Genetics of Childhood Disorders:
XXX. Autoimmune Disorders, Part 3:
Myasthenia Gravis and Rasmussen's Encephalitis

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How can autoimmunity cause illness? Over the years, immunologists have debated this issue regarding many different diseases. The consensus today is that several criteria must be fulfilled to demonstrate an autoimmune etiology. For those disorders in which a humoral etiology is proposed, autoantibodies should be found in the sera of affected individuals. In addition, removal of these autoantibodies should lead to an improvement of symptoms. Finally, transfer of the autoantibodies to an experimental animal should reproduce some of the clinical symptoms of the disorder. In this column, we review the evidence that myasthenia gravis and Rasmussen's encephalitis are autoimmune disorders. Similar strategies will need to be used by investigators attempting to demonstrate that illnesses such as Tourette's syndrome and obsessive-compulsive disorder are caused by an autoimmune mechanism.

Myasthenia gravis is a relatively rare neurological illness that affects approximately 1 of every 200,000 individuals. A key clinical finding is muscle weakness, especially after repeated activity. Although the course of the illness is highly variable, the disease typically affects eye and eyelid muscles leading to double vision and ptosis. In more severe cases, muscles controlling swallowing, speaking, and breathing become affected.

The typical treatment is to administer inhibitors of acetylcholinesterase. Acetylcholine is a neurotransmitter normally found at the neuromuscular junction. To initiate a muscle contraction, the nerve terminal releases the neurotransmitter acetylcholine into the synaptic cleft. The neurotransmitter rapidly diffuses across and binds to acetylcholine receptors on the surface of the muscle cell. The binding of neurotransmitter to its receptor leads to a rapid change in ion flow across the membrane, which in turn propagates a muscle contraction.

Acetylcholine must be rapidly removed from the synaptic cleft to allow for multiple signals to arrive at the muscle and to allow for repeated contractions in sustained muscle activity. The rapid removal is accomplished in part by the action of acetylcholinesterase that degrades the neurotransmitter. The finding that drugs that inhibit this enzyme improved the muscle weakness in patients with myasthenia gravis suggested that the illness was caused by a disturbance in some component of the acetylcholine signaling pathway.

Little progress was made, however, until the chance observations of two investigators at the Salk Institute in 1973. Jim Patrick and Jon Lindstrom were interested in determining the location of the nicotinic acetylcholine receptor within the CNS. In order to do this, they wanted to generate antibodies against the receptor that could be used in immunocytochemical localization studies. Generating antibodies is a time-honored procedure by which investigators produce a probe that will bind to and help visualize a specific protein under investigation.

Typically, rabbits are used to produce such antibodies. When a protein or a portion of the protein is injected into a rabbit, the animal will mount a humoral response against the foreign
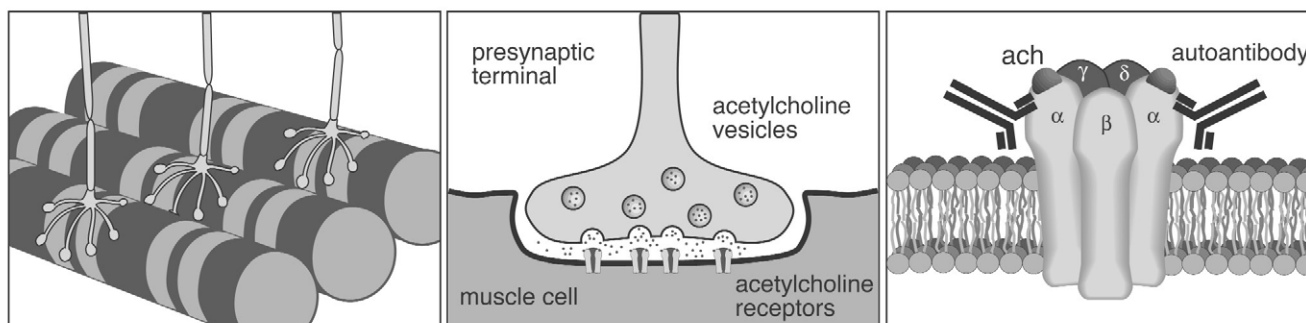
Fig. 1 Muscle cells are innervated by axons with terminal arbors that form synapses at structures called *neuromuscular junctions*. The neuromuscular junction is composed of a nerve terminal that releases acetylcholine neurotransmitter and the postsynaptic component on the muscle cell where the acetylcholine receptors are found. Autoantibodies produced in myasthenia gravis recognize amino acid sequences on the α subunit of the acetylcholine receptor. Binding of the antibody to the receptor is thought to lead to its internalization, leading to a loss of functional receptors at the neuromuscular junction, as well as the eventual attack of the muscle cell by lymphocytes.

