular macrophages sample the CSF in the space between the astrocytic and endothelial basement membrane, within postcapillary venules. They harbor phagocytic properties and might have important implications as antigen presenting cells.

The blood CSF barrier (BCSFB) separates blood from the CSF and is formed by epithelial cells of the choroid plexus, which possess unique apical tight junctions. In addition to serving a barrier function, the choroid plexus epithelial cells secrete CSF. This architecture permits a continuous interchange of CSF and interstitial fluid and has been recently named the glymphatic system ¹⁰. The finding of functional lymphatic vessels, which connect the CSF with the deep cervical lymph nodes, allow passage of immune cells and immune molecules into the CNS. ^{11,12}.

The BBB begins to be formed early in embryonic development, following neovascularization of neural tube at embryonic day 10.5 (E10.5) ¹³. There are many studies that have investigated the timing of BBB development during embryogenesis ¹⁴. It is clear that even during the very early

stages of brain development, there is a limitation on transit of molecules from the blood to brain parenchyma that is more restrictive than in other tissues. Our own studies, however, demonstrate that, in mouse, the BBB is not fully impenetrable to IgG until ~ E17.5 when there is almost total exclusion of IgG in the brain ¹⁵. Presumably a similar window of vulnerability to IgG exists for the developing human brain ¹⁶. Thus, there is a short window during the second trimester of gestation when maternal IgG crosses the placenta to enter the fetal blood stream and may access brain parenchyma. After this brief developmental window, the barrier prohibits IgG access to the brain terminates. We have demonstrated that aspects of maternal physiology can impair the development of a competent BBB in the fetus. For example, female mice lacking gut microbiota harbor fetuses that never develop a proper BBB, and even as adults, the offspring have leaky barrier properties. Reconstitution of the adult offspring with a gut microbiome leads to the proper maturation of the BBB ¹⁵. The spectrum of maternal abnormalities that cause either a transient or permanent deficiency in BBB integrity is not known.

The BBB is a dynamic structure and contact with various molecules can alter barrier integrity. Mechanisms for altering the barrier function include altering the integrity of the tight junctions between the endothelial cells, or affecting the capacity of the endothelial cells to internalize molecules through receptor mediated internalization, for example, in the transport of cytokines or chemokines ¹⁷. Interestingly, many molecules that impair barrier integrity are soluble mediators released during inflammation. In sepsis, characterized by a dramatic activation of an innate immune response, it is well documented that there is a massive breakdown in barrier integrity ¹⁸. Because imaging studies of the BBB are still relatively insensitive to small changes in barrier function, we do not know if chronic inflammatory diseases characterized by a lower level of inflammatory mediators in the plasma might be associated with a low-level, but chronic, barrier breach that might have clinical consequences. It is well documented that barrier

modulators include bacterial and viral infection, systemic inflammation, trauma, brain ischemia, and stress, as well as specific agonists of endothelial receptors like nicotine, caffeine and cocaine ¹⁹. The exact mechanisms by which each of these conditions alters barrier integrity are not known, although many would appear to alter the integrity of the tight junctions. Two additional aspects of BBB function need to be mentioned. First, insults causing inflammation within the brain, with activation of inflammatory properties of microglial cells, can also impair barrier integrity. Second, the microvascular endothelial cells of the brain are specifically designed to shield the brain from IgG and have transport receptors for immunoglobulin, the neonatal FCR (FcRn), usually located at the luminal surface of the endothelial cell and responsible for transporting IgG into tissue, at the abluminal surface where it functions to transport IgG out of the perivascular space and interstitial fluid. Under healthy conditions, IgG is removed from the central nervous system (CNS) by reverse transcytosis across the BBB ²⁰, mediated by the FcRn ²¹. When BBB is compromised, net influx of IgG is increased such that FcRn are saturated, resulting in accumulation of IgG in the parenchyma (Figure 1).

There are also reports of impaired barrier integrity with aging, Alzheimer's disease and atherosclerosis ²². ApoE deficient mice, an important model of both atherosclerosis and Alzheimer's disease, exhibit a BBB that is leaky to small molecules and in some cases to IgG. ²³⁻²⁵. Given the growing list of conditions with altered BBB function, it is probable that we will continue to identify even more conditions associated with altered BBB properties. Whether antibody can enter the brain without traversing tight junctions is not established, although the mechanism of transcytosis has been exploited for therapeutic antibody delivery in model systems ²⁶.

In contrast, only a few mechanisms are known to maintain BBB integrity. Glucocorticoids appear to support barrier properties ²⁷, as do interferons ²⁸⁻³⁰. Type 1 interferon prevents barrier

disruption through multiple mechanisms. It reduces neutrophil invasion following inflammatory insults and diminishes their production of matrix metalloproteinase 9 (MMP9) ³¹. It also increases expression of ecto-5'-nucleotidase (CD73) on endothelial cells, thereby increasing the local concentration of adenosine, an anti-inflammatory molecule ³². Indeed, the therapeutic efficacy of β interferon in multiple sclerosis may, in part, reflect its effects on barrier integrity ³³.

