Investigating the role of phosphatidylethanolamine esterified to n-3 and n-6 fatty acids on RAW 264.7 macrophages-mediated immune response: A Lipidomics analysis

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Exacerbated inflammation is a common cause of cellular and tissue damage, triggering or exacerbating conditions with an inflammatory component. Macrophages are effector cells of the immune system, with an essential role in the activation and resolution phases of the inflammatory response. Recent evidence suggests that phospholipids (PL) can act as immunomodulators of these immune cells. Therefore, this study aims to investigate the influence of phosphatidylethanolamine (PE) esterified with *n*-3 (PE 18:0/22:6) or *n*-6 (PE 18:0/20:4) polyunsaturated fatty acids, on the activation and orchestration of the immune response mediated by murine macrophages (RAW 264.7) using an *in vitro* model of inflammation induced by lipopolysaccharides (LPS). The first stage of our research was to determine cell viability, as well as to evaluate the anti-inflammatory potential of PL. The highest concentrations tested (100 and 200 μM) of PE 18:0/22:6 (*n*-3) and 18:0/20:4 (*n*-6) significantly reduced nitrite production by LPS-activated macrophages, suggesting a pro-resolving effect. To investigate the lipidome of macrophages following PE exposure, a lipidomic analysis based on mass-spectrometry was undertaken. Macrophage exposure to PE *n*-3 and *n*-6 alone and in the presence of LPS resulted in a significant reprogramming of macrophage lipidome, with 319 lipid species significantly different among conditions. A marked increase in PL species linked with the pro-inflammatory response (sphingomyelins and ceramides), was identified in PE *n*-3 and *n*-6 activated-macrophages. Constratingly, in the presence of LPS, PE *n*-3 and *n*-6 activated-macrophages show an increase in PL species, namely phosphatidylinositol, compared to conditions without LPS. Overall, our findings imply that PE supplementation significantly impacts lipid metabolism in macrophages. This study enhances our understanding of the molecular mechanisms that govern the immunomodulatory properties of certain PL species.