T. Springer May 1984

PURIFICATION OF MONONUCLEAR CELLS AND NEUTROPHILS ON DENSITY GRADIENTS.

1. All procedures are sterile. Collect blood into a syringe with 10 U preservative-free heparin/ml whole blood or 0.14ml/ml anti-coagulant citrate phosphate dextrose adenine (CPDA). The latter is a good preservative for storage or shipment of whole blood. Typically we collect 50 ml of blood into a 60 ml syringe. Typical yields are 0.5 - 1 x 10^6 lymphs/ml and 1 - 2 x 10^6 granulocytes/ml. A more accurate estimate of the amount of blood necessary is calculated as follows:

$$\text{Volume of Blood} = \frac{\text{\# of lymphs needed}}{\text{WBC count} \times 10^3 \times \% \text{ lymphs} \times 0.3}$$

2. Add 20% by volume 6.0% dextran T-500 (Pharmacia) in phosphate-buffered saline (0.01M phosphate pH 7.2, .13M NaCl). Clamp the syringe vertical, with the needle upright, and allow the RBC to settle for 30 min (not longer) at room temp. Small syringes need less settling time.

3. Bend the needle so the bevel is pointed down and expel the leukocyte-rich supernatant. Place ~25 ml in a clear 50 ml polystyrene or glass tube.

4. Underlay carefully with 12 ml d = 1.08 ficoll-hypaque using pipet-aid. Underlay further with d = 1.106 ficoll-hypaque.

5. Centrifuge 2500 RPM (1,000-1,200g) 25 min. at 25° or 4°. Remove cells from interfaces with Pasteur pipette. Mononuclear cells are found in upper interface, neutrophils in bottom interface. The pellet contains RBC and white cells which are enriched in (~40%) eosinophils. Platelets will be in upper interface, and can be mostly removed by careful washing.

6. Dilute interfaces at least 2x with RPMI 1640 and centrifuge 1000 RPM x 7 min. Use 2.5 mM EDTA in the RPMI to prevent platelet aggregation and pipet up and down to thoroughly resuspend pellets during washes of mononuclear cells. Mononuclear cells should be washed at least 3x, granulocytes 1 or 2x. Resuspend with RPMI 1640 + 10% FCS, 1,000 RPM x 10 min. Count mononuclear cells and neutrophils.

Ficoll-Hypaque: 200 ml of 50% Hypaque-M (Winthrop Labs, NY) (measure, do not trust vol in vials) + 75 g Ficoll 400 (Pharmacia) + 325 ml DH_2O, dissolve and dilute to 600 ml (16.7% Hypaque, 12.5% Ficoll). Measure density by placing 10 ml in volumetric flask and weighing on analytic balance (should be 1.125 - 1.135). Dilute entire batch to d = 1.106. Confirm by weighing in volumetric flask. Dilute 3/5 of entire batch to d = 1.08. Confirm density. Millipore filter and store protected from light; store most of batch at 4° and working solution at 20°.