Studies in rodent models have demonstrated that the BBB is not a homogeneous structure and that receptors for modulators of barrier integrity are present at different density on the lumen of endothelial cells in different regions of the brain. For example, we have demonstrated that systemic administration of bacterial lipopolysaccharide (LPS) will permit IgG to access primarily the hippocampus ^{34,35} while systemic administration of epinephrine will expose cells mainly in the amygdala to blood-borne substances ³⁶. It is possible that the regional distribution of TLR4 and TLR5 may mediate the specific hippocampus impairment by LPS ³⁷, while epinephrine might cause selective leak of the BBB by increasing differentially the cerebral blood flow in the amygdala ³⁸ and therefore induce changes in vascular permeability ³⁹. Insufficient information currently exists of the regional distribution of TNF receptors, complement component receptors, and more that modulate BBB integrity. The regional specificity of BBB compromise allows the same circulating antibody to have different effects on brain function depending on the region of exposure to antibody. In addition, certain areas of the brain, the circumventricular organs, lack an intact BBB, and thus might be important regions for antibody passage ⁴⁰.

Antibodies may also alter brain function if they are produced locally within the CNS or the meninges, by infiltrating B cells. In MS, where oligoclonal IgG bands in the CSF are a hallmark of the disease ⁴¹. B cells have been identified in tertiary lymphoid structures of the meninges and Virchow Robin spaces, where antigen specific B cells clonally expand ⁴². Understanding whether brain-reactive antibodies are produced by B cells sequestered in the brain or by

peripheral B cells has important therapeutic implications, however, most brain pathology caused by antibody appears to reflect transit of IgG into the brain rather than local production.

Antibodies in the adult brain

Over the last decade, several antibodies have been described which bind to different extracellular or intracellular central nervous system (CNS) antigens (Table 1; Key Table). For example, a substantial number of patients with neoplasms that trigger production of antibodies to oncofetal proteins and elicit neuro-muscular symptoms also suffer from cognitive impairment associated with antibodies that bind to neuronal antigens (for review see ³). In many of these patients, the development of antibodies is secondary to the presence of a primary tumor that expresses neuronal antigens. Antibodies that bind brain antigen are also present in patients with autoimmune disease, including celiac disease, type 1 diabetes and thyroiditis ⁴³⁻⁴⁵. Many of these appear to bind antigens that are not restricted to the brain or to bind antigens in other organs and cross-react with brain antigens. In many patients, there is no discernible brain pathology, but more and more there is awareness that these diseases may be associated with cognitive impairment. Whether, and to what degree, antibodies are responsible for the neurocognitive symptoms requires further study.

For the present discussion we focus on SLE and NMO, two autoimmune diseases that have been shown to be B cell-dependent ^{46,47}. Neuropsychiatric SLE (NPSLE) is characterized by autoantibodies directed to neuronal antigens in at least a substantial group of patients. These include anti-N-methyl-D-aspartate (NMDA) receptor (NMDAR) antibodies, which are a cross-reactive subset of anti-DNA antibodies, the hallmark autoantibody in SLE, and anti-ribosomal P protein (anti-P) antibodies. Both these antibody specificities can induce NPSLE in mice ^{35,36,48}. In contrast, as we will discuss further in later sections, in NMO, antibodies are directed to antigen on glial cells.

Autoantibodies in SLE and the induction of behavioral abnormalities

Anti-DNA/NMDAR antibodies

SLE patients are known to harbor autoantibodies of multiple specificities, such as anti-phospholipid, anti-Ro and anti DNA antibodies. Anti DNA antibodies are the most frequent autospecificity in SLE ⁴⁹. A subset of anti-dsDNA autoantibodies has been demonstrated to cross-react with NMDAR (anti-DNA/NMDAR antibody) ⁵⁰. The NMDAR is a glutamate receptor and ion channel protein found in nerve cells. Glutamate is a major excitatory neurotransmitter in brain involved in synaptic plasticity and memory function. The anti-DNA/NMDAR antibodies bind to GluN2A and GluN2B subunits of the NMDAR, which contain the consensus peptide sequence D/E-W-D/E-Y-S/G (DWEYS) ⁵¹. Both murine and human anti-DNA/NMDAR antibodies, the latter cloned from B cells of SLE patients, cause dose-dependent excitotoxic neuronal death when injected directly into the hippocampus ^{50,52} of a non-autoimmune, unmanipulated mouse. The anti-DNA/NMDAR antibodies preferentially bind the active receptor, presumably prolonging the time when the channel is open to calcium influx ⁵². Overstimulation of NMDAR leads to high influx of calcium into the cell, causing neuronal dysfunction and cell death.

