PROTOCOL FOR BONE MARROW TRANSPLANTATION

Preparation of Recipient Mice for Bone Marrow Transplantation:

- One week before irradiation, the recipient mice are given acidified, antibiotic water. Water is first adjusted to pH 2.6 with concentrated HCl and then autoclaved in 1L bottles. Then, to 1L of acidic H_2O, add 10 ml of 10 mg/ml neomycin in saline (Sigma N112, 20 ml vials) and 400 l 25 mg/ml polymyxin B sulfate in water, sterilized by filtration (Sigma P4932; 5,000,000 units).

- The mice are kept on acidic, antibiotic water for 2 weeks after irradiation and then switched to acidic water without antibiotics for the rest of their lives.

- Our irradiation protocol is 1000 rads (we use a ^{137}Cesium Gammacell source) for a C57BL6/J mouse. This is given in one dose. Then 4 hours later the mice are injected (via tail vein) with bone marrow cells.

- For mice that will be fed an atherogenic diet, start the diet 6 weeks after transplantation

Preparation of Donor Bone Marrow Cells for Transplantation:

- Place the femurs from the donor mice in a small dish (on ice) containing the following medium: RPMI 1640 + 2% FBS + 10 units/ml heparin + penicillin and streptomycin (the usual concentration you use for tissue culture cells).
  The heparin (Sigma H3393 250,000 units) is prepared as a 20 mg/ml solution in sterile water, filtered, and stored at 4^{o}C. From the certificate of analysis you receive from Sigma, you can calculate how many units/ml this is equivalent to. For 500 ml, you need to add 5000 units.

- Flush the femurs with this medium using a 25G 5/8 needle. To remove bits of bone, etc., put the solution through a sterile 40-µm nylon Cell Strainer (Falcon 352340) and collect in a 50-ml tube. Bring the volume to 50 ml with medium and then centrifuge at 2000 rpm (900 x g), 10 min, 4^{o}C.

- Wash the cell pellet twice with 50 ml of serum-free RPMI (RPMI 1640 + 20 mM Hepes + penicillin and streptomycin, adjusted pH to 7.4 before filtering). Centrifuge at 2000 rpm, 5min, 4^{o}C.

- Resuspend the cells in 25 ml of serum-free RPMI. Determine cell number by mixing 20 l cells and 20 l 2% acetic acid, waiting 1 minute, and then counting using a hemocytometer. (This acetic acid lyses the cells, leaving only nuclei, which are much easier to count.)

- Re-centrifuge the cells. Resuspend in serum-free RPMI to give a final concentration of 1 x 10^7 cells/ml. Dispense about 1 ml of the cell suspension into 1.5 ml Eppendorf tubes and keep these on ice.

- Inject 0.5 ml, *i.e.*, 5 x 10^6 cells, by tail vein into each of the recently irradiated recipient mice.