IL4 and IL6 induce pro-survival reactivity and ALOX12 activation in human astrocytes.

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Introduction

Parkinson’s disease (PD), a progressive neurodegenerative disease that affects mostly individuals over 60 years of age, represents a growing economic and social burden as the size of the elderly population increases. The hallmark of PD is the death of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), a structure of the midbrain that is crucial for modulating the initiation of motor movement among other specific cognitive and emotion processing functions. Currently, there is no cure for PD and palliative therapies provide only temporary relief of symptoms as neurodegeneration progresses. Astrocytes, major players in energetic neuronal support, synaptic maintenance, and ion and neurotransmitter homeostasis, contribute to the etiology of PD either by gain of toxic function or loss of survival support for the DA neurons (Escarin et al, 2021). There is an inflammatory component in the PD pathology, with activation of microglial cells and astrocytes noticed in postmortem brains (Badanjak et al, 2021). Maresin 1 (Mar1), a bioactive lipid and derivative of DHA, is a signaling molecule that was shown to exert its anti-inflammatory function on reactive microglial cells (Yin et al, 2019). Mar1 is synthesized by ALOX12, an enzyme expressed in astrocytes, neurons, and microglia and can be used by the three types of cells to convey a pro-survival message. In addition, PLA2G6 is highly expressed in SNpc, providing the precursor, free DHA, to ALOX12 to be converted to Mar1 (Fig.1). We hypothesized that astrocytes up-regulate and release Maresin-1 in response to certain cytokines to promote homeostasis. Here we uncover the effects of IL6 and IL4 in the transformation of astrocytes into pro-survival mode.

Results

![Figure 2: Mar1 restores proliferation in astrocytes undergoing phenotypic changes induced by TNFa and IFNy.](image)

- TNFa and IFNy induced the activation of NFkB/p65 and Mar-1 prevented the transcription factor nuclear translocation.

- IL4 increased the transcription of IL6 in human astrocytes.

- IL6 increased the transcription of HMGBl but not its own expression.

- IL6 also activated ALOX12 that was noticed in the augmented production of 14-HDHA and 12-HETE, products of the enzyme from two different substrates: Docosahexaenoic acid and Arachidonic acid.

- Overall, IL4 induced the expression of IL6 which activates the synthesis of Mar-1 via activation of ALOX12.

Materials & Methods

**Human and rat astrocyte culture:** primary cultures of astrocytes (Cell Applications Inc., San Diego, CA) were cultured following media and directions provided by distributors. Briefly, rat and astrocyte cultures were thawed and plated at passage 2 and expanded up to passage 5. The cells were plated and treated as described in each figure.

**Immunocytochemistry:** Immunostaining took place as follows: cells were fixed using 4% paraformaldehyde solution for 20 min, washed with PBS and permeabilized with 0.1% Triton-X. After blocking with 1% BSA and 10% Donkey normal serum, primary culture was added overnight in humid chambers at 4°C. The cells were washed and incubated for one hour with secondary antibody conjugated with Alexa-fluor 555. DAPI (Thermo Fisher cat# DI306) was used for nuclear, and cell mask (Thermo Fisher cat# C10046) for cell membrane staining. Z-stacks were obtained for 5 random fields in a FluoView 3000 laser confocal microscope. Images were converted to and processed using IMARIS 9.9 using the Cell module.

**Real-Time PCR:** Cells were scraped using RLT buffer and processed for total RNA extraction using RNAeasy plus kit (Qiagen, Germantown, MD). Total RNA was measured using Nanodrop. First strand synthesis of cDNA was performed using iScript Mastermix cDNA synthesis kit (BioRAD, Hercules CA). Real-Time PCR was performed using Taq-Man pre-design primers from LifeTechnologies (ThermoFisher, Waltham, MA).

**Statistical analysis:** q-PCR CT were processed by the deltaCT method. Plots and statistical treatment were performed using GraphPad 10.0. The data was first analyzed using one-way analysis of variance (ANOVA) and multiple comparisons via Tukey’s honest significant differences test. The pairwise comparison was performed using a two-tailed Student’s t-test.

**Future Directions**

In future studies we will determine the mechanisms by which IL6 induces activation of ALOX12 and if that counteracts the inflammatory effects of TNFa and INFy.

**References**


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