Neurotrophin receptor ligands modulate select immune functions of microglia

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Introduction

Alzheimer's disease

- Alzheimer's disease (AD) is a severely debilitating brain disorder characterized by progressive cognitive decline and dementia.
- An accumulation of amyloid beta (Aβ) protein plaques and neurofibrillary tangles, as well as a state of chronic neuroinflammation, are the main hallmarks of AD pathology.

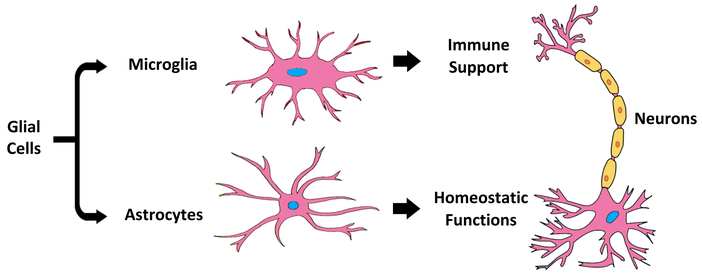

Figure 1. In healthy central nervous systems, neurons are supported and protected by glial cells, such as microglia and astrocytes, allowing optimal conditions for function and survival.

- Microglia, the resident immune cells of the brain, respond to cellular debris and pathogenic stimuli by becoming activated and mounting an immune response.
- Upon activation, microglia are phenotypically altered and release various inflammatory molecules, including proinflammatory cytokines and cytotoxic molecules.
- In AD, microglia are activated by Aβ, causing a release of proinflammatory cytokines and cytotoxins, resulting in further activation of microglia through a positive feedback mechanism. This leads to over-activation of microglia and neuron death due to increased cytotoxin secretion.
- In addition, the phagocytic activity of microglia is down-regulated in AD

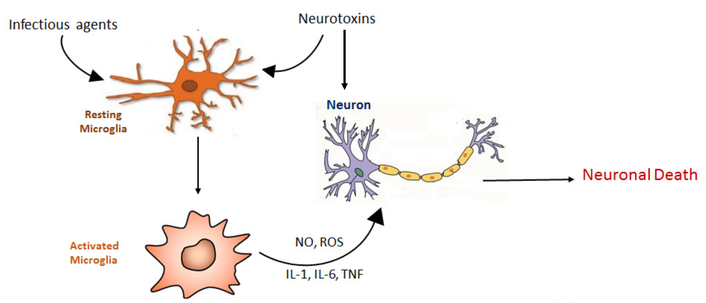

Figure 2. Chronic activation of microglia by molecular structures such as amyloid beta (A*β*) protein plaques and neurofibrillary tangles cause release of neurotoxins. Abbreviations: *α*-synuclein (*α*-Syn), nitric oxide (NO), reactive oxygen species (ROS), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF)
https://www.sigmaaldrich.com/technical-documents/articles/biology/microglia-in-neuroinflammation.html

- Microglia express the neurotrophin receptors (NTRs) TrkA, TrkB and p75NTR that respond to neuroprotective neurotrophic factors (NTFs) such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF).
- Microglial immune responses are modulated by p75NTR, TrkA and TrkB activation.
- BDNF and NGF reduce activation of microglia by binding to TrkA and TrkB
- BDNF precursor proBDNF and LM11A-31, a nonpeptide similar to NGF loop 1, are ligands of p75NTR.
- The effects of these NTR ligands on microglia activation are not characterized.

Hypothesis

LM11A-31 and proBDNF reduce the neurotoxic effect and secretion of ROS and RNS by activated microglia, as well as modulate microglial phagocytic activity

Methods

- Murine BV-2 microglia: Stimulated with lipopolysaccharide (LPS) 24 hours after NTR ligand treatment → Supernatant removed for detection of RNS using the Griess assay
- Human THP-1 microglia-like cells: Stimulated with interferon-gamma (IFN-γ) and LPS 24 hours after treatment with NTR ligands → Supernatant tested for level of inflammatory cytokines using the enzyme-linked immunosorbent assay (ELISA).
- SH-SY5Y neuronal cells: Cell viability tested with the MTT and propidium iodide assays 72 hours after treatment with above mentioned THP-1 supernatant.
- Murine BV-2 microglia: Stimulated with lipopolysaccharide (LPS) 15 min after NTR ligand treatment → incubated with fluorescently labelled latex beads for 1 hour → imaged and analyzed for internal fluorescence to assess phagocytic activity.
- HL-60 microglia-like cells: Treated with NTR ligands 6 days after differentiation with dimethyl sulfoxide (DMSO) → Primed with 500 ng/ml LPS 15 minutes after treatment → Respiratory burst induced by N-formyl-L-methionyl-L-leucyl-phenylalanine (FMLP) 24 hours later to detect ROS secretion as increased chemiluminescence signal.

