Isolation and culture of murine peritoneal mast cells (PMCs):

Materials

6 to 9 moth old mouse

1 x Hanks" balanced solution (HBSS) without Ca^{2+} or Mg^{2+} (ice cold) (Invitrogen)

ice box

sterile 50 ml centrifuge tube (Falcon)

disposable plastic pipette

70% Ethanol

Ethrane

vacuum chamber

pair of small sharp scissors

forceps

pin nails (at least 4)

23-G needels

polystyrene platter

10 ml syringe

1 ml Pipette and pipette tips

Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen) containing 10% FBS and 1% Penicillin/Streptomycin (PMC media)

2 petri dishes

8 - 10 poly-D lysine coated cover slips

NOTE: All culture incubations are performed in a humidified 37°C, 5% CO_2 incubator.

NOTE: All solutions and equipment coming into contact with living cells must be sterile and an aseptic technique should be used accordingly.

Prepare Petri dishes with cover slips

Prepare 2 Petri dishes containing 4 - 5 cover slips coated with PDL with 2 ml PMC media and put them in a humidified incubator (37°C, 5% CO_2).

Prepare syringes loaded with 1 x HBSS

Fill one 10 ml syringe with 1 x Ca^{2+} and Mg^{2+}-free HBSS and attach 23-G needle. Put aside until required.

Perform peritoneal lavage

Anesthetize mouse with 2 ml Ethrane in a vacuum chamber for 5 – 10 min. Thereafter make a cervical dislocation by holding the mouse down with the back of a pair of scissors at the neck fold and pull at the tail until you feel the dislocation of the spines.

Then pin the mouse backward with its legs onto a polystyrene platter. With small sharp scissors dissect a square (about 2 cm in diameter) of free skin (carefully prepare off the fur) at the abdomen right above the peritoneum and disinfect it with 70% EtOH (make sure that there is no fur left anywhere in this area). Also disinfect the scissors and the forceps. Pinch and lift the abdominal wall away from the internal organs using forceps. Be sure to keep the peritoneal cavity intact.

NOTE: This will create an empty space within the peritoneal cavity where the needle can be placed for the injection of HBSS

Take the HBSS-preloaded syringes, turn needle beveled side up and pass it through the still intact peritoneal cavity directly under the forceps where the space was created in the previous step. Gently push on the plunger of the syringe until all of the HBSS has been expelled.

NOTE: Be careful not to puncture any internal organs with the needle or over-inflate the cavity ith HBSS, since this can cause internal bleeding. The peritoneum should be left intact during this step.

Make a lavage by massaging the fluid-containing area by gently squeezing the top of the cavity (below the diaphragm) and the bottom of the cavity (above the hips) and recover then as much fluid as possible with the syringe without damaging any internal organs. Collect the PMCs into the 50 ml centrifuge tube.

NOTE: Be careful not to catch any internal organs or fat, when recovering the HBSS from the peritoneal cavity, as this would contaminate the cell suspension. The recovered liquid should be cloudy, indicating the presence of cells.

Finally, pin up the abdominal wall with the forceps and make a small cut with the scissors (the opening should be just big enough to fit a disposable plastic pipette). Keep the forceps steady so that you can pipette about 2 ml of extracellular solution into the peritoneum. Carefully lavage (massage) the cavity again and recover the liquid with the disposable pipette (be careful not to suck any internal organs into the pipette since this could rupture blood vessels). Collect the PMCs into the sterile centrifuge tube. If necessary, repeat this step to harvest as much of the peritoneal cells containing HBSS as possible and end up with about 5 ml of cloudy HBSS solution.

NOTE: The whole preparation should be without any blood flow.

Count viable cells

Finally, centrifuge the cells at 800 rpm for 10 min. Remove supernatant and resuspend the pellet in 1 ml of 37°C PMC media.

Add 10 µl cell suspension to 10 µl of 0.4% trypan blue solution, then count the total nubers of live and dead cells, respectively, using a hemacytometer.

NOTE: Dead cells will take up the dye and stain blue while viatl cells will exclude the dye and be clear. Cells are routinely >99% viable.

Culture primary peritoneal mast cells

Add the cell suspension to the prepared Petri dishes. Cells can be used for patching the next day.

Alternatively, cells can be cultured in DMEM containing 10 % FBS and 1 % Penicillin/Streptomycin in a humidified incubator at 37°C and 5% CO_2 and subsequently utilized for experiments up to the second day after isolation.