

Glutamic acid decarboxylase autoimmunity in Batten disease and other disorders

David A. Pearce, PhD; Mark Atkinson, PhD; and Danilo A. Tagle, PhD

Abstract—Degenerative diseases of the CNS, such as stiff-person syndrome (SPS), progressive cerebellar ataxia, and Rasmussen encephalitis, have been characterized by the presence of autoantibodies. Recent findings in individuals with Batten disease and in animal models for the disorder indicate that this condition may be associated with autoantibodies against glutamic acid decarboxylase (GAD), an enzyme that converts the excitatory neurotransmitter glutamate to the inhibitory neurotransmitter γ -aminobutyric acid (GABA). Anti-GAD autoantibodies could result in excess excitatory neurotransmitters, leading to the seizures and other symptoms observed in patients with Batten disease. The pathogenic potential of GAD autoantibodies is examined in light of what is known for other autoimmune disorders, such as multiple sclerosis, SPS, Rasmussen encephalitis, and type 1 diabetes, and may have radical implications for diagnosis and management of Batten disease.

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Recent studies have identified an autoimmune component in individuals with juvenile neuronal ceroid lipofuscinosis, or Batten disease, thereby raising the question of whether anti-self-immunity could represent a contributory element to the development of this disease.^{1,2} Specifically, autoantibodies against the 65,000 D_r form of the neuroinhibitory enzyme glutamic acid decarboxylase (GAD) and other as yet unidentified neuronal proteins were observed in individuals with Batten disease and in mouse models for the disease. An example of the immunoreactivity of autoantibodies in serum drawn from an individual with Batten disease to neuronal cell populations is illustrated in the figure. Batten disease is usually characterized by the accumulation of autofluorescent storage material. A predominant component of the lysosomal storage material has been identified as mitochondrial ATP synthase subunit *c*.^{3–6} Autosomal recessive inheritance of deletions or point mutations in the *CLN3* gene or both underlie this disorder (re-

viewed by Weimer et al.⁷). However, the exact pathogenic mechanism(s) underlying the disease remains unknown. Western blot analyses show that sera from patients with Batten disease and *CLN3* knockout mice have autoantibodies against GAD and that these sera can inhibit enzymatic activity of GAD in vitro. Therefore, further evaluation of this autoimmune phenomenon may reveal a novel pathogenic mechanism underlying Batten disease. However, because many questions remain in our understanding of other diseases that also have potential autoimmune components, some the subject of decades of research, these same challenges will be faced in any investigation into a potential autoimmune pathogenic mechanism in Batten disease. What has been learned from investigations of those disorders is that when considering an autoimmune etiology for Batten disease, a seminal component will be to establish whether the autoimmune component is actually a functional facet of the disease process or whether it represents an epiphenomenon that lies downstream of the primary causation of disease (i.e., a separation of autoimmunity from autoimmune disease). This is

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From the Center for Aging and Developmental Biology and Department of Biochemistry and Biophysics (Dr. Pearce), University of Rochester School of Medicine and Dentistry, Rochester, NY; Department of Pathology (Dr. Atkinson), University of Florida, College of Medicine, Gainesville, FL; and Neurogenetics (Dr. Tagle), National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

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Address correspondence and reprint requests to Dr. David A. Pearce, Center for Aging and Developmental Biology, Department of Biochemistry and Biophysics, Box 645, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642; e-mail: david_pearce@urmc.rochester.edu

