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A model for lupus brain disease

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Summary

Systemic lupus erythematosus is an autoimmune disease characterized by antibodies that bind target autoantigens in multiple organs in the body. In peripheral organs, immune complexes engage the complement cascade, recruiting blood-borne inflammatory cells and initiating tissue inflammation. Immune complex-mediated activation of Fc receptors on infiltrating blood-borne cells and tissue resident cells amplifies an inflammatory cascade with resulting damage to tissue function, ultimately leading to tissue destruction. This pathophysiology appears to explain tissue injury throughout the body, except in the central nervous system. This review addresses a paradigm we have developed for autoantibody-mediated brain damage. This paradigm suggests that antibody-mediated brain disease does not depend on immune complex formation but rather on antibody-mediated alterations in neuronal activation and survival. Moreover, antibodies only access brain tissue when blood-brain barrier integrity is impaired, leading to a lack of concurrence of brain disease and tissue injury in other organs. We discuss the implications of this model for lupus and for identifying other antibodies that may contribute to brain disease.

Keywords

autoimmunity; systemic lupus erythematosus; antibodies

Systemic lupus erythematosus and anti-DNA antibodies

Systemic lupus erythematosus (SLE) is an autoimmune disease occurring primarily in women of child-bearing age. It is characterized by high serum titers of antibodies to nuclear antigens, the most common antigen being double-stranded (ds) DNA. Antibodies to nuclear antigen are present in essentially all patients with SLE but are present also in 5–10% percent of the healthy population. Anti-dsDNA antibodies are present in approximately 70% of SLE patients and, when present, are diagnostic of the disease. As anti-DNA antibodies are the most common autoantibody in lupus, much effort has been expended to understand their origins and potential pathogenicity.

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The molecular characterization of anti-DNA antibodies derived from mouse strains that spontaneously develop SLE, NZB/W, and the MRL^{lpr} has shown that there is extensive somatic mutation of the immunoglobulin variable region genes (1–5). This is a characteristic of B cells activated in a T-cell-dependent fashion to form germinal centers, where heavy chain class switching and somatic mutation occur at high frequency at immunoglobulin gene loci (6). More detailed analysis of the antibodies has shown that back mutation to the germline-encoded immunoglobulin most commonly generates an antibody that does not bind DNA (7–10). The implication is that an antigen that is not chromatin or DNA itself triggers the activation of the B-cell. Similar studies, performed over decades, analyzing anti-DNA antibodies from patients with SLE have yielded similar observations. Efforts to identify one or more triggering antigen have shown that anti-DNA antibodies often cross-react with bacterial antigen (11–14). Thus, anti-DNA antibodies may arise by a failure to regulate B cells that acquire autoreactivity by somatic mutation during the response to microbial antigen.

Renal disease is present in 50% of patients with SLE (15). The pathogenesis of the renal injury appears to begin with immune complex-mediated glomerulonephritis, although a substantial subset of patients with kidney disease also has interstitial inflammation (16). Numerous studies of immunoglobulin sequestered in kidneys of patients and lupus-prone mice have shown DNA reactivity to be present. Chromatin can bind to glomerular basement membrane providing antigen for the deposition of anti-DNA antibodies (17). However, anti-DNA antibodies often bind *ex vivo* to glomeruli that have been treated with DNase; thus it has become clear that at least some anti-DNA antibodies bind to non-DNA, non-chromatin antigen in the kidney (18–20). Many studies have identified renal antigens that can be bound by anti-DNA antibodies, including laminin, heparan, or α actinin (21, 22). These studies showed that anti-DNA antibodies not only cross-react with microbial antigen (23–26) but also with non-nucleic acid self-antigen (27–29). As it will become important below, these studies more generally demonstrate that antibodies often display physiologically significant cross-reactivities. Antibodies can be elicited by a particular antigen and bind one or more structurally related self-antigens.

Probing the specificity of R4A

Our interest in autoantigenic cross-reactivity of anti-DNA antibodies arose from a structure: function analysis of a mouse monoclonal, glomerulotropic anti-DNA antibody (30). Mutation of three amino acids in the heavy chain variable region of the R4A antibody generated an antibody with a 10-fold higher apparent affinity for DNA. Surprisingly, unlike R4A itself, this antibody no longer deposited in glomeruli when injected into severe combined immunodeficient mice (20). The implication of this observation was that the parental R4A antibody was not binding DNA in the kidney, but rather a cross-reactive antigen. We therefore probed a decapeptide library for R4A binding and identified a consensus sequence D/E W D/E Y S/G within several decapeptides bound by the antibody. An inhibition enzyme-linked immunosorbent assay (ELISA) confirmed that the peptide, composed of either L or D amino acids, was bound by the R4A antibody (31).

Analysis of serum from NZB/W mice showed that approximately 60% of the DNA reactivity was peptide inhibitable, demonstrating this cross-reactivity to be frequent among murine anti-DNA antibodies (32, 33). A study of SLE patients with anti-DNA antibodies and renal disease showed that essentially all had some proportion, from 15% to 90%, of DNA reactivity that was peptide inhibitable, demonstrating this cross-reactivity to be reasonably common in SLE patients also. Subsequent studies have shown that about 40% of SLE patients have anti-DWEYS peptide antibodies. These antibodies are rarely present in the absence of anti-DNA antibodies and are present in about half of SLE patients with anti-DNA antibodies (34–36). Thus, the antibody specificity appeared to be sufficiently prominent to warrant further study.

A search of protein databases revealed the consensus peptide to be present in the NR2A and NR2B subunits of mouse, rat, and human N-methyl-D-aspartate receptor (NMDAR). ELISAs performed on the extracellular domains of NR2A and NR2B showed that the R4A antibody did indeed bind these antigens in a dose-dependent fashion (37, 38) (Fig. 1).