antigen. Large amounts of antibodies are thereby produced that are capable of recognizing different portions of the injected protein. Repeated boosts with the antigen lead to the production of large amounts of antibodies in the sera of these animals. The antibodies can then be separated from other components of the sera and used in immunocytochemical studies within the CNS.

Unexpectedly, the rabbits that were immunized with the acetylcholine receptor developed severe muscle weakness. Patrick and Lindstrom saw a similarity between the animals' behavior and the weakness seen in patients with myasthenia gravis. When they treated the animals with an acetylcholinesterase inhibitor, the rabbits got better. For the first time, the specific hypothesis that the acetylcholine receptor was the target of autoantibodies could be tested.

Relatively quickly, researchers established that the sera of patients with myasthenia gravis contained antibodies and that these antibodies recognized a subunit of the acetylcholine receptor complex of proteins (Fig. 1). This was an important first step. It is currently believed that the immunogenic portion of the acetylcholine receptor lies on one of the five subunits that assemble to form the receptor. The α subunit is not only the site for binding of acetylcholine, but it also contains the amino acid sequence that elicits the antibody reaction. When the autoantibody binds to the receptor, it is believed that the receptor is internalized by the muscle cell and degraded. In some rare cases, the binding of autoantibody to the α subunit blocks access of acetylcholine to its binding site. The binding of autoantibodies, however, initiates a series of events that promotes complement binding and focal lysis of the postsynaptic membrane. The net result is that there are fewer functional acetylcholine receptors at the neuromuscular junction. Muscle cells are innervated by axons with terminal arbors that form synapses at structures called *neuromuscular junctions*. The neuromuscular junction is composed of a nerve terminal that releases acetylcholine neurotransmitter and the postsynaptic component on the muscle cell where the acetylcholine receptors are found. Autoantibodies produced in myasthenia gravis recognize amino acid sequences on the α subunit of the acetylcholine receptor. Binding of the antibody to the receptor is thought to lead to its internalization, leading to a loss of functional receptors at the neuromuscular junction, as well as the eventual attack of the muscle cell by lymphocytes.}]

If true, this decline in receptors would explain why treatment with inhibitors of acetylcholinesterase is effective. The increase in the relative amount of neurotransmitter at the neuromuscular junction compensates for the loss of functional receptors. Removal of the antibodies from sera of affected individuals leads to improvement of clinical symptoms. In fact, plasmapheresis has now become a standard treatment for myasthenia gravis.

It is interesting how history repeats. Twenty years later, in the same neurobiology department at the Salk Institute, investigators discovered the target of a second autoimmune disorder. Once again they did so by immunizing rabbits with a receptor. This time they were interested in localizing one of the glutamate receptors within the CNS. Surprisingly, some of the immunized rabbits developed intractable seizures and neuropathological brain lesions indistinguishable from those seen in humans with Rasmussen's encephalitis.

Rasmussen's encephalitis is a rare form of epilepsy that is associated with progressive neurological dysfunction and destruction of a single cerebral hemisphere. The disorder usually begins during the first decade of life with the appearance of seizures, hemiparesis, and severe cognitive and language impairments. The seizures are often unresponsive to standard antiseizure medications, and surgical removal of the affected hemisphere is the standard treatment. It was recently discovered that the etiology of this devastating disease is an autoimmune response to one of the glutamate receptors, GluR3.

