Concanavalin A-Elicited Mouse Peritoneal Macrophages

Basic Procedure: [Smith CW, Goldman AS. Effects of concanavalin A and pokeweed mitogen *in vivo* on mouse peritoneal macrophages. Exp Cell Res. 1972 Aug;73(2):394-8]

1. Concanavalin A: L-7647 from Sigma. Make 2 mg/ml stock in sterile PBS and store aliquots at –70^{o}C.

2. Injection: Add 20 µl of stock (40 µg) to 480 µl sterile PBS and inject i.p.

3. Harvesting: Harvest peritoneal macrophages 3-4 days later and plate in DMEM containing 10% FBS and 20% L-cell conditioned medium. Discard any harvests that have contamination from the intestinal lumen or blood. Wash away non-adherent cells (non-macrophages) after 30-60 min of culture.

Important Notes from Tabas Laboratory:

1. L-cell conditioned medium: At the time fresh medium is added to the L-cells for the 24-h period of conditioning/collection, the monolayer of L-cells should not be too sparse or too confluent: ~80% confluency is ideal.

2. Age of mice and confluency of wells:

(a) The minimum age is 6 weeks old—these are very small and technically very difficult to work with, but with experience cells can be obtained from one mouse to plate one well of a 12-well dish. Mice that are 8 weeks old are still small but routinely used in our lab—the cells from an 8 wk/o mouse can be used for 1.5 wells of a 12-well dish with proper care. Mice that are 10-12 weeks old are technically easier to work with and may yield enough cells for two wells of a 12-well dish.

(b) Ideally, the wells should be 70-80% confluent with adherent cells on the day of harvesting, and the cells should be used the next day. If the cells are more sparse, they can be grown in medium for few days and then used when the monolayers are more confluent. However: (i) if the cells are plated too sparsely (<50% confluency), they will not grow well and may die; and (ii) if the cells are >100% confluent (*i.e.*, overcrowded), biological artifacts can occur, but the experiment can be salvaged by washing the cells soon after harvest to dislodge the over-crowded cells.

3. Sex and genetic background of mice: We routinely use female mice, but we get similar results in our projects with male mice. We usually use C57BL6J mice; when we use other strains, we design a pilot experiment to determine if the biological effect being studied is similar to our C57 results.

4. Harvesting: Cells can be harvested 3 or 4 days after ConA injection—we have not found major differences between these two intervals. **Important Don'ts:** Do not harvest more than 5 mice at a time. Do not allow the collected cells to sit in cold PBS for more than 1 hour before plating.