Mechanisms of tissue damage in SLE

SLE can affect every organ in the body, but preferentially affects kidneys and skin. In both of these organs, it has been shown that immune complexes engage complement and activating Fc receptors and initiate an inflammatory cascade. In the skin, antibody and complement deposition can occur in both affected and apparently unaffected skin (39); thus complement binding alone does not trigger skin inflammation. In the kidney, antibody and complement deposition most commonly initiate an inflammatory response and subsequent tissue injury (40). Activation of the complement cascade and engagement of Fc receptors on Fc receptor-bearing cells amplifies tissue inflammation and ultimately leads to tissue damage. Immune complexes appear to be involved in all solid organ inflammation in SLE. Immune complexes containing nucleic acid, either DNA or RNA, are perhaps particularly pathogenic because they can be internalized by Fc receptor engagement and then activate Toll-like receptor 9 (TLR9) (DNA) or TLR7 (RNA) to activate inflammatory pathways in multiple cell types within tissues and to enhance antigen presentation by dendritic cells (41, 42), as well as contributing, through the same pathway, to systemic inflammation and accelerated atherosclerosis (43, 44).

Solid organ injury in SLE therefore is mediated by immune complexes. In contrast, some other manifestations of disease are mediated directly by antibody (Fig. 2). Hematologic cytopenias are mediated through cellular opsonization and destruction (45, 46). Thrombosis may be mediated by direct activation of the clotting cascade through antibody binding to β2-glycoprotein I (47, 48) or by direct endothelial cell activation mediated by anti-cardiolipin or anti-phospholipid antibodies (49, 50). The ensuing induction of inflammatory and vasoconstrictive mediators increases the possibility of thrombosis, which is particularly dangerous in the heart and the brain (51, 52).

SLE causes a number of neuropsychiatric syndromes (53). Some occur due to pathology in the blood vessels. Vascular disease, at times with inflammation of the perivascular space, is often associated with accelerated atherosclerosis or with thrombosis (54, 55). Antibodies

interacting with platelets or clotting factors contribute to local brain ischemia (56–58). Some \microinfarcts may be secondary to non-deformability of red blood cells coated with immune complexes (59). Other microinfarcts are secondary to thrombotic events that originate in the vasculature and are largely caused by anti-cardiolipin/anti-phospholipid antibodies, as described above. Peripheral neurologic syndromes may be caused, rarely, by vasculitis. More commonly they results from antibodies to glycolipids, myelin-associated proteins, or nicotinic ganglionic acetylcholine receptor leading to an inflammatory demyelinating polyneuropathy (60, 61).

Central nervous system manifestations of SLE have been recorded in extensive multi-center studies (62), but the mechanism for these are largely unexplained. There are multiple central nervous system manifestations of disease, but the three most common are cognitive impairment, mood disturbance, and headache (62). The cognitive impairment is most commonly memory impairment (63). Among the explanations for central nervous system disease that have been considered are medications. Patients with SLE are often treated with corticosteroids to suppress inflammation. Chronic steroid use carries a slight increased risk of stroke (64). There are compelling data supporting a role for steroids in neuropsychiatric symptoms. There are no convincing data to relate steroids to irreversible cognitive decline or mood disorder. The longitudinal studies to relate steroid use to persistent cognitive decline were based on mixed model analyses and a causal connection was not established (63, 65). The correlation between steroid use and emergent psychiatric symptoms, mania, depression, and delirium, and, less commonly, hallucinations, anxiety, and panic disorder is dose related, and symptoms abate with decreasing steroid dose (66). Thus, these symptoms are far more commonly ascribed to the disease itself.

The toxic side effects associated with steroid treatment, especially the increased risk of infection (67), have led to increased use of cytotoxic drugs that permit steroid taper (65). SLE patients often receive cytotoxic medications to eradicate autoreactive lymphocytes. These medications have been implicated in intellectual impairment when used to treat various malignancies. The doses of these medications used in SLE, however, are less than the doses used in patients with malignancy. Moreover, the clinical data suggest no relationship between brain disease in SLE, especially fixed cognitive impairment, and disease severity necessitating the use of cytotoxic medications.

SLE is characterized by high levels of a variety of cytokines in the circulation (68). There are data in both rodents and humans that high levels of cytokines can affect central nervous system function and lead to constitutional symptoms, such as fever, fatigue, and anorexia (69, 70). The clinical studies in SLE suggest that central nervous system disease may progress in the absence of these symptoms, and no studies have reproducibly associated high serum cytokine levels with central nervous system lupus (53).

While both cytokines and medications may yet be found to contribute to neuropsychiatric lupus other explanations must be sought. In SLE, it would be difficult to consider pathogenesis of tissue injury without considering a potential role of antibody. Thus, the observation that some anti-DNA antibodies might cross-react with the N-methyl-D-aspartate receptor (NMDAR) on neurons was of great interest. NMDARs are composed of 2NR1

subunits and two of any of 4 NR2 subunits (A–D) (71). The NMDAR pore permits influx of calcium on ligand binding initiating a downstream signaling cascade. Indeed, our interest in potential antibody reactivity with NMDARs was enhanced because NMDARs expressed on neurons in the hippocampus are known to be critical in learning and memory (72). NMDARs expressed on neurons in the amygdala, in contrast, are known to modulate behavior in fear conditioning paradigms in rodents. Thus, the potential binding of antibody to NMDARs might provide insight into two common damages of neuropsychiatric lupus, memory impairment and mood disturbance.

Anti-DNA, anti-peptide antibodies bind NMDARs on neurons

To determine whether anti-DNA, anti-peptide cross-reactive antibodies actually bind the NMDARs on the neuronal surface, we first used the R4A monoclonal antibody to immunoprecipitate intact NMDAR from differentiated PC 12 cells and from brain lysates (37, 38). We next asked whether the R4A antibody would bind to and affect NMDARs in live brain tissue. We injected R4A into the hippocampus of a living mouse, as NR2A and NR2B containing NMDARs are highly expressed on hippocampal neurons (38). The antibody-mediated neuronal cell death in the hippocampus suggesting that it was functioning to amplify receptor function; overstimulation of the NMDAR with its natural ligand causes a form of cell death termed excitotoxic or glutamatergic (73). Human antibodies derived from serum of SLE patients and isolated on a peptide affinity column also caused neuronal death when injected into mouse hippocampus (37). Prior treatment of the mouse with the NMDAR antagonist, MK801, blocked neuronal death, confirming that the antibodies were activating the NMDAR pathway (Fig. 3). Thus, these antibodies are clearly cross-reactive with the NMDAR and are hereafter called anti-NMDAR antibodies. Moreover, Fab^1_2 fragments of the antibody, lacking the Fc portion of the antibody molecule and therefore unable to activate Fc receptors or the complement cascade, also mediated neuronal death when injected into mouse hippocampus. Thus, the anti-DNA, anti-NMDAR antibodies did not cause tissue injury in the brain through immune complex formation as in other tissues, but rather through direct activation of a cell signaling pathway.

