# Genetics of Childhood Disorders: XXXV. Autoimmune Disorders, Part 8: Animal Models for Noninflammatory Autoimmune Disorders of the Brain

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Useful animal models for the investigation of autoimmune disorders that involve the brain are essential, especially in children in whom access to the CNS is limited. The paucity of such models can hinder an understanding of causation, pathogenic mechanisms, and treatments. This is particularly true when one is investigating postulated antibody-mediated disorders in which evidence supporting pathogenesis is derived from in vitro studies. The interpretation of such studies is confounded by the frequent nonpathogenic antineuronal antibody binding associated with chronic disorders, such as autism, type 1 diabetes mellitus, as well as normal aging. Differentiating pathogenic from nonpathogenic antibodies cannot be done without a biological assay such as an animal model. This need is seen in the ongoing discussion of the pathogenesis of autoantibodies in childhood Tourette syndrome (TS), obsessive-compulsive disorder (OCD), pediatric autoimmune neuropsychiatric disorders associated with streptococcus (PANDAS), and Sydenham chorea (SC).

An animal model should reflect the type of immune response occurring or postulated to occur in the brain, utilize brain regions involved in the disorder, and maintain the brain's unique immune environment. When studying putative immune brain disorders, it is useful to initially group disorders by the presence or absence of acute inflammation. This distinction is important because
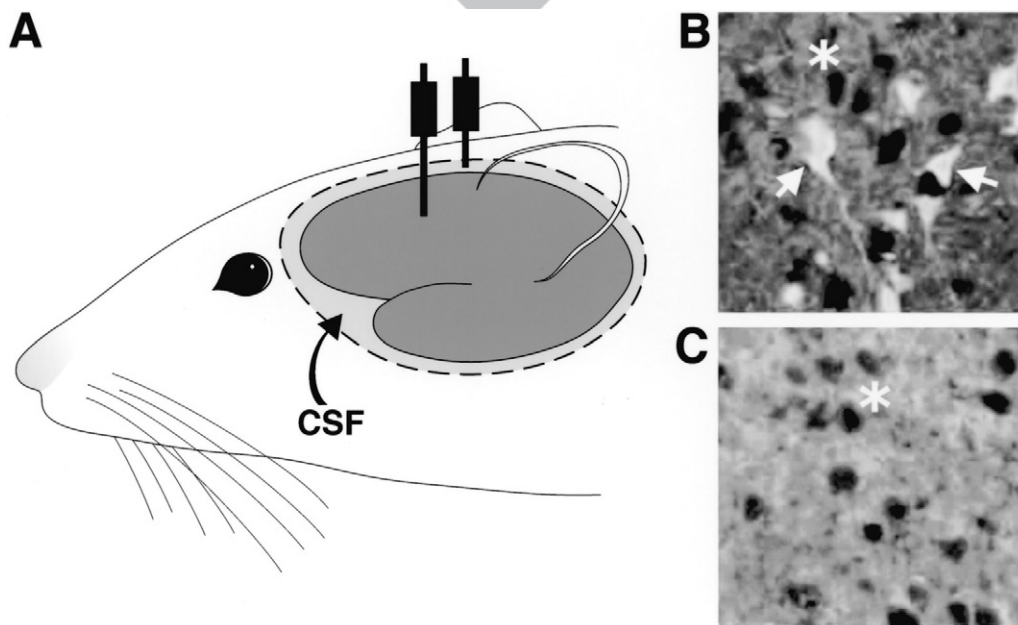
Fig. 1 Implanted cannulae (A) and photomicrographs of coronal sections of rat striatum at the level of cannula placement (B, C). (A) Cannulae are implanted bilaterally in the brain or CSF for infusion of serum or antibodies so that their effect on neuronal function may be investigated. The location of brain-implanted cannula can be adjusted to permit infusion into selected regions of the brain, allowing investigation of regions associated with specific behaviors. Infusion into CSF is less specific because it is distributed over a wider area by the normal flow patterns of CSF with variations in the rate of penetration into the brain parenchyma. (B) A photomicrograph of coronal section of rat striatum at the level of cannula placement after infusion of antibodies from a child with Tourette's syndrome. The fluorescent probe, CY3 conjugated anti-human IgG antibodies, was used to localize the antibodies bound to neurons. Fluorescent-labeled neurons are seen in the brain microinfused with TS-IgG at high magnification (arrows). (C) No neuronal fluorescence is observed in the brain microinfused with control antibodies from healthy children. Myelinated axonal bundles, characteristic of the striatum, are marked with an asterisk.

it determines whether the normal immunosuppressive environment of the brain parenchyma is maintained. Acute inflammation is associated with the disruption of the blood-brain barrier (BBB), a major anatomical and physiological barrier for peripheral immune responses. Loss of this barrier allows the peripheral immune cascade to operate within the brain parenchyma without its normal down-regulating influences. This inflammatory disruption of the BBB in humans is most often associated with vasculitis or perivasculitis of the cerebral vessels.

