Extracellular cardiolipin regulates select immune functions of astrocytes

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INTRODUCTION

- 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

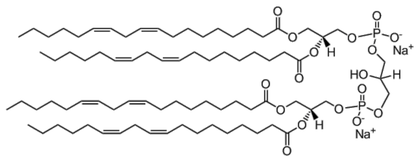

Figure 1. The most abundant structure of cardiolipin in animals including humans and mice

- 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 chemoattractant 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

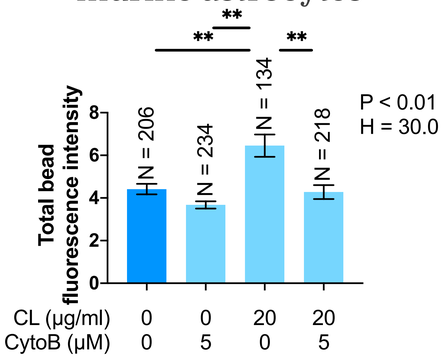

Figure 2. Extracellular CL (20 µg/ml) upregulates phagocytosis of latex beads by primary murine astrocytes. This effect is blocked by the addition of cytochalasin B (CytoB), an inhibitor of actin polymerization. The average total fluorescence of 134-234 randomly selected cells (means ± SEM) from four independent experiments are presented. The selection and analysis of the cells were blinded. ** P<0.01 according to the Dunn's post-hoc test. P and F values for the Kruskal-Wallis test are shown.

Extracellular CL upregulates the secretion of MCP-1 by astrocytes

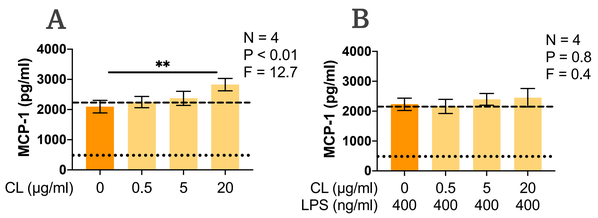

Figure 3. **(A)** Extracellular CL (0.5-20 µg/ml) alone upregulates the secretion of MCP-1 by U118 MG astrocytic cells; **(B)** however, it has no effect on the secretion by lipopolysaccharide (LPS)-stimulated cells. MCP-1 was measured using ELISAs. Data (means ± SEM) from four independent experiments 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

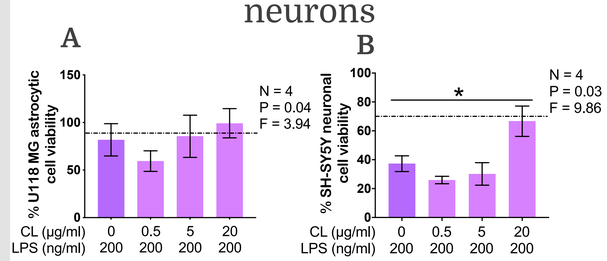

Figure 4. Extracellular CL (0.5-20 µg/ml) inhibits cytotoxicity of **(A)** LPS-stimulated human U118 MG astrocytes towards **(B)** human SH-SY5Y neuronal cells. Viability was measured using the MTT assay. Data (means ± SEM) from four independent experiments are presented. * P<0.05 according to the Dunnett's 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

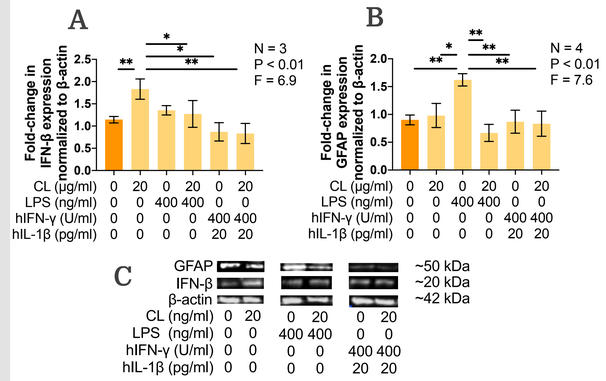

Figure 5. **(A)** Extracellular CL (20 µg/ml) upregulates the secretion of IFN-β from unstimulated U118 MG astrocytic cells and **(B)** downregulates the expression of GFAP by LPS-stimulated U118 MG astrocytes. Immunoblotting was used to measure the expression of IFN-β and GFAP. **(C)** Representative 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<0.05 and **P<0.01 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 chemoattractant 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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