To confirm the effects of the antibody on hippocampal neurons and to understand their potential impact on memory function, we explored the effect of antibody in the *ex vivo* hippocampal slice. We asked whether the R4A antibody could enhance excitatory postsynaptic potentials (EPSPs). The antibody increased EPSPs in a dose-dependent fashion (74). Interestingly, and to our initial surprise, there was no effect of antibody in brain slices not treated with an NMDAR agonist. This demonstrated that the antibody did not function strictly as a pharmacologic agonist; rather it appeared to enhance the activity of a pharmacologic agonist. We reasoned that antibody might preferentially bind the active configuration of the receptor. If so, antibody would augment ligand activity, but would not lead to an activation of the resting NMDAR. We confirmed experimentally the preferential binding of R4A to the active NMDAR (74). Thus, the antibody modulates only the already activated NMDARs, and quiescent NMDARs are unaffected by the presence of the antibody.

Correlation of antibody titer with central nervous system manifestations of SLE

The next key question was whether we could relate antibody titer to brain dysfunction. Several studies have been performed by several groups of investigators examining serum titers of anti-NMDAR antibodies. Some studies suggested an association of high serum titers with memory impairment or depression, whereas other studies failed to confirm these associations (75–79). Studies were also performed looking for a correlation of antibody titers within the cerebrospinal fluid (CSF) with central nervous system symptoms (80, 81). These studies examined CSF of patients obtained during an acute episode marked by a change in alertness, cognitive function or mood in contrast to the studies of serum titers. Studies of serum titer, in contrast, assessed accumulated damage in clinically quiescent patients rather than focusing on patients experiencing an acute change in neurologic status. All studies of CSF titers of anti-NMDAR antibody have demonstrated a correlation with diffuse non-focal injury (81–86). Moreover, we were able to elute anti-NMDAR antibody from the brain of a patient with brain disease as a terminal event (37). These studies demonstrated a link between antibody in the brain and brain dysfunction and confirmed the importance of the blood-brain barrier in protecting the brain from routine exposure to circulating antibody.

A model for antibody penetration of brain

When BALB/c mice are immunized with an octameric form of a peptide (DWEYS) containing the pentapeptide NMDAR consensus sequence, they develop anti-peptide, anti-DNA cross-reactive antibodies. This response is T-cell dependent and requires the presence of the histocompatibility complex (MHC) class II E^d molecule (87). The titers of anti-DNA antibody in these mice are comparable with those in NZB/W lupus-prone mice. The brains of immunized BALB/c mice lack detectable pathology, and the mice performed comparably to controls in behavioral and cognitive tasks, suggesting in mice as in patients that antibody in the circulation is not noxious to the brain unless it penetrates the brain parenchyma through the blood-brain barrier.

There are many insults to the integrity of the blood-brain barrier (53, 88). Blood-brain barrier compromise has long been demonstrated in infection. More recent studies have shown that stress, epinephrine in particular, can abrogate barrier integrity (89–91). Hypertension, smoking, and various drugs have been reported also to compromise barrier integrity. We decided to explore the effect of infection and stress in the peptide-induced model of lupus serology. We chose to study infection as an insult to barrier integrity using lipopolysaccharide (LPS) administration to mimic the effects of infection. SLE patients experience an increased frequency of infection both because of the immunosuppression intrinsic to the disease and the immunosuppression caused by the medications used to treat the disease-specific autoreactivity and inflammation. When peptide-immunized mice were given LPS by intraperitoneal injection, there was evidence of antibody penetration into the brain with specific binding of antibody to hippocampal neurons. The mice exhibited a loss of hippocampal neurons (92). There was evidence of loss by 48 h, and no increased loss was observed thereafter. Most interestingly, there was no evidence of inflammation, no

infiltration of blood cells into the brain, and no evidence of prolonged activation of glial cells. When these mice were subjected to a battery of behavioral and cognitive assessments, they displayed persistent memory impairment (92). To confirm that antibodies from SLE serum had the same ability to cause impaired memory function, we administered serum containing anti-DNA, anti-NMDAR from SLE patients to naive mice, and subsequently administered LPS. The human antibodies bound preferentially to hippocampal neurons, and the mice displayed memory impairment. If the serum was first depleted of anti-NMDAR antibodies on a peptide affinity column and then given to mice, followed by LPS, there was no specific binding to hippocampal neurons and no memory impairment (92). These studies provided a model for the persistent memory impairment experienced by patients with SLE. Indeed, these studies provided the first mechanistic model for any aspect of central nervous system SLE that is not a consequence of thrombosis.

Many SLE patients experience a transient impairment in cognitive function. The model we described involved a fixed impairment with neuronal death. We therefore explored the antibody concentration required to alter excitatory postsynaptic potentials in the hippocampal slice preparation and the antibody concentration required to mediate cell death (74). Although as little as 10 μg/ml could enhance synaptic potentials, 100 μg/ml was needed for neuronal death. Thus, lower concentrations of antibody might cause acute reversible symptomatology, whereas higher concentrations might lead to irreversible damage through neuronal death. When we determined antibody concentrations within the CSF of patients with acute neuropsychiatric lupus, we found that there was a broad spectrum from 10 to 300 μg/ml of specific anti-NMDAR antibody (74, 81). This spectrum would allow for both reversible and irreversible injury.