In addition to categorizing autoimmune-mediated brain disorders by the presence or absence of acute inflammation, it is useful to separate them further, into one of the four established hypersensitivity responses. In general, autoimmune responses in the nervous system are either type 2 (antibody-mediated, often γ-immunoglobulins [IgG]) or type 4 (cell-mediated) hypersensitivity. The immune-mediated mechanism postulated in TS, SC, OCD, and PANDAS has been conceptualized by most investigators as a noninflammatory antibody-mediated response, a type 2 response. This is a reasonable assumption because the evidence supporting acute inflammation in these disorders is meager. For this reason, further discussion of animal models will be limited to models in which antibodies are the predominant immune effectors. This is not to indicate that other humoral components, such as cytokines, do not have a pathogenic role. However, current evidence suggests that if they have a role, it is complementary.

Several animal protocols are available for studying noninflammatory type 2 hypersensitivity while maintaining the BBB and the brain's immunosuppressive environment. Peripheral immunization with neuroantigens or neural tissue is one approach. Studies of an autoimmune mechanism in Parkinson disease were conducted by immunizing guinea pigs with bovine substantia nigra. Nigral injury was detected in the guinea pigs, although it was not expressed clinically. Similarly, antibodies generated by immunizing rabbits with glutamate 3 receptors (GluR3) bound to cortical GluR3 and resulted in seizures (see this column, XXX). Subsequent identification of anti-GluR3 antibodies in childhood Rasmussen's encephalitis suggests that an immunization protocol is a useful animal model for further investigations of this disorder.

The immunization model is most effectively studied when the neuroantigen involved in a disorder is known. This knowledge is not yet available for many disorders such as TS, PANDAS, or SC. Results need cautious interpretation because immunological processes in this model are only partially understood. How do serum antibodies gain access to brain regions with an intact BBB? Does this protocol affect only cortical surface antigens? Is perivasculitis a prerequisite? How dependent is the neural effect on the peripheral immunization protocol, i.e., use of Freund's adjuvant and booster immunizations? Brain dysfunction has not been reported in rabbits immunized with other glutamate receptors. Why? Immunizing different strains of mice with GluR3 results in a range of postimmunization serum titers, brain lesions, and the absence of seizures. What is the influence of genetic background on the process?

Infusion of antibodies into CSF (subarachnoid or intraventricular) is a second experimental design that is used for studying putative antibody-medicated neural disorders. This is, in essence, a modification of the traditional immunological technique of adoptive transfer. The approach is an effort to determine whether antibodies are pathogenic by passively transferring them from an affected individual to naïve animals, thereby inducing the disorder in the animals. Induction of ataxia in mice after subarachnoid infusion of anti-GluR1 antibodies isolated from patients with paraneoplastic syndromes is an example of this approach.

The simplicity of this protocol is appealing, but the slow movement of IgG, a globular macromolecule, through the dense tortuous cellular architecture and metabolic active brain environment can confound results. Infused antibodies are rapidly diluted and are removed with CSF turnover. If deep brain structures are targeted, the size of the brain will influence the duration of time that a particular concentration of antibodies is maintained in the CSF. Diffusion will facilitate movement over short distances, but movement over longer distances utilizes bulk flow, which can have a heterogeneous distribution. Longer infusions also must control for the possibility that the infusion rate and volume per se may alter the brain environment, leading to abnormal behavior. The specificity of the response to the IgG will also diminish as the duration increases because its movement throughout the brain is not confined. This can be minimized by infusing monoclonal antibodies, an option that is not available for most disorders.

An alternative to CSF infusion is direct infusion of antibodies into specific brain regions. Although CSF infusion is less invasive, direct infusion into the brain offers specificity. Using this approach, we have demonstrated that some TS-IgG cross-reacts with neural antigens and causes behavioral changes in rats after infusion into lateral striata. Direct infusion into a brain region allows an investigator to select regions implicated in a disorder. The lateral striatum was selected for TS-IgG infusion because stereotypic behaviors, analogous to those in TS, had been induced by pharmacological manipulation of this region. Behavioral changes were also induced after the subthalamic nucleus, which is postulated to have a role in SC, was infused with SC sera. During placement of a cannula the BBB is breached, resulting in a temporarily alteration of the brain's environment. Therefore, it is critical to allow adequate time for the BBB to reestablish itself before infusion. As with CSF infusion, the influence of the infusion rate, duration, concentration, and volume on the brain microenvironment must be minimized.

Direct infusion into brain regions has also been used to study mechanisms by which antibodies can gain entry to a brain with an intact BBB. In this approach, an antigen is infused into the brain and animals are subsequently immunized with the same

antigen. This produces high titers of serum antibodies specific for a unique, albeit foreign, brain antigen. The infusion of specific antigens allows investigators to predetermine the location (at the cannula tip) at which neuroimmunological activity should occur (Figure 1). This greatly limits the search for an effect and facilitates the separation of antigen-specific antibodies from constituent antibodies. Using this protocol, we found that antigen-specific B lymphocytes traffic from the peripheral circulation to the striatum and, after encountering their cognate antigen, transform into antibody-secreting plasma cells.

Clinical and experimental interest in antibody-mediated brain dysfunction under noninflammatory conditions is growing. In TS, OCD, PANDAS, and SC, clinical and immunohistochemical studies have uncovered an association with serum antibodies. Emerging animal models are now available for investigation of causal relationships. However, until we understand more about these models and neuroimmunological mechanisms operating in the unique brain environment, results from animal studies require a cautious extrapolation to the human situation.

WEB SITES OF INTEREST

http://www.tourette-syndrome.com/
http://www.tourette-syndrome.com/tourette-syndrome-links.htm

ADDITIONAL READINGS

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