BALB/c mice immunized with the DWEYS peptide in a multimeric configuration (MAP-DWEYS) produce anti-DNA/NMDAR antibodies ⁵⁰. Despite the presence of anti-DNA/NMDAR antibodies in the serum of these mice, there is no detectable binding to neurons or damage in neurons of the brain that could be detected in histologic analyses. In this model, a breach in the integrity of the BBB is required for antibody to access brain tissue and affect neuronal function and viability. LPS injection as a surrogate for infection leads to the extravasation of the antibody into the hippocampus and, consequently, focal neuronal loss and cognitive deficits ³⁵. In contrast, epinephrine injection in mice as a surrogate for stress allows antibody to penetrate the

amygdala, and neuronal loss in that region leads to emotional disturbances that can be assessed in studies of the response to aversive stimuli ³⁶. These data show that regional disruption of the BBB is dependent on the agent used to modify the BBB and, thus, that the same antibody can cause variable behavioral changes.

Two weeks post-BBB breach, FDG-PET imaging of MAP-DWEYS immunized mice revealed a lowered glucose uptake, a surrogate for metabolic activity, in the hippocampus or amygdala respectively, as compared to the baseline signal, whereas control mice, immunized with MAP only and given LPS, showed heightened glucose uptake⁵³. At 4 weeks post-BBB breach, both groups exhibited increasing glucose uptake in the affected region. In control mice, glucose uptake was directly related to neuron number, while in mice with anti-DNA/NMDAR antibodies, there was an inverse relationship between neuron number and metabolism. The increase in glucose uptake that occurs between 2 and 4 week post-BBB breach in the mice harboring the lupus-like antibodies is thought to reflect enhanced synaptic and/or glial activation. Importantly, hippocampal increase glucose uptake in the patients is commonly seen in SLE ⁵⁴ subjected to FDG-PET imaging, suggesting that they might also have sustained glial activation.

Most recently, we have demonstrated that there are late sequelae of anti-DNA/NMDAR antibody penetration of the hippocampus ⁵⁵. After antibody can no longer be detected in brain tissue, neurons in the hippocampus undergo a loss of dendritic processes. Hippocampal neurons possess place cell activity and are responsible for generating spatial maps of the environment. Each neuron fires when the mouse is in a particular location (place field). Two-4 weeks after antibody penetrates the hippocampus and cues the acute death of some neurons, surviving neurons display decreased dendritic complexity and enlarged place field size along with diminished spatial memory. We speculate that the alteration in dendritic processes may also reflect glial activation ⁵⁵.

Many reports have indicated an association of autoantibodies with mechanism of psychiatric manifestations in human SLE patients. In some studies, anti-DNA/NMDAR antibodies are elevated in serum from patients with active non focal NPSLE ^{56,57}, such as confusional state or psychosis. However, in other studies the presence of these antibodies in serum was not associated with active diffuse NPSLE ⁵⁸⁻⁶³. These results suggest that when anti-DNA/NMDAR antibodies are present only in serum, they do not mediate neuropsychiatric manifestations. However, the level of anti-DNA/NMDAR antibodies in the cerebrospinal fluid (CSF) was significantly elevated in patients with active non focal NPSLE, as compared to NPSLE patients with focal NPSLE or to patients with non-inflammatory disease in the CNS ⁶³. These results suggest that the direct access of pathogenic autoantibodies to the CNS is a key factor for the development of neuropsychiatric manifestations in SLE. In SLE, antibodies appear to access the brain secondary to BBB compromise, which can be assessed by the presence of albumin in the CSF ⁶⁴. There is little evidence for infiltration of antigen-specific B cells into brain parenchyma.

Interestingly, anti-DNA/NMDAR antibodies have been reported to react with human umbilical vein endothelial cells, resulting in the production of inflammatory cytokines ⁶⁵. Thus, anti-DNA/NMDAR antibodies might directly damage brain vessels, and contribute to the breakdown of the BBB ⁶⁶.

Anti-ribosomal P antibodies

Anti-P antibodies were first identified by the binding to C-terminal regions of three ribosomal P proteins (P1,P2 and P0) ⁶⁷. The binding site on the ribosomal proteins has been characterized and termed P epitope. Recently these antibodies were shown to cross-react with a high molecular weight integral plasma membrane protein of unknown function, called neuronal

surface P antigen (NSPA), which possesses a P epitope at the cell surface ⁶⁶ and is expressed exclusively in neurons. Passive transfer experiments in mice showed that anti-P antibodies isolated from SLE patients induced smell alterations ⁶⁸, depression-like manifestations ⁶⁹ and memory impairment ⁴⁸. Immunohistochemistry analyses of NSPA expression patterns in the brain revealed that NSPA is present in multiple areas, including the cortex, amygdala, and hippocampus and is expressed exclusively in neurons.⁷⁰ When added to primary neuron cultures, anti-P antibody induces calcium influx followed by apoptosis, and similarly, direct injection of anti-P antibodies into the brain of normal mice results in neuronal death through an excitatory glutamatergic pathway ⁶⁶.