Results

proBDNF upregulates phagocytosis of latex beads by BV-2 microglia

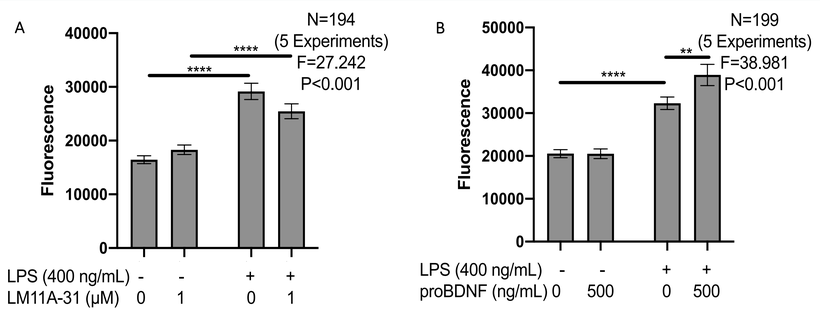

Figure 3. (A) 1μM LM11A-31 had no effect on BV-2 cell phagocytic activity, and **(B)** 500 ng/mL proBDNF causes a significant increase in BV-2 microglial phagocytic activity, as shown by an increased ingestion of fluorescent beads.

LM11A-31 and proBDNF have no effects on RNS production and the secretion of select cytokines and neurotoxins (data not shown)

- LM11A-31 and proBDNF do not affect RNS secretion by murine microglial BV-2 cells as shown by the Griess assay
- LM11A-31 and proBDNF do not affect MCP-1 secretion by human THP-1 microglia-like cells as shown by ELISA
- LM11A-31 and proBDNF have no effect on the viability of SH-SY5Y neuroblastoma cells following their exposure to cytotoxic THP-1 microglia-like cell supernatants.

NTR ligands modulate ROS secretion by DMSO-differentiated human HL-60 microglia-like cells

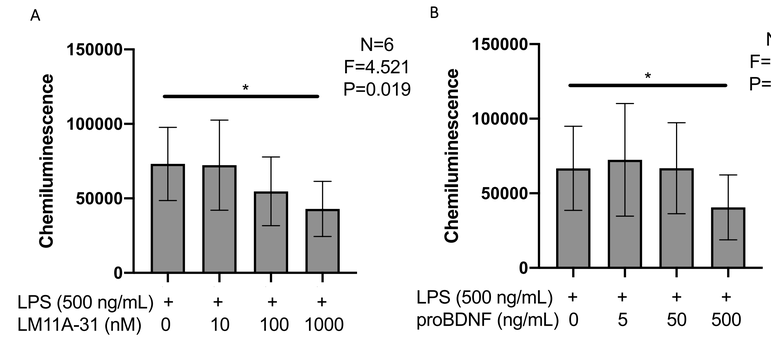

Figure 4. (A) 1μM LM11A-31 and **(B)** 500 ng/mL proBDNF cause a significant decrease in ROS secretion by LPS-primed and stimulated HL-60 microglia-like cells compared to vehicle treated controls, as shown by reduced chemiluminescence signal.

Conclusions

- LM11A-31 and proBDNF have no effect on RNS secretion by BV-2 murine microglia or secretion of MCP-1 and cytotoxins by THP-1 human microglia-like cells.
- LM11A-31 has no effect on the phagocytic activity of BV-2 murine microglia but proBDNF increases the phagocytic activity of these cells.
- LM11A-31 and proBDNF significantly decrease ROS secretion by LPS-primed and FMLP-stimulated HL-60 human microglia-like cells.
- These NTR ligands may have potential therapeutic applications in neuroinflammation and AD.

Abbreviations

AD	Alzheimer's disease	IL	Interleukin
Aβ	Amyloid beta	LPS	Lipopolysaccharide
BDNF	Brain-derived neurotrophic factor	NGF	Nerve growth factor
DMSO	Dimethyl sulfoxide	NTFs	Neurotrophic factors
ELISA	Enzyme-linked immunosorbent assay	NTRs	Neurotrophin receptors
FMLP	N-formyl-L-methionyl-L-leucyl-phenylalanine	ROS	Reactive oxygen species
IFN-γ	Interferon-gamma	RNS	Reactive nitrogen species

Acknowledgements

Figure 1 was created with BioRender.com

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