The glutamate neurotransmitter system has captured the attention of so many investigators for several reasons. Glutamate is the most abundant transmitter within the CNS. It has been implicated in a wide range of complex neuronal processes including development, apoptosis, learning, and memory. Its capacity to participate in these processes is due to its ability to stimulate a wide variety of intracellular signals, and this is due in part to the large number of distinct receptors through which glutamate acts.

There are at least 20 different genes that encode for glutamate receptors. These receptors can be classified into two broad groups: the metabotropic receptors and the ionotropic receptors. The metabotropic receptors are members of the G-coupled family of receptors that are membrane-associated proteins capable of stimulating a cascade of intracellular pathways when activated. The ionotropic receptors also bind glutamate directly, but these receptors act as ion channels. Binding to glutamate rapidly changes ion currents across the cell membrane. The ionotropic receptors can be further divided through their distinctive response to pharmacological reagents that activate them: NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid), and kainic acid.

The ionotropic glutamate receptors are formed by several subunits that associate with each other to form a pore through the cell membrane. Each receptor subtype is formed by combinations of five protein subunits. In this way, a large number of receptor subtypes can be constructed. It is in fact the varying subunit compositions that determine exactly which ions are allowed through the channels. The central pore formed in AMPA/kainate receptors primarily allows passage of Na^+ and K^+ ions and in some cases small amounts of Ca^{++}, while activation of NMDA receptors results in the influx of large calcium currents.

Once it was discovered that many different subtypes of glutamate receptors existed, investigators became interested in determining their localization patterns. Why were there so many different glutamate receptors, and could localizing them

within the CNS clarify their varying functions? The group at the Salk was particularly interested in studying the GluR3 receptor, a subtype of the AMPA family. Rabbits were immunized with the GluR3 protein in order to make specific antibodies against the receptor. As mentioned above, several of the immunized rabbits developed intractable seizures and, on histopathological examination, the brains showed a similar pattern of perivascular lymphocytic infiltrate and microglial nodules that is commonly seen in Rasmussen's encephalitis.

This initial study suggested that perhaps humans with Rasmussen's encephalitis developed their illness as a consequence of an autoimmune response directed against the GluR3 receptor. Investigators quickly determined that anti-GluR3 antibodies were in fact present in the sera of affected individuals. In addition, several patients responded dramatically to the removal of the autoantibodies through plasmapheresis; this finding suggests that the autoantibodies contributed at least in part to the progressive neuronal loss and hemispheric atrophy so characteristic of the disease. Attempts to create an animal model have not been successful to date.

One question that has puzzled investigators is the fact that tissue destruction occurs in only one cortical hemisphere. Circulating autoantibody that crosses the blood-brain barrier should not show unilateral specificity, especially as the antigen, the GluR3 receptor, is expressed at many sites in both cortices. A proposed model is that the illness is initiated through a local traumatic event (such as a blow) to the head that disrupts the blood-brain barrier in a very limited region. Autoantibody is then able to leak into a limited area of a single hemisphere.

Exactly how the autoantibody causes cellular damage is also a matter of considerable debate. One hypothesis is that the GluR3 autoantibody is excitotoxic. This would occur if the binding of the antibody to the glutamate receptor activates the receptor and leads to a massive influx of ions. Activation of glutamate receptors is a well-known mechanism that precedes neuronal cell death. Lymphocytic infiltration then occurs and causes local inflammation and a further disruption of the blood-brain barrier which permits entrance of additional damaging autoantibodies.

A competing hypothesis is that the autoantibody that binds to GluR3 receptors attracts specific components of the complement system. Complement cascades are activated and lead to neuronal death and lymphocytic infiltrations. Both hypotheses are being tested and would explain the progressive neuronal death that occurs in this degenerative seizure disorder.

WEB SITES OF INTEREST

http://www.chem.uci.edu/~archambe/glutamate.html
http://www.ucl.ac.uk/~smgxt01/frameh.htm?page = collicul.htm
http://www.myasthenia.org/

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