- Cardiolipin (CL) is a mitochondrial phospholipid that may be released into the extracellular space from dying cells, where it can interact with nearby cells including astrocytes.

- Astrocytes are the main support cells in the central nervous system (CNS), and they have a role in regulating immune responses.
- Astrocytes carry out phagocytosis, an essential homeostatic function.
- Astrocytes secrete both pro-inflammatory cytokines, such as monocyte chemotactic protein (MCP)-1, and anti-inflammatory cytokines, such as interferon (IFN)-β.
- Activated astrocytes are characterized by an upregulated expression of glial fibrillary acidic protein (GFAP).
- In a state of chronic neuroinflammation, astrocytes become overactivated, leading to neurodegeneration in Alzheimer's disease (AD).

**HYPOTHESIS**

We hypothesize that extracellular CL reduces astrocyte activation, resulting in an altered neurotoxic secretome and phagocytic profile.

**RESULTS**

Extracellular CL increases the phagocytic activity of primary murine astrocytes.

Figure 2. Extracellular CL (20 µg/ml) upregulates phagocytosis of latex beads by primary murine astrocytes. This effect is blocked by the addition of cytochalasin B (CytoB), an inhibitor of actin polymerization. The average total fluorescence of I34-234 randomly selected cells (means ± SEM) from four independent experiments are presented. **P<0.01 according to the Dunnett’s post-hoc test. P and F values for the one-way randomized blocks ANOVA are also shown.

Extracellular CL upregulates the secretion of MCP-1 by astrocytes.

Figure 3. (A) Extracellular CL (0.5-20 µg/ml) alone upregulates the secretion of MCP-1 by U118 MG astrocytic cells; (B) however, it has no effect on the secretion by lipopolysaccharide (LPS)-stimulated cells. MCP-1 was measured using ELISAs. Data (means ± SEM) from four independent experiments are presented. **P<0.01 according to the Dunnett’s post-hoc test. P and F values for the one-way randomized blocks ANOVA are also shown. The detection limit of the ELISAs are represented as the dotted line. The secretion of MCP-1 from control cells is represented as the dashed line.

Extracellular CL inhibits astrocyte-mediated cytotoxicity towards neurons.

Figure 4. Extracellular CL (0.5-20 µg/ml) inhibits cytotoxicity of (A) LPS-stimulated human U118 MG astrocytes towards (B) human SH-SY5Y neuronal cells. Viability was measured using the MTT assay. Data (means ± SEM) from four independent experiments are presented. *P<0.05 according to the Dunnett’s post-hoc test. P and F values for the one-way randomized blocks ANOVA are also shown.

Extracellular CL induces the secretion of IFN-β and downregulates GFAP expression.

Figure 5. (A) Extracellular CL (20 µg/ml) upregulates the secretion of IFN-β from unstimulated U118 MG astrocytic cells and (B) downregulates the expression of GFAP by LPS-stimulated U118 MG astrocytes. Immunoblotting was used to measure the expression of IFN-β and GFAP. (C) Representative bands of GFAP, IFN-β and β-actin for each treatment are shown. Data (means ± SEM) from (A) three or (B) four independent experiments performed on different days are presented. The data are expressed in fold-change in expression compared to control cells exposed to growth medium only and are normalized to β-actin. **P<0.01 and ***P<0.001 according to Tukey’s post-hoc test. P and F values for the one-way randomized blocks ANOVA are shown.

**CONCLUSIONS**

- Extracellular CL increases primary murine astrocyte phagocytosis which is commonly viewed as a protective function.
- Extracellular CL inhibits the release of cytotoxins and reduces neuronal death.
- Extracellular CL increases the expression of IFN-β and downregulates the expression of GFAP, thus indicating that CL may reduce chronic neuroinflammation.
- Extracellular CL upregulates the secretion of MCP-1, suggesting its complex regulation of neuroinflammation.
- Overall, we demonstrate that cardiolipin interacts with astrocytes to regulate their select immune functions dysregulated in AD.

**ABBREVIATIONS**

AD: Alzheimer’s disease
GFAP: Glial fibrillary acidic protein
ANOVA: Analysis of variance
IFN: Interferon
CL: Cardiolipin
IL: Interleukin
CytoB: Cytochalasin B
LPS: Lipopolysaccharide
CNS: Central Nervous System
MCP: Monocyte chemotactic protein
ELISA: Enzyme-linked immunosorbent assay
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
SEM: Standard error of the mean

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