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Antibodies and the Brain: Lessons from Lupus

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Anti-DNA Abs in systemic lupus erythematosus

My laboratory has been studying lupus for ~25 y. I was first introduced to autoimmunity as a medical student. Kurt Bloch gave five lectures on all of immunology, and they were mesmerizing. I became committed to lupus research when I learned that Flannery O'Connor, one of my favorite authors, died of the disease.

Lupus is a disease characterized by autoantibodies, and autoantibodies initiate the inflammatory cascades in this disease. In the 1980s, we knew that essentially all patients with lupus exhibited antinuclear Abs. We also knew that many patients had anti-DNA Abs, and when these were present, they were essentially diagnostic of the disease (1). Moreover, titers of anti-DNA Abs correlated with renal disease. In the 2000s, we learned that anti-DNA Abs not only contribute to renal disease, but they also induce systemic inflammation through a TLR pathway (2).

Over the years, our laboratory has contributed to the current understanding of the induction and pathogenicity of anti-DNA Abs in systemic lupus erythematosus (SLE). We showed that anti-DNA Abs can be elicited by microbial Ag (3–6). We showed that somatic mutation can lead to the acquisition of DNA binding. Because much autoreactivity is generated in the germinal center response, we asked what mechanisms might regulate post germinal center selection and showed that receptor editing in post germinal center B cells acts as a tolerance mechanism following somatic hypermutation (7). We also showed that renal pathogenicity of anti-DNA Abs may reflect cross-reactivity with renal Ags (8).

A peptide mimetope of DNA

In the course of searching for cross-reactive Ags, we screened the nephritogenic R4A Ab for binding to a peptide library (9). Our goal was to use peptides to identify proteins that might be involved in the induction of an anti-DNA response or that might be the tissue target of that response. From the sequences bound by the R4A Ab, we deduced D/E W D/E YS/G to be a peptide mimetope of DNA. Indeed, both the L peptide DWEYS and the D peptide can inhibit the binding of R4A to dsDNA (10).

When we next examined protein databases to find proteins containing the peptide consensus, we were excited to discover that both the NR2A and NR2B subunits of the N-methyl-D-aspartate receptor (NMDAR) contained the consensus sequence in an exposed region of the extracellular domain (9).

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The NMDAR is a membrane receptor present on neurons throughout the brain. It is composed of two NR1 subunits and two of any of four NR2 subunits (A–D). Its natural ligands are glutamate and glycine. On ligand binding, the pore of the receptor opens to calcium. Activation of the NMDAR has been shown to be critical in learning and memory, but prolonged stimulation can result in high calcium influx, causing an apoptotic death of the neuron. Our interest in this molecule stems from a growing awareness of the prevalence of neuropsychiatric manifestation of SLE. As patients live longer, we have come to appreciate some late sequelae of disease, including cognitive impairment and mood disorder, which are both frequent and debilitating (11,12).

Cross-reactivity of anti-DNA Abs with NMDAR

We wondered if anti-DNA, anti-peptide cross-reactive Abs might bind NMDAR and so potentially affect both cognitive functions and mood. We demonstrated that R4A could bind the extracellular domain of both NR2A and NR2B (13). We next demonstrated that R4A could induce neuronal cell death when injected into the hippocampus of a mouse. Moreover, F(ab')₂ fragments of R4A also mediated neuronal death, demonstrating that neither complement nor the activity of FcR-bearing cells is necessary for neurotoxicity (13). R4A also enhanced glutamate-induced excitatory postsynaptic potentials. Interestingly, R4A alone did not induce excitatory postsynaptic potentials in a mouse hippocampal brain slice; thus, we reasoned that R4A might preferentially bind the open configuration of the NMDAR, enhancing the effects of a true agonist but not functioning as one. We therefore treated ex vivo hippocampal slices with MK801, an NMDAR antagonist that fixes the receptor in the open configuration but blocks the pore. R4A bound better to MK801-exposed neurons than to neurons not exposed to MK801, confirming our hypothesis that R4A preferentially binds a receptor with an open pore. Thus, R4A functions as a receptor modulator.

Based on these studies showing that R4A cross-reacts with DNA and NMDAR, we asked whether NZB/W lupus-prone mice had Abs with this antigenic specificity. Approximately 60% of DNA binding by NZB/W serum was peptide inhibitable, demonstrating that the antigenic specificity of R4A is present in a high percentage of the polyclonal anti-DNA response in serum. We, therefore, asked whether this specificity was present in the serum of patients. Cross-reactive anti-DNA, anti-peptide Abs were present in 30–50% of lupuspatients (14–18). This has been observed in several geographically distinct cohorts.