In an effort to confirm the antibody-mediated neuronal loss that followed abrogation of blood-brain barrier integrity by exposure to LPS, we treated peptide-immunized mice with epinephrine. These mice displayed a normal hippocampus and normal memory function. In contrast to LPS treated peptide-immunized mice, they displayed neuronal loss in the amygdala and impairment in a fear conditioning paradigm (93). This study demonstrated that neurons in both the amygdala and the hippocampus were vulnerable to antibody-mediated cell death and that both cognitive and behavioral impairments might be secondary to antibody-induced damage. Moreover, they demonstrated for the first time that insults to the blood-brain barrier have regional effects with LPS, compromising barrier integrity in the hippocampus and epinephrine in the amygdala (Fig. 4).

This model fulfilled several conditions of the human disease. The model requires two hits; the first is neurotoxic antibodies and the second an insult to blood-brain barrier integrity. The compromise to barrier integrity is not mediated by the disease itself, but by a disease-independent insult, such as infection or stress. Neuropsychiatric SLE also appears to require two hits, as the presence of memory or mood impairment is unrelated to concomitant disease activity or indeed to cumulative disease activity. Furthermore, the neuronal loss appears to be non-inflammatory. Most SLE patients with documented cognitive or behavioral impairment have never had a clinical episode of brain inflammation. Thus, the model reflects key aspects of the clinical disease.

Examining brains of SLE patients for damage to the amygdala and hippocampus

We examined brain function in SLE patients by functional magnetic resonance imaging (MRI), using paradigms to activate the hippocampus or the amygdala (94). We studied patients with less than 2 years disease duration and patients with more than 10 years disease duration. We used the Sternberg test, which assesses memory for shapes and depends on hippocampal function (95). In brief, there is an initial phase when the individual sees 1, 2, or 3 objects on a screen. The screen goes dark during a consolidation phase. One figure, either previously displayed or a novel figure, is then shown to the subject in the recall phase of the experiment. Patients with disease duration of less than 2 years showed little brain activity during the consolidation phase and increased blood flow not only in the hippocampus but also in the cingulate gyrus, prefrontal, and somatosensory cortex during the recall phase, a pattern associated with neurodegenerative disease (96). In contrast, patients with long disease duration exhibited diffuse activity during the consolidation phase and no specific hippocampal activity during the recall phase, a pattern likely associated with more extensive brain injury.

The test of amygdala function employs a fearful faces paradigm. When a fearful face is shown to an individual, there will be increased blood flow in the amygdala. If an individual is shown the fearful face very briefly, a subliminal exposure, followed by a neutral face, there will be increased activity in the amygdala in some individuals who are sensitive to the fearful face but not in all individuals (97). Patients with <2 years of disease behaved like a normal cohort with most responding to the subliminal exposure to the fearful face. Fifty percent of patients with more than 10 years of disease did not respond to the subliminal exposure. Thus, the functional MRI assessments showed that SLE patients accumulate damage to the hippocampus and the amygdala over time. One team of investigators showed that damage to the amygdala assessed by a volumetric analysis correlated with the presence of elevated serum titers of anti-NMDAR antibodies (98). Our study also showed that abnormal brain function did not correlate with lupus damage assessed by the SLICC damage index which measures accumulated, fixed damage in organs throughout the body. The discrepancy between the SLICC damage index and measures of damage in the brain can be explained by the blood-brain barrier, which protects the brain from antibody-mediated disease. Thus, the brain is protected while antibodies are destroying other tissues. Damage accrual in most organs therefore proceeds at a different rate from damage accrual in the brain; some non-SLE dependent insults to the blood-brain barrier must occur to initiate damage or at least antibody-mediated damage in the brain.

Recent studies of the CSF of SLE patients have shown increased interleukin-6 (IL-6) during active diffuse disease of the central nervous system (99–101). Another recent study has shown that the antibodies present in the CSF can bind apoptotic debris, and the resulting immune complexes can activate TLRs in dendritic cells (102). Thus, it is possible that anti-DNA antibodies, including those cross-reactive with NMDAR, activate myeloid or glial cells in the brain and induce local cytokine production, especially IL-6.

Antibody-mediated damage to the fetal brain

It has been reported in several studies, each relatively small, that the children of mothers with lupus display an increased incidence of learning disabilities (103–106). As this increased incidence is apparently not present in the children of men with lupus, we reasoned it reflected some aspect of the *in utero* environment.

Maternal antibody can cross the placenta after the first trimester of pregnancy. It is not known exactly when the blood-brain barrier forms, but it appears to be somewhat porous to immunoglobulin until almost to the end of pregnancy. Thus, throughout the second and much of the third trimester of pregnancy, maternal antibody can penetrate brain tissue. Our studies show that 50–70 times more immunoglobulin per gram of tissue is present in fetal than maternal brain. It is important to note, however, that even the porous blood-brain barrier restricts antibody access to brain, and more immunoglobulin is present in other fetal organs than in the brain.

On the basis of the information we had obtained in studies of antibody-mediated brain disease in adult mice, we asked whether maternal anti-NMDAR antibody might alter fetal brain development (107). BALB/c female mice were immunized with octameric peptide or irrelevant antigen. When antibody levels were high, the females were mated to male mice. Thus, we could examine mice that developed *in utero* in the presence or absence of anti-NMDAR antibodies. Those mice exposed *in utero* to lupus-like antibody displayed abnormal brain histology with a thin cortical plate, increased apoptosis, and increased numbers of mitotic cells during fetal development. Moreover, the mitotic cells were found not in the usual location, at the subventricular zone, but throughout the developing cortex. These mice displayed a delayed acquisition of neonatal reflexes. Once they became adults, they performed normally on a standard neurological battery (108–111) and a number of standardized cognitive and behavioral tasks (37, 74, 92, 93, 107). They did, however, exhibit significant impairment on three tasks that depend on cortical function. The first task was a novel object recognition that depends on the function of the rhinal and perirhinal cortex (112). The second task was a spatial recognition task that depends on the parietal cortex (113). The third task required intact fear conditioning, but focused on the extinction of the conditioned response that depends on the infra-limbic areas of the prefrontal cortex (114). The neuropathological characterization showed abnormal cortical thickness and organization present in the adult brain of mice exposed *in utero* to anti-NMDAR antibodies. These impairments cannot be termed learning disorders, as there is no murine correlate of a learning disability, but they are restricted performance deficits that reflect murine cortical dysfunction and in that way they are comparable to learning disorders. It will be important to study prospectively whether female SLE patients with anti-NMDAR antibodies are more likely to have a child with a learning disorder.

