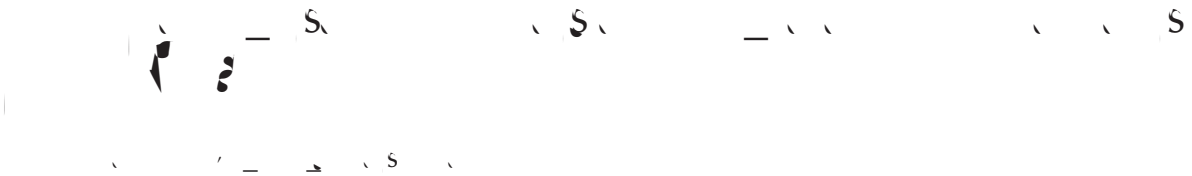
Protocol



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Adoptive cell transfer from inbred adult Xenopus to inbred tadpoles is a useful way to study the dissemination of immune cells or pathogen-infected immune cells in tadpoles. For example, Xenopus peritoneal leukocytes (PLs) can be readily infected by pathogens such as Frog virus 3 (FV3) and Mycobacterium marinum (M. marinum). By transferring fluorescently labeled, FV3-infected PLs into tadpoles, we observed infiltration of these cells into the tadpole's brain, which indicates that FV3-infected PLs can cross blood brain barrier. Taking advantage of tadpoles' transparency, fluorescently labeled immune cells can be tracked in real time using fluorescence microscopy.dua.MATERIALcellsScroscoocol

Texas red dextran (Sigma-Aldrich)
Trypan blue solution (Thermo Fisher Scientific)
Trypsin-EDTA (0.25%; Gibco)
Tween 80 (optional; see Step 2)
Viral (FV3) or bacterial (*M. marinum*) pathogens (optional; see Step 2)

Equipment

Beckman Coulter Allegra 21R Centrifuge
Conical tubes (15-mL)
Glass capillaries
Hemocytometer
Incubator (27

5. Fluorescently label the cells with either CFSE or PKH26.

i. Resuspend up to 2 × 107 cells in each 1 mL of Diluent C (a component of the PKH labeling kit). Then mix cells with an equal volume of 4 µM PKH26. Incubate the mixture for 15 min at 27°C.

ii. Stop the reaction by adding 2 mL of ASF with FBS.

iii. Pellet the cells by centrifugation at 9000g for 30 sec at room temperature. Decant the supernatant, and wash with 1 mL of APBS.

iv. Repeat Step 5.iii two more times.

v. Resuspend the pellet in 1 mL of APBS. To determine if the cells are properly labeled with red fluorescence, place an aliquot of cells on a glass microscope slide and look for red signal under the fluorescence microscope.

vi. Resuspend up to 2.5×10^6 cells in 50 µL of 40 µM CFSE (diluted in DMSO; included in supplier package) in a microcentrifuge tube. Incubate for 15 min at 27°C.

vii. Quench CFSE by adding 250 µL of ASF with FBS, and incubate for 7 min at 27°C. Flick with fingers every 2 min.

viii. Add 1 mL of APBS and pellet the cells by centrifugation at 9000g for 30 sec at room temperature. Decant the supernatant.

ix. Repeat Step 5.viii two more times.

x. Resuspend the pellet in 1 mL of APBS. To determine if the cells are properly labeled with green fluorescence, place an aliquot of cells on a glass microscope slide and look for green signal under the fluorescence microscope.

6. Count a sample of the cells using a hemocytometer and determine cell death by Trypan blue exclusion test. Note the percent of cell death as you proceed further.

7. Inject 100,000 labeled cells intraperitoneally into J inbred tadpoles of stage 55 (3 to 4 wk of age) as follows.

i. Centrifuge the fluorescently labeled cells at 9000g for 30 sec at room temperature, and resuspend the cells in 10 µL of sterile APBS.

ii.

TROUBLESHOOTING

Problem 1 (Step 5) Poor fluorescence signal of the labeled cells is observed.

Solution When CFSE is purchased, it comes in several aliquots. Because CFSE is sensitive to humidity, reusing aliquots is not recommended. Check the fluorescence before the adoptive transfer to make sure that the fluorescence can be detected in culture.

Problem 2 (Step 5) Adoptively transferred cells not found.

Solution Ensure that the donor and recipient are both inbred J strains by skin grafting or MHC class Ia gene typing (Flajnik and Du Pasquier 1990). Since some cells are fragile and die rapidly, it is a good idea to save some cells in culture as a backup and measure cell death after a few days. If necessary, it is also possible to transfer more cells (e.g., 1×10^6 cells).

DISCUSSION

This technique can be used to study homing or infiltration of adoptively transferred cells into particular organs or tissues. For example, we used this adoptive cell transfer system to study the ability of FV3-infected PLs to cross the blood brain barrier. We have successfully visualized the donor's peritoneal macrophages infected with FV3 infiltrating into the recipient tadpole's brain at stage 55 when the BBB is fully functional (De Jesus Andino et al. 2016). In another example, adoptive cell transfer in *Xenopus* has been used to study the immunization role of heat shock proteins (hsp). When PLs pulsed with gp96 chaperoning minor H-Ag were adoptively transferred from minor H-Ag-mismatched donors to recipients, the transferred cells were successfully detected in the recipient's spleen and the appearance of tumors were delayed in these immunized recipients (Maniero and Robert 2004).

Using confocal microscopy instead of conventional fluorescence microscopy is an attractive approach to trace fluorescently labeled cells in real time. Confocal microscopy can locate the adoptive transferred cells in three-dimensional structure with high resolution. Recently, we successfully used confocal microscopy to study the dynamic structure of cell aggregation (e.g., granulomas) following *M. marinum* infection. In parallel, it is also possible to label blood vessels by injecting Texas Red Dextran into the heart (25 mg/mL; 10 µL of volume) to provide a better localization of infiltrated cells.

RECIPES

Phosphate-buffered saline (PBS)

Sodium chloride (NaCl)	6.6 g/L
Sodium phosphate (Na_2HPO_2)	1.15 g/L
Potassium phosphate (KH_2PO_4)	0.2 g/L

Reagent	Quantity
Iscove's DMEM basal medium, powdered (Gibco 12200-036)	1 package
Insulin (Sigma-Aldrich 19278-5mL)	10 mL
Nonessential amino acids (Gibco 11140-050)	10 mL
Penicillin-streptomycin (Gibco 15070-063)	10 mL
Primatone (Sheffield Products Division)	

Adoptive Transfer of Fluorescently Labeled Immune Cells in *Xenopus*

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Cold Spring Harb Protoc; doi: 10.1101/pdb.prot097592 originally published online July 6, 2018