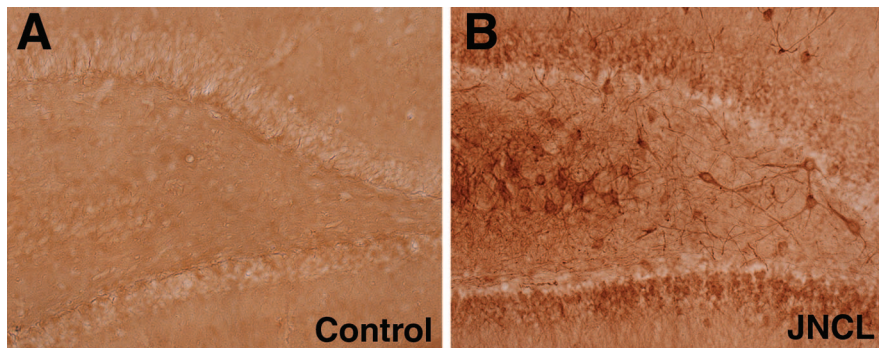


Figure. Serum from individuals with juvenile neuronal ceroid lipofuscinosis reveals immunoreactive neuronal populations when used as a primary anti-serum for immunohistochemistry (IHC) on normal rat brain. An example of the staining that is apparent in the hilus of rat brain when performing IHC using serum from an age-matched control individual with no history of a neurologic disorder (A) and an individual with Batten disease (B). Batten disease serum reveals immunoreactivity predomi-

nantly but not exclusively to GABAergic neuronal populations, whereas the control reveals no reactivity. Figure provided by Dr. J.D. Cooper and Dr. M.J. Lim, Pediatric Storage Disorders Laboratory, Institute of Psychiatry, King's College, London, UK. Methods for IHC followed are as described by Chattopadhyay et al.¹

particularly relevant in Batten disease, given its characteristic lysosomal storage component.

We evaluate herein a potential role for autoimmunity to GAD65 in Batten disease. A key element of this is the belief that one can learn considerably from other autoimmune diseases and, in particular, from other diseases that have been shown to have an autoimmune response to GAD65.

Immunology of the nervous system and immune mechanisms underlying autoimmune neurologic diseases. In reviewing the state of autoimmune disease that affects the brain, one has to consider that within the CNS, the brain itself is considered to be immune privileged. Hence, the key initial questions to address are **how does the immune system actually access the brain, and how would the brain be affected potentially by an autoimmune component that would be attacking the brain?** To address these questions, several salient findings with respect to the brain being immunologically privileged are available. First, transplants of tissue and/or cells appear to survive better in the intracerebral environment than outside the brain. Second, the brain lacks lymphatic channels, and third, the brain (most likely) lacks major histocompatibility expression. Finally, peripheral leukocytes are normally excluded from the brain because of the presence of the blood-brain barrier. Microglia, a specific type of cell that occurs within the brain, could represent the brain's immune system. Microglia are activated when neurons are injured, and they protect the injured nerve cells by producing neuronal growth factors, which enable some neurons to regenerate. Microglial activation is a reactive glia response that is induced as a consequence of neuronal injury. However, examination of microglia processes is extremely important if one desires to propose some aspect of immune compromise within the CNS. Therefore, when one examines diseases affecting the CNS in which an inflammatory response is present, or perhaps an autoimmune response is suspected, microglial activation is one of the first things that must be examined. One such example

would be the juvenile or childhood cerebral form of X-linked adrenoleukodystrophy with inflammatory demyelination.

Perhaps one of the best characterized diseases as having autoimmune pathology is myasthenia gravis, a disorder characterized by autoantibodies to acetylcholine receptors that appear to result in the loss of neuromuscular junction receptors in muscle, precipitating weakness and fatigue (reviewed by Antozzi⁸). Often, the role of the autoantibodies in a disorder is confirmed by clinical response to plasma exchange of patients with conditions such as myasthenia gravis. Such a treatment removes the circulating autoantibodies. Another example of when plasma exchange has been used is in some individuals with Rasmussen encephalitis. In this condition, autoantibodies to certain glutamate receptors (anti-GluR3) are present, and it has been shown that there is dramatic recovery in individuals who have undergone plasma exchange.⁹ Another criterion by which a role for autoantibodies in disease can be confirmed is to passively transfer the disease (i.e., its symptoms or pathogenic features) to mice by injection of patient serum or immunoglobulin isolated from these subjects. Studies involving transmission of the disease to experimental animals by passive transfer of immunoglobulins are now ongoing for a variety of other neurologic-based disorders to see whether they do create the physical disease condition. The transfer of disease to mice by injection of serum from patients with Batten disease has not as yet been performed. However, studies are ongoing to address whether removal of autoantibodies by plasma exchange, or by genetic manipulations of CLN3 mouse models by crossing them with mice that have impaired immune systems, will have an influence on disease outcome.