To determine whether human lupus Abs cross-reactive with DNA and peptide also exhibited neurotoxic potential, we isolated Abs on a peptide affinity column and injected them into the hippocampus of a mouse (13). Again, we observed neuronal death. Mice pretreated systemically with MK801, an NMDAR antagonist, were protected from the effects of the Ab, thus confirming that the Ab was acting through NMDAR. CSF obtained from a patient with neuropsychiatric SLE (NPSLE) and containing anti-DNA, anti-peptide Abs also mediated neuronal death when injected into mouse hippocampus, whereas CSF from a patient with severe migraines did not. This observation showed that concentration of Ab bathing a patient's brain was sufficient to cause neuronal death. Indeed, a 1:100 dilution of CSF caused apoptosis of neurons, and only neurons, in a human fetal brain culture. Finally, Ig isolated postmortem from the brain of a lupus patient also caused neuronal death when injected into a mouse hippocampus (13). Thus, anti-DNA, anti-NMDAR cross-reactive Abs are present in serum, CSF, and brain tissue of lupus patients and have neurotoxic potential in mice.

Several studies have been performed asking whether serum titers of these Abs correlate with symptoms of NPSLE. The results have been contradictory; however, a small number of studies have addressed whether the presence of these Abs in the CSF correlates with symptoms of

NPSLE. All have observed a significant relationship between CSF anti-DNA, anti-peptide Ab and CNS symptoms of disease (14,19,20).

In vivo pathogenicity of anti-DNA, anti-NMDAR cross-reactive Abs

Immunization of BALB/c mice with a multimeric form of peptide (a decapeptide containing the consensus pentapeptide, mitogen-activated protein [MAP]-peptide) leads to high titers of anti-DNA Abs, titers similar to those present in NZB/W mice, and to glomerular Ig deposition. We therefore had a model to study brain pathology in a host with high serum titers of these Abs. Perhaps, not surprisingly, the brains of immunized mice were normal. We reasoned that this reflected the integrity of the blood–brain barrier that keeps substances in the circulation from penetrating brain tissue. There are a number of conditions, however, that are characterized by a decrease in barrier integrity (21,22). Two of these are infection and stress, both conditions familiar to patients with SLE.

To mimic the effects of infection on the blood–brain barrier, we injected mice systemically with bacterial LPS (23). Mice immunized with MAP-peptide and making anti-DNA, anti-NMDAR, cross-reactive Abs showed binding of IgG to hippocampal neurons, whereas mice immunized only with the polylysine backbone (MAP-core) showed IgG throughout in the brain but no specific neuronal binding. One week later, hippocampal neuron loss was visible in mice immunized with MAP-peptide, but not in mice immunized with MAP-core. Furthermore, mice with hippocampal neuron loss performed poorly on a T maze test of memory function. When human serum containing anti-DNA, anti-NMDAR Abs was given i.v. to mice followed by systemic administration of LPS, human IgG bound to hippocampal neurons. Mice given normal human serum or lupus serum depleted of anti-peptide Abs on a peptide-affinity column failed to display Ab bound to hippocampal neurons after LPS administration. Mice exposed to human neurotoxic Ab also displayed poor memory function in a paddling maze test. Thus, human lupus Abs can cause a cognitive impairment in mice if they gain access to hippocampal neurons (23).

We expected to replicate this finding using epinephrine to breach the integrity of the blood–brain barrier. We were initially quite surprised to find no damage to hippocampal neurons and no impairment in memory function. Instead, we observed neuronal death in the amygdala and impairment in the performance of a fear-conditioning paradigm (24). This resulted from Ab penetration in the amygdala rather than the hippocampus following epinephrine administration. These studies demonstrated that the regional exposure to Ab determines the behavioral deficit.

