

Orthotopic lung epithelial cell transplantation

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Abstract

This protocol describes a method for orthotopic transplantation of purified lung epithelial cells from uninjured donors into influenza-injured recipient mice.

Introduction

This simple protocol allows for transplantation of putative lung progenitor populations directly into influenza-injured recipient mouse lungs.

Reagents

Materials

Influenza A/H1N1/Puerto Rico/8/34 (PR8)

Sterile PBS

4% PFA VWR 15714-S

OCT Fisher 14-373-65

Ub-GFP (Tg(UBC-GFP)30Scha) mice

mTmG (Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo) mice

Equipment

Anesthesia Machine

P200 Pipettman and sterile filter tips

BD FACS Aria

Procedure

1. Influenza Injury: Recipient mice are administered 280 FFU of Influenza A/H1N1/Puerto Rico/8/34 (PR8) intra-nasally. PR8 virus dissolved in 30 μ l of PBS is pipetted onto the nostrils of heavily anesthetized mice. Proper anesthesia can be visually confirmed by agonal breathing and usually requires about 5 minutes in an isoflurane chamber. Properly anesthetized mice will aspirate the fluid directly into their lungs.
2. Donor cell isolation and transplantation: At 9 days post-infection, isolate donor cells from a ubiquitously fluorophore-expressing mouse, following the Epithelial Cell Isolation protocol. Ub-GFP and mTmG are particularly useful strains for this. After sorting, donor cells are resuspended in 50ul sterile PBS. Recipient mice receive cell solution intranasally, exactly as described above

for influenza administration. After transplantation, we've observed that keeping mice in a nose cone with a flow of 100% oxygen can aid in recovery and decrease the chance of accidental death. We observe engraftment using anywhere between 1,000 to 1 million donor cells.

3. Tissue Harvest: Endpoint analysis is performed at day 21 post-infection (or desired experimental endpoint). Mice are anesthetized and lungs perfused and lavaged three times with 1 ml PBS. Lungs are inflated with about 0.75 mls of 4% PFA and tied off at the trachea, being careful not to overinflate. Place lungs in an additional 20 ml of 4% PFA and rock at room temperature for 1 hour. Decant PFA and rinse with PBS. Decant PBS and replace with fresh PBS and incubate 1-4 hours rocking at room temperature. This allows for the diffusion of remaining PFA inside the lungs to prevent over-fixation. Incubate in 30% sucrose in PBS + 0.02% azide at 4 degrees on a rocker overnight. The following day, decant sucrose solution and replace with 50% OCT / 15% sucrose / PBS for 2 hours at room temp. Pour out the lung and carefully dissect each lobe and trachea apart. Arrange the lungs and trachea in a cryomold containing 100% OCT and incubate on ice for 1 hour. Freeze entire cryomold in a dry ice alcohol bath.

Timing

21 days. For the day of transplant, estimate an 8 hour procedure that includes harvest and sorting of donor cells through intranasal transplant.

Troubleshooting

1. Mice that are under-anesthetized will often fail to breathe solely through their nose, resulting in "bubbling" of cell suspension. Insure agonal breathing as described to avoid this.
2. We hold anesthetized mice in one hand and use our thumb to push their heads back at a ~60o angle, and then slowly pipette the cell suspension onto their nostrils, where it is aspirated. This can be difficult at first, and we recommend practicing with

uninjured mice and saline. Other methods of manipulating the animal may also work and should be determined by the researcher.

3. If transplanting a small number of cells (<25,000), we recommend “spiking” the fluorescent cells of interest into a non-fluorescent bulk cell population to act as a carrier. One good option is to isolate multiciliated cells (EpCam+ CD200- β 4+) from C57BL6 mice. Multiciliated cells are terminally differentiated and will not divide upon engraftment.
4. Due to prefixation with PFA, background may be a bit higher. Alleviate this by reducing with sodium borohydride. Apply sodium borohydride at 1mg/ml in PBS to cut slides for 3 x 10 minute intervals.
5. Some antigens may be masked. Usually a light antigen retrieval (15 min at 70o with 150 mM Tris-Cl, pH 9.0) will help a lot without denaturing endogenous fluorophores.

Anticipated Results

Results are dependent on the population transplanted. For instance, purified progenitor cells (Epcam+ β 4+ CD200+ CD14+) will engraft with as few as 3000 cells, and will evidence multilineage differentiation potential. Specifically, expect to observe SPC+, CC10+, and Krt5+ donor-derived cells depending on the region of engraftment.

Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury

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