Glutamic acid decarboxylase: Role in autoimmune disease and autoimmunity. In addition to the question as to whether the autoantibodies are pathogenic, another question is **whether the GAD65 protein is the predominant autoantigen in Batten disease.** Many researchers have studied the pathogenic role of GAD65 autoantibodies in

other diseases such as type 1 diabetes and stiff-person syndrome (SPS). In type 1 diabetes, the beta cells of the pancreas are destroyed, and it has long been proposed that autoimmunity to GAD65 may play a key pathogenic role in this attack.¹⁰ One powerful model that has been used for the study of type 1 diabetes is the nonobese diabetic (NOD) mouse, which is recognized for the model for T cell-mediated autoimmune type 1 diabetes. This model has pancreatic beta cell autoimmunity as a default, which results from impaired communication between antigen-presenting cells and T cells.¹¹ Recent studies of NOD mice suggest that the autoreactivity within type 1 diabetes could possibly begin at the level of peri-islet Schwann cells.¹² This suggests a potential for the neuronal element to type 1 diabetes autoimmunity. This mouse model also develops autoimmune responses to GAD. Despite multiple studies indicating the potential for GAD therapy to influence disease outcomes when administered in a therapeutic modality in NOD mice, most studies have recently concluded that anti-GAD immunity most likely represents an indirect response and is not required for the formation of the disease.

How does one determine whether autoantibodies to GAD65 or other neuronal proteins are pathogenic in Batten disease? To address this question, one first needs to consider what Batten disease is. Batten disease results from defects from mutations in the *CLN3* gene product. CLN3 is a novel transmembrane protein that has been localized to different subcompartments within the cell by different techniques. Growing evidence indicates the CLN3 protein to be predominantly located within the lysosomal and late endosomal membrane, being trafficked through the golgi.¹³ Interestingly, a small amount of CLN3 may traffic through the plasma membrane.¹⁴ Batten disease is characterized as a lysosomal storage disorder and is a progressive neurodegenerative disorder. Individuals who inherit two defective copies of the *CLN3* gene/protein develop normally up to age 5 years, at which point they begin to experience slow retinal degeneration, eventually leading to blindness. From ages 7 to 8 years, progressive cognitive decline ensues. Typically intractable seizures continue through this decline. Ultimately, after cognitive decline and loss of motor function, the outcome of this disease is almost always fatal.

It has been previously reported that there is an apparent selective vulnerability of certain neurons in Batten disease.¹⁵ The selective loss of γ -aminobutyric acid (GABA)ergic neurons or loss of GABAergic function has been proposed by several groups. Originally it was suggested that loss of mitochondrial function could result in cell death, possibly through an excitotoxic response.¹⁶ This notion represents an interesting possibility because the presence of autoantibodies in mice with Batten disease may lead to an accumulation of glutamate, which one would predict could result in excitotoxicity. Taken a step further, one would also

predict that exposure to these elevated glutamate levels would be most damaging to inhibitory GABAergic neurons. Glutamate levels may also result in selective effects on mitochondrial function.

What is the status of studying autoimmune response in Batten disease itself? As has been previously discussed, mouse models for CLN3 knockout and individuals who have Batten disease clearly have circulating antibodies to GAD65 and other neuronal proteins. Importantly, the availability of the mouse model has meant many biochemical studies have followed up on the potential contribution of these autoantibodies to the disease, in particular, on GAD function. Cln3-knockout mice, for example, have a decrease in activity of glutamic acid decarboxylase/GAD65. Furthermore, Cln3-knockout mice also have an elevation of glutamic acid decarboxylase/GAD65 substrate, glutamate.¹ This provides biochemical proof of principle that the GAD enzyme has a decreased activity and concomitant accumulation of its substrate. This supports the hypothesis that glutamic acid decarboxylase enzyme activity is affected by the presence of this autoantibody early in the disease process, which leads to decrease in activity and possibly degeneration of GABAergic neurons in Batten disease. Accumulating evidence further suggests that there is an early microglial response in Batten disease and that lymphocytic infiltration occurs as the disease progresses (Cooper and Pearce, unpublished observation). These observations may or may not be directly related to an autoimmune insult of the brain. Hence, it will be important to determine whether CLN3 protein is potentially involved in maintaining the integrity of the blood-brain barrier.