Anti-DNA/NMDAR antibodies and anti-P antibodies both recognize neuronal cell surface antigens that enhance glutamate induced neuronal activation, but they act through different mechanisms. At low concentrations, anti-DNA/NMDAR antibody increases excitatory postsynaptic potentials (EPSPs) by directly interacting with an epitope of NMDAR that becomes accessible in the open channel configuration once the Mg^{2+} plug has been removed. At higher concentrations, an excess of calcium flux into the cell leads to neuronal death ⁵². In contrast, anti-P antibody enhances glutamatergic transmission in the presence of Mg^{2+} through a mechanism mediated by NSPA and involving α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and NMDAR activation ⁷⁰. Like anti-DNA/NMDAR antibodies, intravenously injected anti-P antibodies impaired memory function in mice after LPS administration ⁷⁰. Anti-P antibodies can potentiate the pathogenic effects of anti-DNA/NMDAR, promoting the active configuration of the NMDAR, and the concomitant presence of both antibodies might constitute another factor in brain dysfunction variability.

Anti-P antibodies are major autoantibodies that are present in serum of 40-50% of active non-focal NPSLE patients ^{71,72}. In vitro, anti-P antibodies from patients with diffuse NPSLE can

directly react with the surface of human peripheral blood monocytes, which results in TNF- α production ⁷³. Therefore, anti-P antibodies could be associated with autoantibody-mediating inflammation in the CNS through the mechanism of BBB rupture by monocyte-derived TNF- α with or without infiltration of activated macrophage into CNS ⁷³. Accordingly, anti-P antibodies might also activate the immune system, access the CNS, and directly damage neurons in SLE patients ^{48,73}.

Taken together, these findings suggest that two phases are required for the development of neuro- psychiatric or neurocognitive manifestations in human lupus patients. The first phase involves the direct access of pathogenic autoantibodies to the CNS, mainly as a consequence of influx from the systemic circulation to the CNS following the breakdown of BBB. The second phase involves either direct or microglia-mediated damage of neurons by autoantibodies in the CNS. It should also be noted that whereas anti-DNA/NMDAR and anti-ribosomal P antibody specificities have been studied extensively, other brain-reactive antibodies have been reported in SLE ⁷⁴. Furthermore, there is a growing awareness that antibodies implicated in other autoimmune diseases may cross-react with brain targets. For example, anti-gliadin antibodies present in patients with celiac disease have been shown to cross-react with antigens expressed by purkinje cells in the cerebellum ⁷⁵, and to bind to neuronal synapsin I ⁴⁴. Thus, the paradigm described above for anti DNA/NMDAR and anti-P antibodies may hold true for antibody-associated neuropsychiatric and neurocognitive impairment in many autoimmune diseases.

Neuromyelitis Optica (NMO) – cognitive impairments resulting from antibodies targeting non-neuronal cells

Brain-reactive antibodies that bind to non-neuronal cells can also alter neuronal viability or function through mechanisms that are not yet well described. Antibodies that result in glial cell death can impair neuronal survival. Patients afflicted with the autoimmune disease NMO have

antibodies directed to astrocytes in their serum ⁷⁶. Lennon and colleagues ⁷⁷ discovered that those astrocyte antibodies in patients with NMO bind the antigen aquaporin-4 (AQP4), a water channel protein that is expressed on astrocytic foot processes surrounding blood vessels and that controls the flow of water in and out of the brain. A pathogenic role of AQP4 antibodies was first suggested due to pathological findings of a massive deposition of antibody and complement components surrounding blood vessels⁷⁸. The mechanism through which the loss of astrocytes leads to demyelination and neuronal loss is not fully understood; however, there is emerging evidence that astrocytes can regulate myelination ⁷⁹, maybe by modulating the function of oligodendrocytes. Alternatively, ex vivo data suggest that patients' antibodies which target astrocytes can damage oligodendrocytes secondarily possibly due to a bystander effect caused by glutamate-mediated excitotoxicity ⁸⁰.