A peptide therapeutic

Current therapies for SLE involve immunosuppression. Indeed, approximately one-third of SLE patients die of infection, often secondary to immunosuppressive therapy. We reasoned that a decoy antigen would prevent antibody-mediated tissue damage and would not be

immunosuppressive (80). As the anti-NMDAR antibody binds to the D-peptide as well as the L-peptide, we could use the D-peptide as a decoy antigen as it has a longer half-life than the L-peptide. D-DWEYS is not vulnerable to serum proteases and has a half-life of 6–8 h. We therefore tested whether it could protect kidneys from binding by the monoclonal R4A antibody *in vivo*. The D-peptide, but not the L-peptide which is immediately degraded *in vivo*, prevents tissue binding by R4A. The peptide was also able to protect neurons from antibody-mediated damage when given systemically to peptide-immunized mice, prior to epinephrine administration (115). We have demonstrated that the peptide can cross the blood-brain barrier, but it may also serve as a decoy antigen to protect neurons by binding antibody in the circulation, prior to penetration of brain tissue. While other investigators have considered the use of an NMDAR antagonist as a therapeutic strategy in neuropsychiatric lupus (116), the incapacitation of the NMDAR chronically over years will clearly have a significant impact on brain function. Thus, the strategy of a decoy antigen seems preferable. It is also of importance that the D-peptide is not immunogenic in mice, perhaps because a 5 amino acid peptide is too small to fit snugly in the peptide binding groove of a major MHC class II molecule. Even mice given prolonged treatment with peptide delivered by intraperitoneal injection, followed by a rest and then re-exposed to peptide, a regimen designed to induce an immune response, failed to generate detectable antibody titers to peptide.

A peptide mimetope as a therapeutic agent

While the therapeutic efficacy of D-DWEYS in protecting kidney and brain from antibody-mediated injury confirms the contribution of antibody to tissue injury and provides proof of principle for the therapeutic use of a decoy antigen, the D-DWEYS peptide, like most peptides, cannot be given orally. While individuals do self-inject daily or even more frequently in the case of some therapeutic agents, such as insulin, it may be difficult for patients to self-inject peptide 2 or 3 times daily. We therefore synthesized a small molecule mimetope of the DWEYS peptide, FISLE 412 (117). This molecule is orally absorbed and, like the D-peptide, will inhibit a significant percent of DNA reactivity in multiple lupus sera. It can prevent both R4A, the mouse monoclonal anti-DNA, anti-NMDAR antibody and G11, a human monoclonal antibody with the same cross-reactivity and cloned from a peripheral blood B-cell of a lupus patient, from binding glomeruli *ex vivo* or from causing neuronal death *in vivo*. FISLE 412 is an attractive therapeutic not only because it can be taken orally but also because it binds even more avidly to R4A and G11 than DWEYS, suggesting that it may have a competitive advantage over tissue antigen despite being monomeric.

It should now be possible to perform clinical trials to protect the brain from antibody-mediated damage and maintain cognitive function and mood stability. The remaining obstacle is to identify a metric for neuroprotection. Neuropsychiatric testing does not exhibit fixed and reproducible changes in lupus patients over a 6-month period of time, a reasonable time frame for a clinical trial. Moreover, fatigue, motivational status, steroid use may all influence test results. Similarly, functional MRI cannot be used to assess neuroprotection for all the same reasons. It is critical to identify an objective parameter of brain function that changes in many patients over a period of 6 months and is not affected by current steroid use or by aspects of performance that may change day to day.

Implications for brain disease: antigen targets

These studies have implications for brain disease. They demonstrate that autoantibodies that bind to a target in the brain and a cross-reactive target in another organ may mediate damage in each organ through different effector mechanisms. Anti-DNA, anti-NMDAR antibodies activate inflammatory cascades in the kidney; in the brain, they function as modulators of NMDAR activation. There is no evidence that they induce inflammation in the brain.

These studies also remind us that antibodies can bind a cellular receptor or other protein in one configuration and not another. The anti-NMDAR antibodies we study, preferentially bind the active configuration of the NMDAR. This means that *in vivo*, the antibodies react only with activated neurons. *In vitro*, antibody binding may be absent in some assays; for example, lupus-like anti-NMDAR antibodies will not bind to quiescent cells. Many brain-reactive autoantibodies bind neurotransmitter receptors or channel proteins. Whether these antibodies recognize epitopes that are unchanged by neuronal activation state is not currently known.

Implications for brain disease: the critical role of the blood-brain barrier

These studies emphasize the importance of the blood-brain barrier in limiting or permitting antibody access to brain tissue. An intact blood-brain barrier renders the antibodies harmless, as little antibody penetrates the central nervous system on a routine basis. It is important to remember, however, that if the antibody binds a target that is also present on peripheral nerves, the brain may be spared while the antibodies destroy or impair function of peripheral targets.

These studies have serendipitously shown that the compromise of barrier integrity is regional. An understanding of insults to barrier function is still in its infancy. Nevertheless, these studies provide precedent for broad, even ubiquitous expression of target neuronal antigen, and yet regional specificity of antibody-mediated disease. This is an important caveat in studies of central nervous system autoimmunity. While the antibody may bind throughout the brain, toxicity *in vivo* may be local and depend on the nature of the compromise of barrier integrity.

The importance of the blood-brain barrier in defending against autoantibody-mediated brain damage highlights the transient vulnerability of the fetal brain. The fetal brain is exposed during the second and third trimester of gestation to maternal antibody. Thus, the mother may show no untoward effect of harboring brain-reactive antibody in her circulation as she may have an intact blood-brain barrier, but her fetus may experience transient or permanent alterations in brain function. Importantly, the function of surface molecules on neurons many change over the lifespan. NMDARs in the developing brain have been shown to regulate neuronal migration; this is not a function that persists into adulthood. The implications of this observation are that the cellular alterations mediated by antibody in fetal brain may differ from those mediated by the same antibody in adult brain. How commonly a disparity in molecular function exists between fetal and adult brain is not known.