Our observations in mice seemed, in some respects, quite consistent with clinical features of NPSLE. Cognitive impairments and mood disturbances occur independent of disease flares and often with no evidence of CNS inflammation. Our data suggest that unfavorable serology and a breach in blood–brain barrier integrity are both needed for CNS damage, and the insult to barrier integrity need not be a lupus flare. Moreover, the neuronal loss we observed was noninflammatory. In patients, the CNS symptoms are often transient, yet our model exhibited irreversible neuronal death. To investigate this conundrum, we determined the concentration of R4A Ab needed to alter synaptic potentials and the concentration needed to mediate cell death. A 10-fold higher concentration was needed to induce neuronal death than to augment excitatory postsynaptic potentials. This provides a possible explanation for transient as well as fixed alterations in brain function.

Pathogenicity in fetal brain

Overall, these studies showed that: 1) Ab can cause both cognitive and behavioral disorders in SLE; 2) the same Ab can cause more than one manifestation of CNS lupus; and 3) the nature

of the manifestation depends on the brain region exposed to Ab, which in turn, depends on the insult to barrier integrity.

Anti-DNA, anti-NMDAR Ab can be toxic to adult brain if there is a breach to the blood–brain barrier. Fetal brain, however, is routinely exposed to maternal Ab, as IgG is transported across the placenta after the first trimester of pregnancy, and the blood–brain barrier does not develop until some time around birth. There are several studies of the children of mothers with SLE, showing that these children have an increased frequency of learning disabilities (25–27). We therefore examined whether the offspring of mothers harboring anti-NMDAR Ab displayed brain deficits (28). At day 15 of fetal development, fetuses from dams immunized with MAP-peptide having high titers of anti-NMDAR Ab displayed a thin cortical plate, whereas fetuses of dams immunized with MAP-core or scrambled peptide displayed a normal cortical plate. Moreover, mice exposed to high titers of anti-NMDAR Ab in utero, but not low or absent titers, exhibited a delay in acquisition of the negative geotaxis reflex. As adults, these offspring displayed impairment on only three of a battery of tests of cognitive, behavioral, and motor function, novel object recognition, topologic recognition, and fear extinction. Thus, maternal lupus Ab can cause fetal brain damage.

A paradigm for altered brain function

Based on these studies, we speculate that neurotoxic Abs may mediate brain dysfunction more often than previously suspected (29). The B cell repertoire does not diversify until after the blood–brain barrier is formed; therefore, there may be brain-specific Ags that do not mediate negative selection of developing B cells. If in response to microbial Ag, Ab cross-reactive with brain Ag is generated, it may cause brain damage if it penetrates brain parenchyma. Usually this will not happen, but on occasions, there may be an insult to blood–brain barrier integrity at the moment the titer of such cross-reactive Ab is high. Thus, there may be conditions of behavioral or cognitive impairment mediated by Ab even in nonautoimmune individuals. Similarly, some women may have high titers of brain-reactive Ab during pregnancy; these Abs may alter fetal brain development. We predict that future studies will identify many brain-reactive Abs and an increased array of insults that modulate blood–brain barrier integrity. In this way, we will identify syndromes that may be Ab-mediated, but arise even in nonautoimmune individuals.

Peptide as therapeutic

Because anti-DNA, anti-NMDAR Ab binds a D -pentapeptide, it is possible to consider that the peptide can function therapeutically. Indeed, D -peptide given systemically to MAP-peptide–immunized mice will protect against neuronal damage after epinephrine exposure (24). Similarly, D -peptide given to gestating mice with high titers of anti-DNA, anti-NMDAR Ab will protect fetal brain (28). The peptide can also prevent glomerular Ab deposition. D -peptide may therefore be a novel nonimmunosuppressive approach to lupus therapy (10). Because peptide is not well absorbed following oral administration, we recently generated a small molecule mimotope of the DWEYS peptide that also confers both renal and neuronal protection. This molecule is orally absorbable and therefore more practical as a therapeutic than peptide.

Overall, these studies demonstrate how understanding fine antigenic specificity of lupus Abs can lead to a new understanding of disease pathogenesis and to the development of novel therapeutics. As new syndromes of Ab-mediated cognitive or behavioral impairment are identified, the approach of using peptide or small molecules to block Ab from binding to its tissue target can be used therapeutically in at-risk individuals. It would be supremely gratifying if these studies indeed lead to new therapeutics and a new understanding of the delicate interaction between the immune system and the brain.

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Biography



Betty Diamond

Abbreviations used in this paper

MAP	mitogen-activated protein
NMDAR	N-methyl-D-aspartate receptor
NPSLE	neuropsychiatric systemic lupus erythematosus
SLE	systemic lupus erythematosus

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