Serum AQP4 antibodies can help to diagnose the disease at an early stage, and thereby can enable early treatment. This is particularly important as NMO can closely resemble other diseases such as Multiple sclerosis (MS), which often require different treatment approaches ⁸¹. Confirming the importance of autoantibodies in NMO pathology, antibody or B cell depleting treatment are beneficial for NMO patients, whereas interferon β , commonly used for treating MS, can exacerbate the disease ⁸¹. Clinical assays for detection of AQP4-IgG have high sensitivity and specificity, and thus antibody serology is included as a diagnostic criterion for NMO ^{82,83}. Approximately 70-90% of NMO patients are seropositive for AQP4-IgG ^{84,85}, and this range is thought to depend on differences in the detection assays. AQP4-IgG recognizes conformational epitopes and therefore, antibodies can be detected with highest sensitivity and specificity using cell-based assays using a transfected cell line expressing AQP4 on their surface ⁸⁶⁻⁸⁸. NMO was initially described as a disease which selectively targets astrocytes in the optic nerve and spinal cord and spares the brain ⁸⁹; however, brain MRI abnormalities are very common in patients with AQP4-IgG ⁹⁰ and brain lesions are found in areas of high AQP4 expression, most

prominently in the grey matter. Indeed, NMO patients exhibit cognitive impairment and cortical neuronal loss ⁹¹.

Intravenous injection of AQP4-IgG to rodents, without disrupting the BBB, does not result in a neuropsychiatric phenotype ⁴⁷. This finding is consistent with the observation that AQP4 IgG can be detected in the serum of NMO patients many years prior to the full onset of disease ⁹². However, the inflammatory setting that leads to the breach of the BBB in NMO is not well understood. It is also not clear how the antibodies exert their pathologic effect. Some studies have suggested that AQP4 antibodies damage the brain in concert with pathogenic T cells; others studies suggest that complement or antibody dependent cellular cytotoxicity are the critical mechanisms ⁹³⁻⁹⁵.

In vivo models of NMO either require induction of experimental autoimmune encephalitis (EAE) to activate BBB disrupting pathogenic T cells reactive to antigens expressed in the brain ^{47,96}, or direct intracerebral injection of antibodies together with human complement to mediate CNS injury ⁹⁷. It is possible that there is not a single mechanism of BBB insult in NMO. Different mechanisms of BBB compromise might be specific to distinct regions of brain or spinal cord and thus might correlate with the heterogeneous pattern of lesions that have been described in NMO ⁹⁸. Moreover, some mechanisms might permit only IgG to enter the brain as we have seen in our LPS-induced model of BBB compromise while others such as EAE induction permit cellular entry and in situ production of IgG ^{99,100}. Evidence, albeit rare, supporting the latter model comes from the identification of transient oligoclonal IgG bands in CSF of some patients⁹³, suggesting that the antibodies in the CSF may be made from B cells residing in brain parenchyma where they might locally produce antibodies, as in MS.

In vivo models differ in terms of where the breach of the BBB occurs, and the time it takes for this to happen. For example, the choice of the antigen specific T cells for in vivo NMO/EAE

models results in different regional brain lesions and varying disease severity ¹⁰¹. Whether specific brain-reactive T cells, perhaps AQP4 reactive T cells ^{94,102,103}, are necessary to initiate or propagate the disease remains unresolved ^{94,102}. However, T cells are present in brain lesions of NMO patients ¹⁰¹. A role for cytokines has been demonstrated by injecting cytokines directly into the striatum of AQP4 seropositive rats ¹⁰⁴. Interleukin-1 beta (IL1b) was the only cytokine capable of inducing NMO lesions outside the needle track. Interestingly, IL-1b is also found in active human NMO lesions ¹⁰⁴. These rodent NMO models partially mimic human disease, specifically the initial stages of the disease ¹⁰⁵.

Overall, these studies demonstrate that antibodies capable of causing brain pathology can remain in the circulation for long period of time. So long as they do not penetrate the BBB, there are no CNS sequelae. Mechanisms of BBB breach in the adult organism can permit only soluble molecules to penetrate brain parenchyma or cells as well as soluble molecules. The mechanism, and the location, of BBB breach dependent on the particular stressor. Once antibodies penetrate the CNS, they may alter brain function through damaging cells, either neurons or glial cells, or through altering cell function. Finally there are both immediate and late sequelae of antibody exposure. The availability of appropriate animal models is critical as studies of patients may be confounded by cohorts that express similar antibodies but differ with respect to mechanism of barrier breach and state of disease.

Maternal antibodies and the fetal brain

During pregnancy, the placenta provides a barrier between maternal and fetal circulation while simultaneously allowing the transfer of a wide range of substances to the fetus, including nutrients. Among these factors are maternal IgG molecules, which start to cross the placenta around the second trimester and provide the developing fetus with humoral immunity, since the fetal immune system is immature at this stage (for a review see ¹⁰⁶). Nevertheless, there are

circumstances in which placental transmission of antibodies is deleterious to the infant. For example, neonatal lupus is a rare disorder in which maternal anti Ro/SSA and/or La/SSB antibodies can lead to transient symptoms including skin rash, liver problems, or low blood cell counts, which typically disappear after six months with no lasting effects ¹⁰⁷. These antibodies can lead also to congenital heart block with a permanent structural heart defect ¹⁰⁷. Transient and/or permanent tissue injury secondary to maternal antibodies has also been reported in myasthenia gravis ¹⁰⁸, anti-phospholipid syndrome and in other autoimmune diseases ¹⁰⁹.