The blood-brain barrier not only limits access of molecules in the circulation to the brain, it also limits access of brain antigens to the systemic immune system. The B-cell repertoire is incredibly diverse, designed to protect the organism from a vast world of microbial antigens. The diversity of the repertoire is limited to a B-cell subset termed follicular B cells. B1 cells and marginal zone B cells have a relatively limited repertoire of antigen receptors. Moreover, they undergo limited mutation of their immunoglobulin genes, following antigen exposure. Follicular B cells, in contrast, have a much more diverse repertoire of antigen receptors and undergo a germinal center reaction on antigen activation that involves extensive mutation of their immunoglobulin genes, further diversifying the repertoire. As B cells develop, much autoreactivity is generated. Some studies show as many as 70% of immature B cells are autoreactive (118). Exposure to antigen at an immature stage tolerizes the B cells and effectively eliminates the autospecificity from the repertoire of mature, immunocompetent B cells. Obviously, tolerance is most effective to antigens that are present in the periphery at sufficiently high concentration to extinguish low as well as high affinity autoreactivity. The generation of follicular B cells begins after the blood-brain barrier is formed. Thus, there may be many brain antigens that are not present outside the brain at high enough concentration to tolerize B cells. Brain-reactive B cells may be present in the immunocompetent naive B-cell repertoire. We speculate that during the routine exposures to microbial challenge, B cells that recognize microbial antigen and cross-react with brain antigen are generated. In contrast, B cells that recognize microbial antigen and cross-react, for example, with liver or kidney antigen will have been tolerized. Thus, brain-reactive antibodies may routinely arise during the protective response to injection. Identifying these antibodies and determining whether they are potentially pathogenic requires further investigation.

There are a growing number of anti-brain antibodies in SLE and a growing number of diseases in which anti-neuronal antibodies contribute to symptoms. These include antibodies that engage the peripheral nervous system, the spinal cord, and the central nervous system (119). In most, it is not yet understood why the antibodies arise. In others, such as celiac disease, it appears the antibodies are triggered by a cross-reactive antigen (transglutaminase). In rheumatic fever, the cross-reactive antigen is clearly bacterial with antibodies to bacterial N acetyl glucosamine cross-reacting with the dopamine receptor and triggering a movement disorder (120).

We do not know how many microbial antigens elicit anti-brain cross-reactivity, or how often such antibodies can traverse the blood-brain barrier. We would suggest, however, that many acquired changes in cognition behavior or motor function may reflect antibody-mediated damage. In parallel, many congenital, non-genetic alterations in fetal brain development may be antibody-mediated (121). The role of antibodies in human pathobiology may be far greater than currently appreciated. The model of preventing antibody-mediated brain injury through the use of a decoy antigen suggests that as brain-reactive antibodies are identified, a therapeutic strategy is readily available.

Summary

Antibodies can mediate both cognitive and behavioral alterations in adults by binding to neuronal antigens. In SLE, some anti-DNA antibodies cross-react with the NMDAR to mediate insults to brain function that can be either transient or permanent. However, in the adult brain, anti-brain antibodies alone do not cause disease. There must also be an insult to blood-brain barrier integrity to provide the antibody access to brain tissue. Insults to barrier integrity display regional specificity, thereby accounting for the specificity of histologic damage and the nature of the cognitive or behavioral impairment. Antibodies can likewise alter fetal brain development. Maternal antibody has access to fetal brain tissue during the second and third trimesters of gestation.

The spectrum of antibody-mediated brain disease is probably greater than we currently appreciate. While identifying brain-reactive antibodies and relating their presence to clinical symptomatology is complex, the road from identification to therapy is relatively straightforward, making the effort immensely worthwhile.

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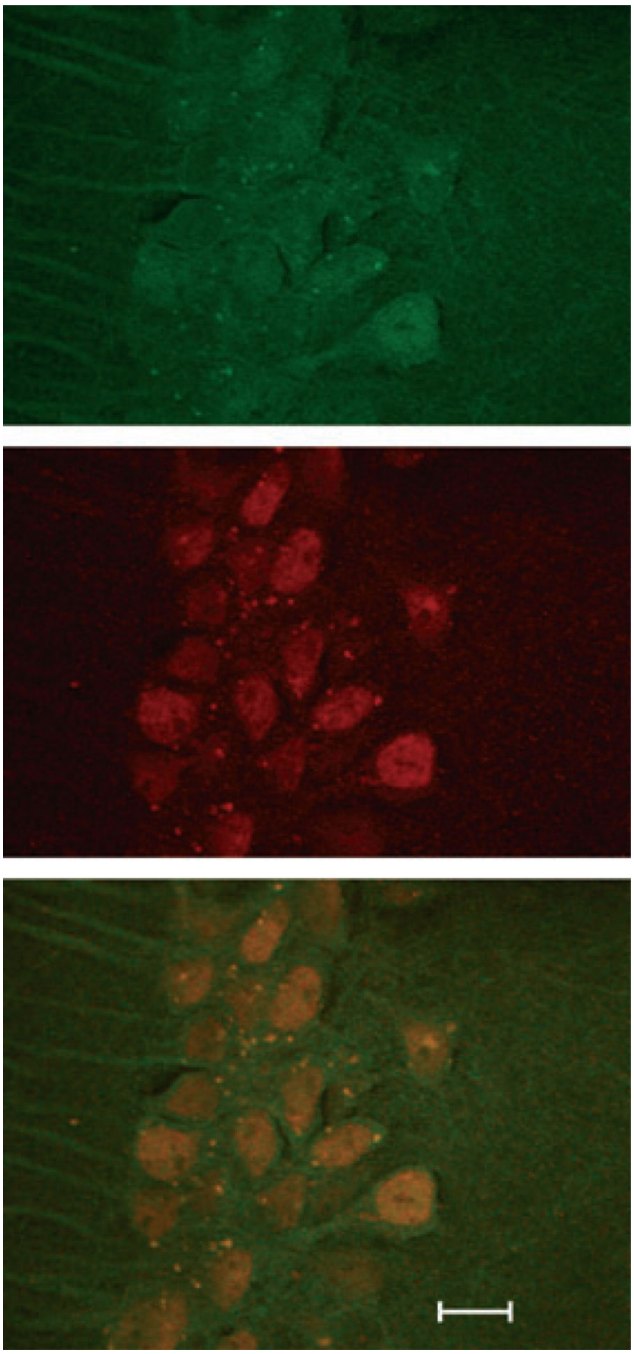

