Video Article

Induction of Experimental Autoimmune Hypophysitis in SJL Mice

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Abstract

Autoimmune hypophysitis can be reproduced experimentally by the injection of pituitary proteins mixed with an adjuvant into susceptible mice¹. Mouse models allow us to study how diseases unfold, often providing a good replica of the same processes occurring in humans. For some autoimmune diseases, like type 1A diabetes, there are models (the NOD mouse) that spontaneously develop a disease similar to the human counterpart. For many other autoimmune diseases, however, the model needs to be induced experimentally. A common approach in this regard is to inject the mouse with a dominant antigen derived from the organ being studied. For example, investigators interested in autoimmune thyroiditis inject mice with thyroglobulin², and those interested in myasthenia gravis inject them with the acetylcholine receptor³. If the autoantigen for a particular autoimmune disease is not known, investigators inject a crude protein extract from the organ targeted by the autoimmune reaction. For autoimmune hypophysitis, the pathogenic autoantigen(s) remain to be identified⁴, and thus a crude pituitary protein preparation is used. In this video article we demonstrate how to induce experimental autoimmune hypophysitis in SJL mice.

Protocol

Experimental Protocol

Once the pituitary immunogen is prepared (see companion article), it is injected subcutaneously into SJL mice (The Jackson Laboratories, stock 000686). A second identical injection is repeated 7 days later. Mice are then used to collect *in vivo* and post-mortem outcomes.

Step 1. Subcutaneous injection of pituitary immunogen into SJL mice

Mice are first anesthetized by injecting 0.5 ml of 20 mg/ml 2,2,2-Tribromoethanol (Avertin, TCI America, T-1420) intraperitoneally. Mice are then injected subcutaneously with 100 μ l of the pituitary emulsion that, as indicated in the companion JoVE 2181 article, contains 1 mg of pituitary extract and 0.25 mg of complete Freund's adjuvant. The emulsion is injected in the left dorsal hind leg region ((50 μ L) and the right inguinal region (50 μ L). The pituitary emulsion is injected again on day 7 into the opposite sites (50 μ l in the right dorsal hind leg region and 50 μ l in the left inguinal region). Mice are monitored daily for the first 3 days and then weekly until the day of sacrifice, which is typically 28 days after the first immunization.

Step 2. Collection of *in vivo* outcomes: Blood assays

The tissue most commonly used to assess experimental outcomes while the mice are still alive is blood. Blood is routinely drawn 14 days after the first immunization to measure the serum levels of pituitary antibodies or other markers like cytokines and chemokines.

Blood is collected from live mice as follows. Mice are held securely by the scruff of the neck and the back of the jawbone is located. Once the back of the jawbone is located, 4 mm lancets are used to pierce the submandibular vascular bundle. After blood is collected, place pressure on the on the area using an alcohol swab to avoid a hematoma. Collected blood is centrifuged at 2,000g for 20 minutes, and stored at -80°C until used.

Step 3. Collection of post-mortem outcomes: Pituitary histopathology

Mice are typically sacrificed 28 days after the first immunization. The cardinal feature necessary to establish the diagnosis of EAH is the infiltration of the pituitary gland by hematopoietic mononuclear cells.

Mice are euthanized by placing them in a sealed plastic chamber that is perfused with CO₂. Mice are kept in the chamber for a total of 8 minutes. After euthanasia, the pituitary gland is collected as described in the companion article. A diseased pituitary gland appears swollen and more firmly adherent to the surrounding dura mater. Sharp forceps are used to loosen the meninges surrounding the pituitary. Once the pituitary moves freely on the sphenoid bone, one end of the pituitary gland is grasped with forceps and the pituitary gland is carefully lifted. The gland is then placed in a microcentrifuge tube containing Beckstead's fixative⁵. The pituitary gland is fixed overnight, wrapped in lens paper, and placed in a cassette. The glands are then processed using the following protocol:

Station Number	Reagent	Time in Minutes	Temperature (Celsius)
1	70% alcohol	5	25
2	70% alcohol	15	25
3	95% alcohol	15	25

4	95% alcohol	15	25
5	100% alcohol	15	25
6	100% alcohol	7.5	25
7	100% alcohol	7.5	25
8	xylene	20	25
9	xylene	20	25
10	xylene	20	25
11	paraffin	30	58
12	paraffin	5	58
13	paraffin	5	58

After processing, pituitary glands are embedded in paraffin and at least 5 nonconsecutive sections (5- μ m thick) are cut from each gland. After sectioning, the glands are stained with hematoxylin and eosin, and analyzed to quantify the mononuclear infiltration. The section with the most severe infiltration is selected for scoring. The severity is scored based on a subjective estimate of the extent of anterior pituitary replacement or destruction by immune cells: grade 0, no disease; grade 1, 2%-20% involvement; grade 2, 20%-30%; grade 3, 30%-50%; grade 4, 50%-90%; grade 5, greater than 90%. All sections are scored by two individuals blinded to the source of the sections.

Discussion

Four techniques have been reported to induce hypophysitis in experimental animals. The technique described in this article, that is the injection of pituitary proteins mixed with Freund s adjuvant, is the oldest one, dating back to 1967⁶, and has been improved more recently¹. Other techniques are the injection of soluble rubella virus glycoproteins in 1992⁷, the stereotactic injection of an adenovirus directly into the pituitary gland in 2002⁸, and the transplantation of pituitary glands from one rat strain under the kidney capsule of another rat strain in 2010⁹. The main advantage of the Freund s adjuvant approach is that it is less invasive, more consistent and, above all, more accurate in inducing a disease that closely resembles the human counterpart. The technique described in this video article yields a reproducible model of hypophysitis, which can be used by investigators to improve our understanding of this fascinating disease.

Disclosures

No conflicts of interest declared.

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