Once maternal IgG crosses the placenta, the antibodies can penetrate all tissues including the developing brain. Importantly, although the mature BBB prevents IgG entry into the adult CNS, in the fetus IgG can penetrate the brain ¹⁵, potentially through an immature BBB, through the blood-CSF barrier, or perhaps through the vascular fenestration in the circumventricular organ. In mice, we have recently confirmed that the brain is accessible to maternal antibodies from embryonic day (E) 13.5 to E16.5 ¹⁵. Maternal autoantibodies in patients afflicted with SLE and NMO have been shown to affect fetal neurodevelopment, as we discuss below. In addition, we discuss the evidence for a role for maternal antibodies in autism spectrum disorder (ASD).

Maternal autoantibodies in SLE

Several studies have suggested that children, particularly the sons of mothers with SLE, but not fathers, are at increased risk for learning disabilities ¹¹⁰⁻¹¹³. Other epidemiological studies suggested that children born to mothers with SLE have, in general, a higher risk to neurodevelopmental disorders including ASD ¹¹⁴. Studies in mice have further sustained a potential link between exposure to SLE and neurodevelopmental disorders. We showed cortical abnormalities in fetuses exposed in utero to monoclonal anti DNA/NMDAR IgG, cloned from an SLE patient ¹¹⁵. Moreover, we studied a mouse model in which dams harbor anti-DNA NMDAR specific autoantibodies throughout gestation to demonstrate that fetal brain exposed to high

titers of anti DNA/NMDAR antibodies showed increased neuronal cell death, abnormal neuronal proliferation and thinning of the cortical plate. Live offspring in these studies showed delayed reflexes as neonates and cognitive impairments in cortical dependent tasks as adults ¹¹⁵. Interestingly, these developmental effects were evident primarily in male offspring as exposure in utero to high titers of anti DNA/NMDAR antibodies led to a preferential loss of female fetuses, apparently due to the apoptosis effects mediated by these antibodies on neurons expressing GluN2A in the midbrain. GluN2A is expressed earlier in female compared to male midbrain and mice lacking GluN2A exhibited no fetal loss in this model ¹¹⁶. Interestingly, it has been recently confirmed that mothers with SLE are more likely to have a male child ¹¹⁷. This finding further supports the hypothesis that maternal antibodies may have gender-specific effects on brain development.

It should be mentioned that additional maternal anti NMDAR antibodies against GluN1 have been described as leading to cortical dysplasia in the offspring in NMDA encephalitis ¹¹⁸.

Autism Spectrum Disorder (ASD)

The possibility that maternal autoantibodies can lead to a subset of ASD has been entertained for more than a decade. Several investigators have identified the presence of antibodies that bind to human fetal brain tissue in a subset of women who have children with ASD ¹¹⁹⁻¹²².

In the largest study that has been performed so far including 2789 mothers of an ASD child, we have shown that brain-reactive antibodies are present at higher frequency in mothers of an ASD child. 10.5% of mothers of an ASD child harbor antibodies with strong reactivity to mouse brain antigens compared to 2.6% of unselected age-matched women ¹¹⁹. When researchers gave these antibodies to pregnant mice and monkeys, they caused abnormal behavior in their offspring including increased motor activity, stereotypic behavior anxiety-like behavior and

alterations in sociability ¹²³⁻¹²⁸. A recent study showed prenatal exposure to anti-brain antibodies also alters neuronal proliferation, and brain size ¹²⁹.

Unlike the offspring of SLE mothers who have a pre-identified medical condition, in ASD the mothers themselves may have no obvious autoantibody-related pathology; however, brain-reactive antibodies appear to be more common in women with autoimmune disease. Atladóttir and colleagues have shown that mother with rheumatoid arthritis or celiac disease have almost double increased risk to have a child with ASD ¹³⁰. Our own study associated the presence of maternal anti-brain antibodies with increased autoimmune disease in mothers of an ASD ¹¹⁹.

The specificity of the brain-reactive antibodies associated with ASD is an area of active investigation¹²⁰. Several neuronal antigens expressed in the developing brain have already been suggested as possible targets ¹²⁰. Given the heterogeneity of the disease, it is highly likely that different antibody specificities will be associated with distinct symptoms of ASD. For example, Braunschweig and colleagues have shown that maternal serum reactive with a particular molecular weight band on Western blots of brain correlated with lower expressive language scores in the affected offspring while reactivity to a different molecular weight band size correlated with increased irritability ¹³¹.