Fig. 1. R4A co-localizes in CA1 pyramidal neurons and their dendrites with anti-N-methyl-D-aspartate antibody

Demonstrated in the merged figure on the bottom. R4A was visualized (in the top panel) with Alex 488 fluor (Invitrogen, Grand Island, NY, USA), and antibody to NR2A and 2B (Millipore, Billerica, MA, USA) was visualized with an Alexa 594 fluor (in the middle panel). Scale bar is 10 μm.

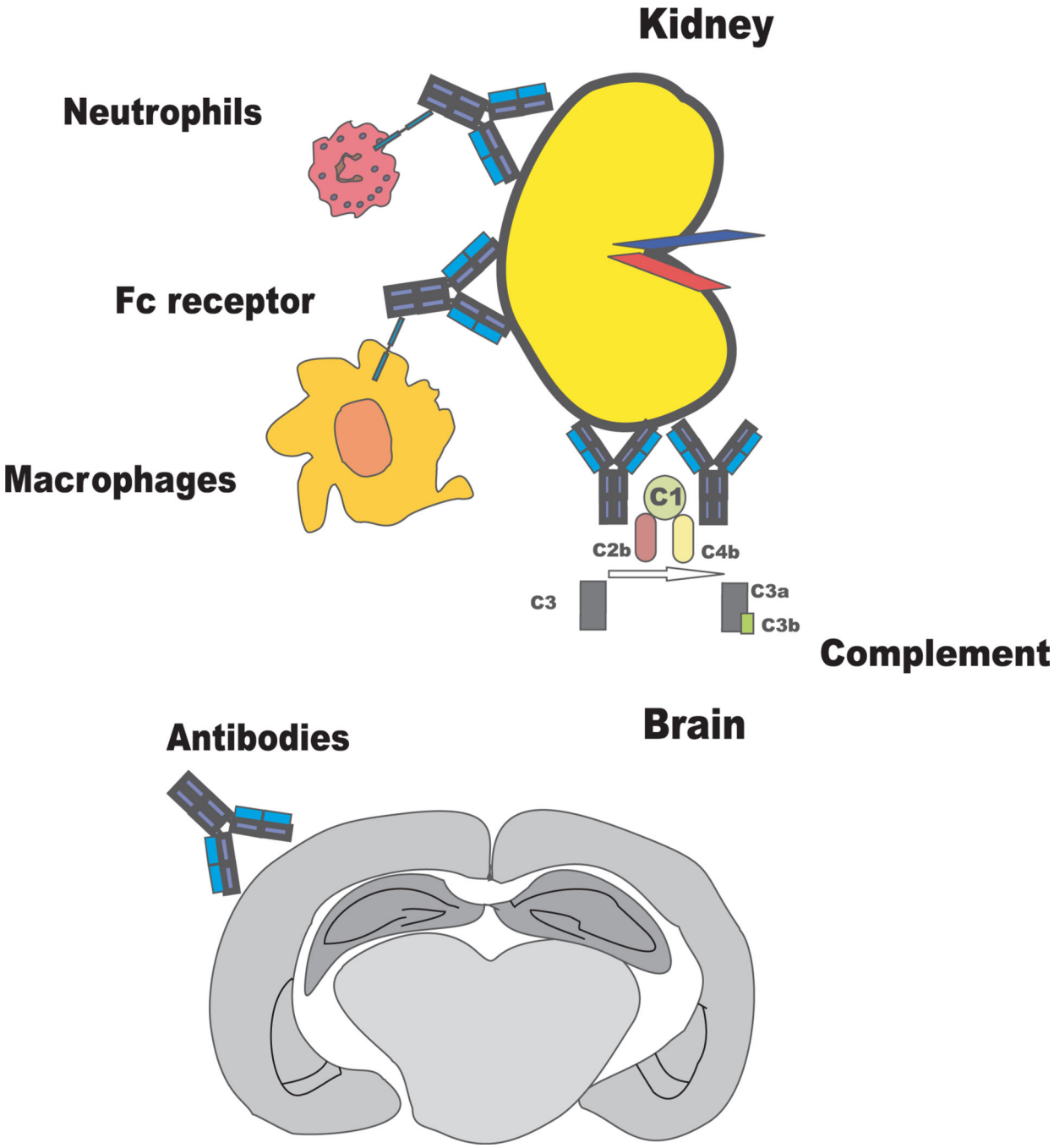

Fig. 2. Kidney tissue injury involves antibody engagement of complement or Fc receptor-bearing cells; brain injury is mediated directly by antibody binding

