Role of Plasmalogens in Macrophage Function

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Macrophages play a key role in the initiation and development of atherosclerosis. Two types of macrophages have been described in atherosclerotic lesions, classically-activated macrophages, which promote the progression of atherosclerosis, and alternatively-activated macrophages, which are characteristic of regressing atherosclerotic plaques. The latter are necessary for removal of apoptotic cells and cell debris from plaques. Macrophage phagocytosis has been shown to be severely impaired in advanced atherosclerotic plaques.

Plasmalogens are a class of membrane glycerophospholipids containing a fatty alcohol with a vinyl ether bond at the sn-1 position, and enriched in polyunsaturated fatty acids at the sn-2 position. Plasmalogens have been proposed to be atheroprotective, mainly because of their anti-oxidant properties, and are involved in the efflux of cholesterol through of HDL in macrophages. In addition, plasmalogens have been found to be decreased in patients with atherosclerosis. To investigate the effect of plasmalogens on macrophage phagocytosis we used the murine macrophage-like cell line RAW264.7 and the mutant cell lines RAW12 and RAW108, both with reduced plasmalogen content. We observed that the plasmalogen-deficient cells showed very low indices of phagocytosis. When the mutant cells were treated with lysoplasmenylethanolamine we observed a partial recovery of the response. Incubation with lysoplasmenylethanolamine induced changes in the plasma membrane, leading to increases in the number and size of lipid rafts. These microdomains are intimately related to phagocytic and cellular signaling processes. Our results suggest that plasmalogens play an important role in the mediation of phagocytosis in macrophages and could be envisioned as a viable therapeutic strategy to prevent the progression of advanced atherosclerotic lesions.


FIGURE 1 – RAW264.7 cells, and plasmalogen-deficient RAW.12, RAW.108 cells were analyzed for phagocytosis of fluorescent zymosan and opsonized zymosan particles (30 min incubations) by confocal microscopy (red color, central columns). DAPI (1μg/ml) was used to stain the nuclei (blue; left columns). Right columns show the Nomarski images. Lower panels are the average of three independent experiments.

FIGURE 2 – LPE plasmalogen restores phagocytosis of opsonized zymosan in RAW.12 and RAW.108 deficient cells and its effect on RAW264.7. The cells, either untreated or pre-treated with 10 μM LPE or LPE plasmalogen for 10 minutes, were analyzed for phagocytosis of fluorescent opsonized zymosan particles (incubated for 30 minutes) by confocal microscopy.

FIGURE 3 – Analysis of lipid raft domains of RAW264.7, RAW.12, and RAW.108 cells. The cells, either untreated or treated with 10 μM LPE or LPE plasmalogen for 30 minutes, as indicated, were analyzed to determine the different characteristics of lipid raft domains, stained with fluorescent cholera toxin subunits β by confocal microscopy (red color, middle columns). DAPI (1 μg/ml) was used to mark the nuclei (blue; left columns). Right columns show the Nomarski images.
FIGURE 4 – Analysis of lipid raft domains analysis of RAW264.7, RAW.12, RAW.108 cells exposed to a phagocytotic stimulus. The cells, either untreated or pre-treated with 10 μM LPE or LPE plasmalogen for 10 minutes, as indicated, were incubated with opsonized latex beads for 15 minutes, and afterward analyzed to determine the different characteristics of lipid raft domains. These were stained with fluorescent cholera toxin subunits β by confocal microscopy (red color, middle columns). DAPI (1 μg/ml) was used to mark the nuclei (blue; left columns). Right columns show the Nomarski images.

FIGURE 5 – Effects LPE plasmalogen on phagocytosis of opsonized zymosan in RAW264.7, RAW.12, and RAW.108 cells. The cells, either untreated or pre-treated with 10 μM LPE or LPE plasmalogen for 10 min, as indicated, were analyzed for phagocytosis of fluorescent opsonized zymosan particles (30 min incubations) by flow cytometry.

FIGURE 6 – Analysis of lipid raft domains analysis of RAW264.7, RAW.12, RAW.108 cells exposed to a phagocytotic stimulus. The cells, either untreated or pre-treated with 10 μM LPE or LPE plasmalogen for 10 minutes, were incubated with opsonized zymosan for 30 minutes, and then analyzed to determine the lipid raft domains by flow cytometry.

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REFERENCES