Although there is still limited evidence correlating the autoantibodies and immune response to Batten disease, it has been suggested that approaches directed at removing the autoantibodies in individuals with Batten disease may be beneficial. As research focuses on understanding the autoimmune component of this disease, manipulation of the existing wealth of reagents that have been generated for type 1 diabetes research, a more exact measure of the autoantibodies, and whether levels alter during disease and relate to disease severity will follow. We already know that the GAD65 autoantibodies in type 1 diabetes and SPS are different (i.e., epitopic specificities, titer, affinity), and it is likely that those in Batten disease are different as well. In particular, early studies suggest that the reactive epitopes for GAD65 autoantibodies in Batten disease are somewhat different to those in the other diseases. The development of better techniques to assay these autoantibodies within this and other diseases is paramount and will clearly allow investigators to discern whether the levels of these autoantibodies actually translate to anything meaningful with respect to the progression and severity of the disease itself.

Human autoimmune disease: Predictive and therapeutic strategies involving GAD. There is no doubt that Batten disease is a devastating neurologic disorder. If it was assumed that this autoimmune component contributed only marginally to the disease, **can a rational and ethical case be made for attempting immune intervention in Batten disease, and, if so, would that a priori link autoimmunity with disease pathogenesis?**

Certainly, if it were proven that GAD65 autoantibodies represent a primary molecular event or pathogenic mechanism in the disease, reducing the levels of these antibodies would, in theory, represent a viable target for intervention in the course of the disease. Interestingly, GAD65 therapeutics in type 1 diabetes in NOD mice have been investigated in a variety of ways, including administration with whole GAD65 or GAD65 peptides via oral, intraperitoneal, IV, thymic, and IM modalities. Such therapies have, in a majority of studies, suggested GAD therapy to represent a remarkably effective means to prevent diabetes in NOD mice. However, this is amid a backdrop of additional investigations suggesting an extensive list of potential therapeutics capable of disease prevention in this animal model and a recent series of investigations calling into question the role for anti-GAD immunity in disease pathogenesis. Despite these latter limitations, there are ongoing efforts toward the use of GAD therapeutics in terms of disease prevention in humans that have undergone preclinical, Phase I, and Phase II testing. With time, the field should know whether GAD immunotherapy represents a safe and effective means to modify the immune response in fashion capable of averting autoimmune activity.

Interestingly, ongoing studies are directed toward altering GABAergic function in the striatum of patients with Parkinson disease using targeted virally expressed GAD65 as a means to alter glutamic acid decarboxylase activity.¹⁷ Thus, there is a precedent for manipulating the activity of this enzyme within the brain itself, as a treatment modality, and potentially removing autoantibodies.

Clearly, much remains to be learned about Batten disease and the potential for an autoimmune component in this disorder. It is clear that circulating neuronal antibodies are associated with the disease and that one of the autoantigens is GAD65. Controversy remains as how to best approach investigations of this disorder. **Does one approach this disorder from the strict viewpoint of it representing a storage disease or an autoimmune disorder whose pathogenic potential could be tested by immune-based interventions?**

Even if autoantibodies are shown to have a high degree of pathogenicity in the disease, the question that will still remain is **what causes the lysosomal storage?** The autoantibodies clearly are not going to contribute to the lysosomal storage component of this disease. However, removal of the autoantibodies

may be a rational therapeutic approach for this disease in the meantime.

What are the future research priorities? We conclude that the concept of Batten disease having a clear genetic origin along with a possible autoimmune component is intriguing, but such a notion still requires certain key follow-up experiments to conclude that Batten disease is an autoimmune disease (see table E-1 on the *Neurology* Web site). Autoantibodies can be present in an individual without causing disease—the fundamental question is whether the GAD autoantibodies are activating T cells to trigger a full-fledged immune response. As discussed previously, there are two gold standard experiments to address this question. The first is to remove the autoantibodies from a patient via plasma exchange and determine whether the patient's condition improves (a test of whether the autoantibodies are *necessary* to develop the disease). The second is to inject the autoantibodies into a healthy mouse and determine whether it then develops symptoms associated with the disease (a test of whether the autoantibodies are *sufficient* to confer the disease). A further more novel approach would be to cross the CLN3 mouse mutants with other mouse models with impaired immune response—if the physical effects of Batten disease are primarily caused by an immune response, these mice should not develop the full Batten phenotype.

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