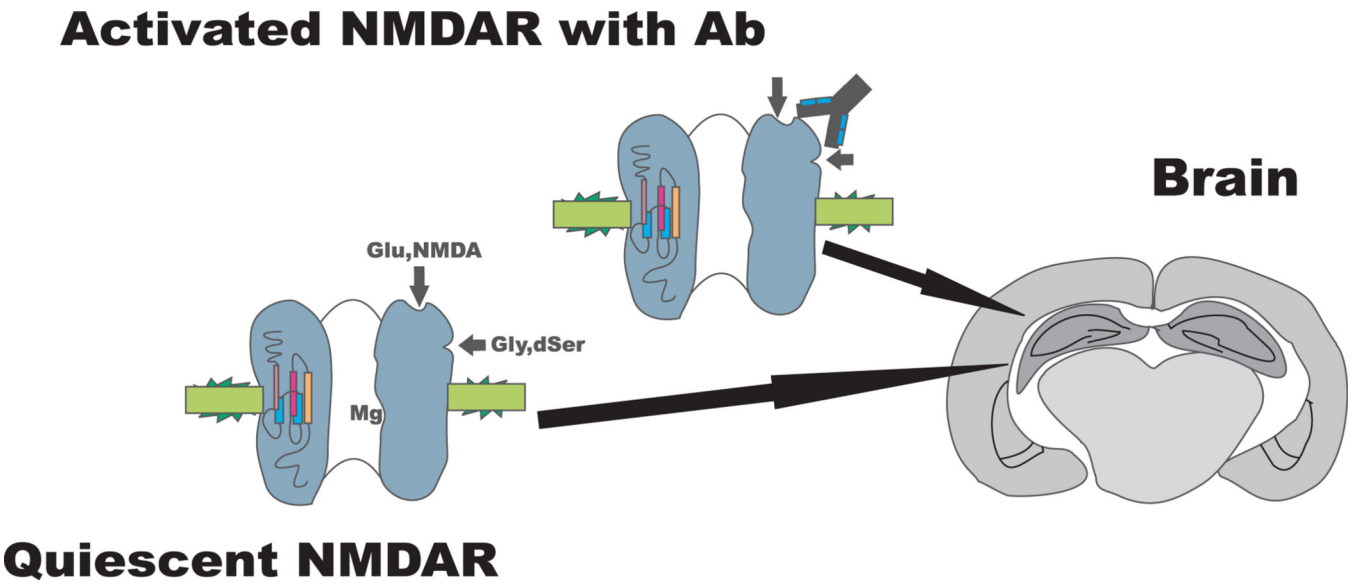

Fig. 3. Systemic lupus erythematosus anti-N-methyl-D-aspartate receptor (NMDAR) antibodies bind activated NMDA receptor

Magnesium (Mg) occupies the pore of the quiescent NMDA receptor.

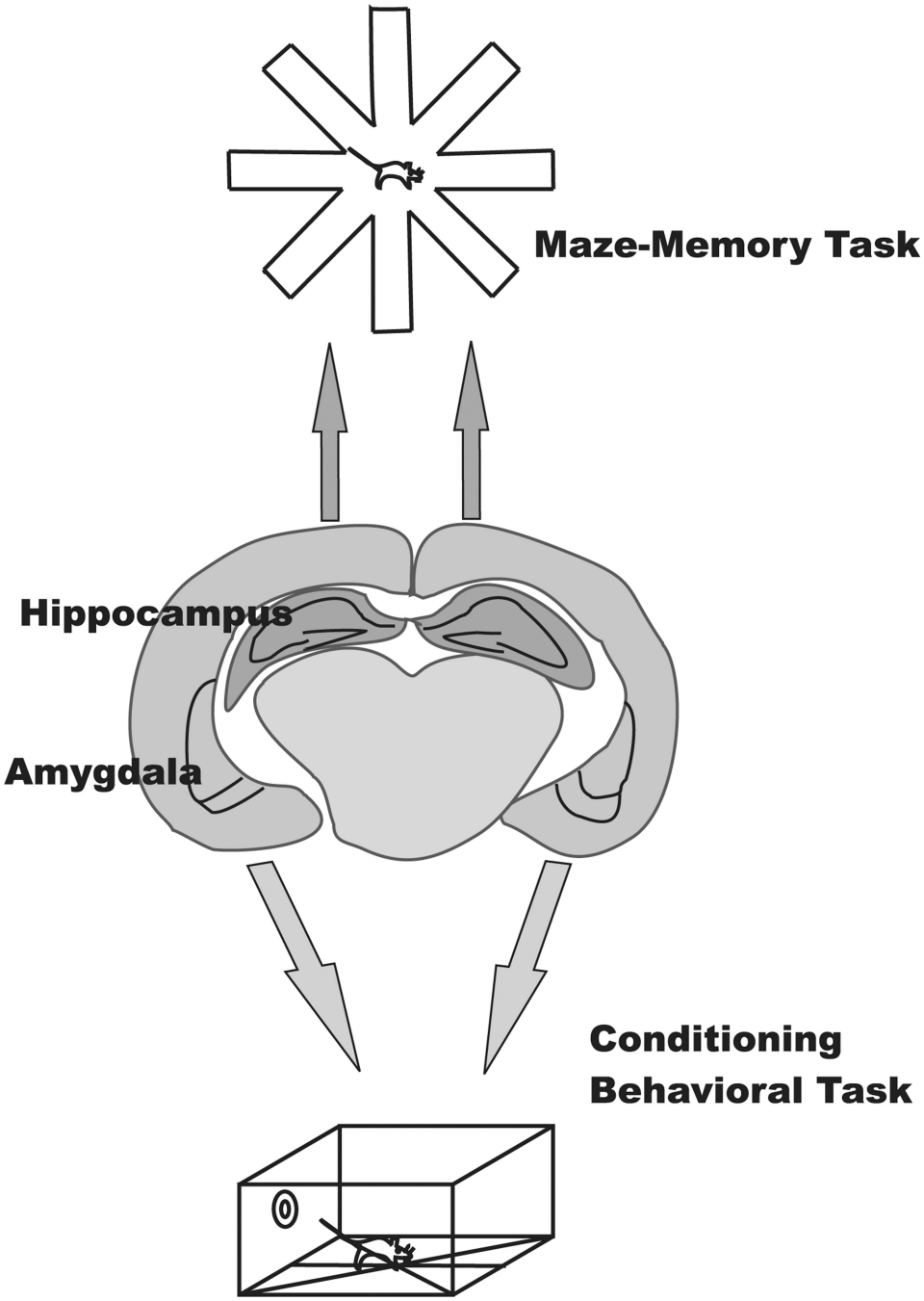

Fig. 4. Performance impairments depend on the brain regions exposed to antibody

Hippocampal exposure leads to isolated memory performance impairments on maze tasks. Amygdala exposure leads to isolated behavioral impairments on conditioning tasks.