Mouse meninges isolation for FACS

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Method Article

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Abstract

Presented is a method for removal of meninges from the brain and interior skull of the mouse yielding tissue suitable for preparing a single-cell suspension amenable to downstream applications such as flow cytometric analysis or short-term cell culture.

Reagents

Nembutal \(or similar, as approved by your governing body) Perfusion apparatus suitable for transcardial mouse perfusion Perfusion Buffer \(0.1M PBS/5u/ml sodium heparin) FACS Buffer \(0.1M PBS \(pH 7.4)/2mM EDTA/1% BSA)

Equipment

Ice bucket Ice Dumont #5 Forceps Dumont #7 Forceps Angled Dissector Scissors 10 mm Petri dish Dissecting microscope 50 ml Falcon tubes 70 um nylon cell strainer Centrifuge

Procedure

1.) Mouse preparation 1.1) Anesthetize mouse with lethal dose of appropriate pharmacological agent \(e.g. Nembutal) to render amenable to surgical manipulation. 1.2) Perfuse transcardially with ice-cold Perfusion Buffer until mouse is exsanguinated. 2.) Skull preparation and brain removal 2.1.) Using large scissors, sever mouse head just above shoulders. 2.2.) Using angled dissection scissors, beginning at caudal aspect, carefully detatch skin and flesh from skull, working rostrally until all skin is removed. 2.3.) Sever eyes at the optic nerve and remove. 2.4.) Turn head over so that ventral aspect of the jaw is facing up. Slide angled dissection scissors through the oral cavity until you feel the resistance of the mandibular junction. Sever the large muscles connecting the lower jaw to the skull; remove. 2.5.) Using angled dissection scissors, remove the lower orbits on each side of the skull \(the squamosae, zygomatic and maxillae). 2.6.) Working quickly, use scissors to all clean flesh from all aspects of skull. 2.7.) With large scissors, remove the nasal bone with a coronal cut along the suture that defines its most caudal point, thus preserving the olfactory lobes. 2.87.) With the tips of the angled dissection scissors, carefully cut shallowly around the skull, being careful to leave the brain within undamaged. Begin and end superior to the external acoustic meatus \(the “earholes”). 2.9.) Remove the lower portion of the skull; discard. Scoop the brain out of the upper half of the skull, and place both brain and skull-cap in a 50 mL falcon tube with 10 mL ice-cold FACS buffer \(or suitable cell culture medium, e.g. DMEM-10, RPMI 1640). 3.) Removal of meninges 3.1.) Place brain and skull-cap, with medium, in petri dish under a dissecting microscope. With a scalpel, separate brainstem/cerebellum from cerebrum. 3.2.) Hemisect the cerebrum with scalpel along the longitudinal fissure. Working from medial to lateral, peel meninges \(pia and arachnoid mater) from the surface of the brain. Pay special attention to the ventral aspect of the brain, as it is a rich source of meningeal tissue. Choroid plexus epithelium and associated leukocytes also lie within the lateral and third ventricles, and can be removed easily if so desired. When the cerebral hemispheres are fully stripped

of meninges and choroid plexus, remove from the petri dish, leaving dissected material in the medium. 3.2.) Separate the cerebellum from the brainstem; the space between houses the fourth ventricle, another source of meningeal tissue. The ventral aspect of the brainstem can generally be peeled off in a single section. The dorsal cerebellar meninges must be removed piecewise, as with the cerebral meninges. When the cerebellum and brainstem are fully stripped of meninges and choroid plexus, remove from the petri dish, leaving dissected material in the medium. 3.3.) Beginning at the rostral-most interior point of the skull-cap, carefully score meninges with the tip of a Dumont #5 forceps, moving along the edge of the interior of the skull-cap until meninges \(dura and arachnoid mater) are scored 360º. If meninges have been completely scored, they can then be peeled whole from the inside of the skull. Remove skull from media and discard 4.) Single cell suspension 4.1.) Place a nylon cell screen \(70 µm) atop a 50 mL Falcon tube in ice. Pipette dissected tissue and media directly onto screen. With the wide tip a plastic plunger \(from a 1 ml syringe), gently but firmly press meningeal tissue with a circular motion \(~40x) through the screen. Connective tissue will not pass through the screen well and will remain; lymphocytes, any glial cells or neurons, and debris will pass through. Do not mash too quickly or heat will be generated by friction of the plunger against the screen. With a clean pipette, pass 20 mL additional media through the screen while continuing to mash with plunger; this will dislodge any cells caught in the screen. 4.2.) Centrifuge at 300 RCF for 10 m at 4ºC. Decant supernatant and resuspend in desired medium for downstream applications. Triturate gently to break up pellet. Yield should be $5X10^5$ viable nucleated cells including T lymphocytes, myeloid cells, and granulocytes per meninges, with 10% purity for leukocytes \(CD45-high cells). Non-leukocytes can be easily gated out in FACS by scatter properties and/or negativity for CD45-high labeling and a viability dye; further purity for cell culture can be achieved by benchtop \(e.g. magnetic columns) or flow cytometric sorting or by ficoll paque gradient. However, we routinely label crude preparations with excellent success–CD45 and live/dead labeling is sufficient to gate out unwanted debris and ensures that as few cells are lost between dissection and analysis as possible.

Timing

Perfusion: 3-5 minutes Skull-cap and brain removal: 5 minutes Meninges removal: 10-15 minutes

Troubleshooting

Be careful to thoroughly exsanguinate mouse prior to dissection. If this is not done, peripheral immune cells will contaminate the preparation.

References

This protocol has been used in the following primary research papers: **Dynamics of the meningeal CD4+ T-cell repertoire are defined by the cervical lymph nodes and facilitate cognitive task performance in mice** A Radjavi, I Smirnov, N Derecki, and J Kipnis Molecular Psychiatry 19 \(5) 531 - 532 11/06/2013 doi:10.1038/mp.2013.79 **Regulation of learning and memory by meningeal immunity: a key role for IL-

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