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Sterol Accumulation in Macrophage Foam Cell Lysosomes Inhibits the Lysosome's Ability to Maintain Acidic pH by Inhibiting Vacuolar-ATPases

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Macrophage foam cells in atherosclerosis develop via massive accumulations of free (FC) and esterified (CE) cholesterol, much of which is within lysosomes. In cell culture, macrophages incubated with mildly oxidized LDL (ox-LDL), small aggregates of LDL (agg-LDL), or CE-rich lipid dispersions (LD) mimic this lysosomal accumulation. Lysosomal CE accumulation is the result of inhibition of lipoprotein CE hydrolysis. CE hydrolysis inhibition was not dependent upon the presence of oxidized lipids and was not immediate, but occurred only after substantial FC accumulation. This suggests a linkage of inhibition to FC accumulation. Using a pH dependant fluorescent dye, we observed a neutralization of the lysosomal pH, suggesting that a factor shared between ox-LDL, agg-LDL, and LD inhibits lysosome acidification and thus interrupts hydrolysis. Furthermore, analysis of lysosomal pH indicated that the inhibition of CE hydrolysis occurred concomitant with a failure of the lysosomes to maintain the necessary acidity for enzyme function. To explore whether lysosomal FC could explain the increased pH and lack of CE hydrolysis, we induced FC accumulation in lysosomes. This was done by incubating cells with 100 μg/ml ac-LDL (which does not induce lysosomal FC accumulation nor failure of lysosomal acidification) in the presence of 10 μg/mL progesterone. Progesterone inhibited FC traffic out of lysosomes producing an increase in lysosomal FC and a concomitant neutralization of lysosomes. The pH inhibition was not seen with ac-LDL alone or progesterone alone. Other aspects of cell metabolism appeared normal. Lysosomal acidification is produced by lysosomal vacuolar ATPases (v-ATPase). The quenching of acridine orange fluorescence in isolated lysosomes measures v-ATPase activity after stimulation with ATP. A two-fold enrichment of lysosomal membrane FC using FC-containing β-cyclodextrins as FC donors significantly inhibited v-ATPase activity. Further increases in FC completely abolished activity. These data indicate that increased lysosomal membrane FC content can inhibit v-ATPase in human cells and suggests that the lysosomal neutralization seen in cholesterol loaded cells may be the result of FC partitioning into the lysosomal membrane. Our data also implies that this is a key step in the lysosomal CE accumulation found in human atherosclerosis. The interruption of lysosome function and sequestration of FC and CE in lysosomes away from normal cholesterol homeostatic mechanisms would be expected to profoundly affect foam cell biology.