Video Article

Isolation of Mononuclear Cells from the Central Nervous System of Rats with EAE

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URL: http://www.jove.com/index/Details.stp?ID=527
DOI: 10.3791/527
Citation: Beeton C., Chandy K.G. (2007). Isolation of Mononuclear Cells from the Central Nervous System of Rats with EAE. JoVE. 10. http://www.jove.com/index/Details.stp?ID=527, doi: 10.3791/527

Abstract

Whether studying an autoimmune disease directed to the central nervous system (CNS), such as experimental autoimmune encephalomyelitis (EAE, 1), or the immune response to an infection of the CNS, such as poliomyelitis, Lyme neuroborreliosis, or neurosyphilis, it is often necessary to isolate the CNS-infiltrating immune cells.

In this video-protocol we demonstrate how to isolate mononuclear cells (MNCs) from the CNS of a rat with EAE. The first step of this procedure requires a cardiac perfusion of the rodent with a saline solution to ensure that no blood remains in the blood vessels irrigating the CNS. Any blood contamination will artificially increase the number of apparent CNS-infiltrating MNCs and may alter the apparent composition of the immune infiltrate. We then demonstrate how to remove the brain and spinal cord of the rat for subsequent dilaceration to prepare a single-cell suspension. This suspension is separated on a two-layer Percoll gradient to isolate the MNCs. After washing, these cells are then ready to undergo any required procedure.

Mononuclear cells isolated using this procedure are viable and can be used for electrophysiology, flow cytometry (FACS), or biochemistry. If the technique is performed under sterile conditions (using sterile instruments in a tissue culture hood) the cells can also be grown in tissue culture medium. A given cell population can be further purified using either magnetic separation procedures or a FACS.

Protocol

1. Deeply anesthetize rats. Spray with 70% ethanol and do a cardiac perfusion with PBS for 10 min to remove cells from blood vessels (cut the right atria and perfuse through the left ventricle).
2. Remove the brain and spinal cord and place in a 50 ml tube containing ice-cold PBS. Cut the brain and spinal cord in a 70 mm cell strainer placed in a 10 cm petri dish containing 10 ml of ice-cold PBS. Press each piece of organ through the cell strainer using the back of a sterile 1 ml syringe plunger. Collect the single cell suspension into a 50 ml tube on ice. Wash the cell-strainer with PBS and add to the tube until the solution is clear.
3. Centrifuge for 8-10 min at 390 g.
4. Resuspend the cells in 20 ml PBS + 30% Percoll and overlay onto 10 ml of PBS + 70% Percoll.
5. Centrifuge at 390 g for 20 min at room temperature.
6. Remove the fat on top of the tube. Collect the cells from the interface and wash twice with PBS. Count.

Discussion

This procedure, as all procedures involving live animals, must be approved by your institution's animal use and care committee. We recommend that a veterinarian or a veterinary technician be present when performing the first cardiac perfusions to ensure a sufficient level of anesthesia is given to the animal before and during the procedure, and that the animals do not undergo unnecessary pain or distress.

References

1. Beeton C. and Chandy K.G. Induction and clinical scoring of chronic-relapsing experimental autoimmune encephalomyelitis, Journal of Visualized Experiments, 5, http://www.jove.com/index/Details.stp?ID=214, doi: 10.3791/214.

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