Maternal antibodies in NMO

In NMO, a disease that affects primarily women of childbearing age, the possible effect of AQP4 antibodies on fetal CNS development has only been recently addressed. During pregnancy, women with AQP4 IgG have a more severe disease, develop preeclampsia more often and have an increased risk of miscarriages ^{132,133}. Case reports have suggested placental inflammation or birth defects in offspring that were exposed in utero to AQP4 IgG ^{132,134}. Our own work, with pregnant mice injected intravenously with a single dose of AQP4 antibody

isolated from a patient with active NMO, suggests a titer dependent effect of maternal AQP4 IgG on offspring (unpublished Mader & Brimberg et al.). At high titers maternal AQP4 IgG impairs survival of offspring and at lower titers AQP4 IgG can affect the offspring's brain.

Concluding Remarks

Classical roles for antibodies are to assist in cellular debris removal, to eradicate harmful pathogens and to neutralize toxins. For the latter two functions especially, a diverse repertoire is required. Although it is clear that antibodies can be responsible for significant brain pathology, we have raised the hypothesis before ³ that negative selection for brain antigens might be deleterious leaving the host with too narrow an antibody varsity and a diminished ability to protect effectively against infections and toxins. Indeed, it is notable that diversification of the B cell repertoire occurs after the BBB is formed, limiting the exposure of developing B cells to brain antigens. This hypothesis predicts that all individuals may develop brain-reactive antibodies as part of a protective immune response, and that the BBB serves two important functions. First, it sequesters brain antigens and limits their contribution to negative selection of B cells. Second, it protects the brain whenever anti-brain antibodies are made. We, therefore, speculate that anti-brain antibodies may be commonly present in healthy individuals. The purging of the immature B cell repertoire of autoreactive cells occurs in the bone marrow and spleen after the BBB has been formed. All mechanisms of B cell tolerance that we understand to date require that antigen to be present and trigger B cell receptor (BCR)-mediated tolerance mechanisms. It is possible therefore, that many brain-reactive B cells mature to immunocompetence. Usually, these B cells may be of no consequence. Even if they are activated, possible through cross-reactivity with microbial antigens, as has been demonstrated for B cells cross-reactive with streptococcus and the dopamine receptor ^{6,7}, the antibodies are not able to bind to the brain antigen so long as the BBB retains it impermeability to antigen. If,

however, the integrity of the BBB is compromised, these antibodies can contribute to alterations in brain function.

The increasing recognition of the potential damaging impact of brain-reactive antibodies on the developing fetal brain and the adult brain requires that we consider therapeutic strategies to protect the brain. Antibody-depletion strategies such as plasmapheresis are impractical as long-term therapy, B cell depletion or blockade of B cell activation is potentially immunosuppressive. A novel, potentially non-immunosuppressive, strategy is to protect or enhance BBB integrity. Most brain-reactive antibodies that cause pathology in adults appear to enter the brain primarily as soluble molecules; thus, more studies of the mechanisms that regulate the BBB are needed as well as more modalities for non-invasive or minimally-invasive assessments of BBB function. Protecting the BBB has the advantage that it does not require knowledge of all pathogenic antigenic specificities.

An alternative strategy might be the use of decoy antigen to neutralize serum antibodies such that they no longer bind to target tissue ¹³⁵. The requirements for this strategy approach are a lack of toxicity or immunogenicity. This approach has been pioneered in ex vivo studies, but has not yet been demonstrated to show efficacy in vivo ¹³⁵. Its advantage is that it is less likely to perturb other aspects of physiology, is non-immunosuppressive, and does not require the knowledge for each disease on how antibodies penetrate the BBB. It is our hypothesis that decoys antigens might be most useful in conditions of transient production of brain-reactive antibodies, such as during infection.

One important question that has not yet been rigorously addressed is whether all brains are equally vulnerable to antibody-mediated pathology or what makes some brains more vulnerable, either during fetal development or in adulthood. We would speculate that vulnerability may

involve genetic factors as well as antibody exposure or the coexistence of other modulators or stressors of brain cell function or viability, respectively.

Finally, it is interesting to ask whether we might use antibodies to improve brain function. It may be that antibodies could enhance neurotransmitter signaling in conditions characterized by a deficiency in these processes. The same antibodies that have pathogenic effects in individuals with normal brain function might improve brain function in some individuals with perhaps diminished NMDAR or dopamine receptor function. This intriguing possibility awaits further investigation.

