Assay for Apoptosis of FC-Loaded Macrophages

(Annexin/propidium iodide kit is from Molecular Probes)

1. 1st day: inject mice i.p. with concanavalin A, 40 µg/mouse, diluted in PBS.

2. 4th day: harvest MΦs in the morning, plate on coverslip dishes @ 10 dishes per mouse in "full medium": DMEM containing 20% L cell-conditioned medium, 10% FBS, 1% glutamine, and pen/strep.

3. Change the medium in the evening, and culture overnight in the same medium.

4. 5th day: MΦs should be 80-90% confluent but not more. Incubate the Mϕs under control or FC-loading conditions (below) either for 7-9 h in DMEM containing 1% FBS, 1% glutamine, and pen/strep or for 18-24 h in full medium. With FC loading, the Mϕs should show subtle morphological changes (*e.g.* rounding) by the end of these incubations.

Control: 1% FBS-DMEM medium ± acetyl-LDL (100 µg/ml) alone or 58035 (10 µg/ml) alone. 58035 (ACAT inhibitor) is from Novartis; the stock solution is 10 mg/ml in DMSO.

FC loading: Acetyl-LDL at 100 µg/ml plus 58035 (from Novartis) at 10 µg/ml (stock = 10 mg/ml in DMSO)

5. At the end of the incubations, wash cells gently with PBS 3X.

7. Incubate for 15 min at room temperature in the dark with 100 µl 1X binding buffer (stock sol: 5X, diluted with dH2O), 5 µl fluorescent annexin V, and 1 µl propidium iodide (100 µg/ml; stock sol: 1 mg/ml, diluted with 1x binding buffer)

8. Observe by fluorescence microscopy.