Table 1: Antibodies mediate brain dysfunction

Etiology	Disease	Defined Antigen
Malignancy	Hodgkin's lymphoma	AMPA, mGluR1, mGluR2, and mGluR5, Tr (PCA1) ¹³⁶
	SCLC	AMPA, mGluR1, mGluR2, and mGluR5 Amphiphysin/synaptic vesicle GABA (B1), CV2/CRMP5, Gephyrin, Hu (ANNA1), Ma, Recoverin, Ri (ANNA2) ¹³⁷⁻¹⁴¹
	non-SCLC	GAD (GAD65, GAD67) ¹⁴²
	Ovary dermoid	NMDAR [NR2A/NR2B] ¹⁴³
	Thymoma	AMPA, mGluR1, mGluR2, and mGluR5 GAD (GAD65, GAD67) Caspr2, CV2/CRMP5, Gephyrin ^{141,144}
	Breast	AMPA, mGluR1, mGluR2, and mGluR5 Amphiphysin/synaptic Vesicle, Hu (ANNA1), Ma, Ri (ANNA2), Yo (PCA1), Zic4 (PCA1) ^{141,145}
	Thymic carcinoma	AMPA, mGluR1, mGluR2, and mGluR5, Ri (ANNA2) ^{141,146}
	Endometrial carcinoma (rare)	Caspr2 ¹⁴⁴
	Teratoma	NMDAR [NR2A/NR2B] ¹⁴¹
	Renal carcinoma	CV2/CRMP5
	pancreatic carcinoma	GAD (GAD65, GAD67) Gephyrin ¹⁴²
	Prostate	Hu (ANNA1) ¹⁴⁷
	Ovary	Hu (ANNA1), Ri (ANNA2), Yo (PCA1), Zic4 (PCA1) ¹⁴⁸
	Bladder	Hu (ANNA1) ¹⁴⁹
	Germ cell (testis)	Ma ¹⁵⁰
Infection	Post-Streptococcal Movement Disorders Sydenham's chorea& PANDAS	lysoganglioside Dopamine D1 and D2 receptors ^{6,7,151}
Autoimmunity (Antibodies are known to cause disease)	Neuropsychiatric SLE	NR2A/NR2B ⁵⁰ Neuronal surface P antigen ⁷⁰
	Neuromyelitis Optica	AQP4 ^{76,77}
	ADEM	MOG ¹⁵²
	Limbic Encephalitis	AMPA (GluR1, GluR2) ¹⁴¹ NMDAR NR1/NR2B ^{153,154}
	Rasmussen Encephalitis	GluR3 ¹⁵⁵
	Stiff Person Syndrome	GAD, Gephyrin GABA(A), GABA (B1) ¹⁵⁶

Autoimmunity (Antibodies are NOT known to cause disease)		Anti-Amphiphysin ¹⁵⁷
	Celiac Disease	Synapsin 1 ⁴⁴
		Transglutaminase ¹⁵⁸
	Limbic Encephalitis	Lgi1/Caspr2 ^{144,159,160}
	Hashimoto's Encephalitis	aldehyde reductase, thyroglobulin ^{45,161}
	Autism	LDH, Cypsin, STIP-1, CRMP1, CRMP2, YB-1 ¹²⁰

Abbreviations: AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mGluR, metabotropic glutamate receptor; AQP4, astrocytic aquaporin-4 water channels; GABA, γ -aminobutyric acid; GAD, glutamic acid decarboxylase; Caspr2, contactin-associated protein-like 2; CRMP5, collapsin response mediator protein 5; CRMP1/CRMP2, collapsin response mediator protein 1/2; Lactate dehydrogenase; LDH, Lgi1, leucine-rich, glioma-inactivated 1; NMDAR, N-methyl-D-aspartate receptor; SCLC, small-cell lung cancer; NR1, NR2A, and NR2B, subunits of the NMDAR; MOG; myelin oligodendrocyte glycoprotein; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections; STIP-1, stress-induced phosphoprotein 1; YB-1, Y-Box-Binding protein 1.

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Figure Legend

Figure 1: Mechanism of action of autoantibodies penetrating brain tissue. Autoantibodies that bind to cell surface proteins on glial cells or neuronal cells can induce complement mediated cytotoxicity (CDC), which results in the formation of the membrane attack complex (MAC) (1). In contrast, antibody-dependent cell-mediated cytotoxicity (ADCC) is induced by Fc receptor bearing effector cells that can lyse antibody coated target cells (2) CDC and ADCC can result in cell death. Antibodies can interfere with the crosstalk of receptor signalling by acting as agonistic, antagonistic or co-agonistic antibodies (3). Antibodies can cause receptor internalization following binding of their cell surface antigen, which leads to altered antigen density on the cell surface (4). Some brain reactive antibodies will have no functional effect (5).

Outstanding Questions

- What mechanisms regulate the integrity of the blood-brain barrier?
- What approaches can be used to assess barrier integrity in a non-invasive manner?
- Are there other autoimmune diseases that exhibit antibodies to neuronal antigens? Are there neuronal pathologies or behavioral abnormalities associated with these diseases?
- Are there other infectious diseases where brain pathology and behavioral disorders can be associated with autoantibodies?
- What renders a brain susceptible to antibody-mediated injury? Under circumstances where barrier integrity is broken, some individuals do not exhibit brain pathology. What features of the tissue microenvironment and immune cell types determine a pathogenic response? What features of the autoantibodies determine a pathogenic response?
- Why do some antibodies appear to selectively target cells in the CNS when they are response to antigen that is expressed also in peripheral tissue?

Figure