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

Bailey Haynes[1,2], E. Tendayi Mpofu[1], Tonya Chaney[1,3], Marbella Maristany[1,4], Jorgelina Calandria PhD[1]

[1]Louisiana State University Health Sciences Center, Neuroscience Center of Excellence, New Orleans, LA, [2]Vanderbilt University, Nashville, TN, [3]Xavier University, New Orleans, LA, [4]The Willow School, New Orleans, LA

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 proceeds. 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 (Escartin 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.

Figure 1

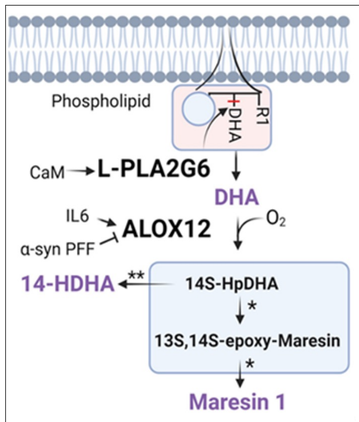

Figure 1: Proposed synthetic pathway of Maresin 1 (Mar1) in astrocytes. DHA is hydrolyzed by L-PLA2G6 from membrane phospholipid. L-PLA2G6 activity is modulated by Calmodulin (CaM) (Zhou et al, 2016). DHA is converted to 14S-peroxyde-DHA (14S-HpDHA) by ALOX12. ALOX12 activity is increased by IL6 and inhibited by α-syn PFF in astrocytes. 14S-HpDHA is converted enzymatically to 13S,14S-epoxy-Mar and subsequently to Mar1 (Serhan et al, 2015).14-HpDHA is highly unstable; what is not enzymatically transformed, will be converted to 14-HDHA spontaneously. Because Mar1 works at very low concentrations endogenously, 14-HDHA increase is an indication that the synthesis was activated. Thus, by LC-MS/MS, we measure free DHA (L-PLA2G6 activity), 14-HDHA (ALOX12 activity) and Mar1 (purple).

Results

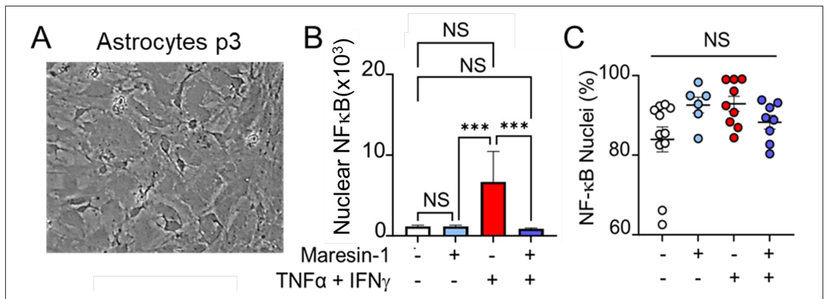

Figure 2: Mar1 restores p65 status in astrocytes undergoing phenotypes changes induced by TNFα and IFNγ. A) Bright field image of rat astrocytes passage 3 (p3) used in this experiments. B-C) Quantification of nuclear NFkB/p65 (intensity B; number of cells C) using Imaris 10.0 software of rat astrocytes treated with 10 ng/ml TNFα and 20 ng/ml IFNγ for 24 hours in the presence or absence of 200nM Mar1 and immunostained using anti-p65 (Genetex cat# GTX102090). ***p<0.0001. ANOVA plus Tukey's HSD.

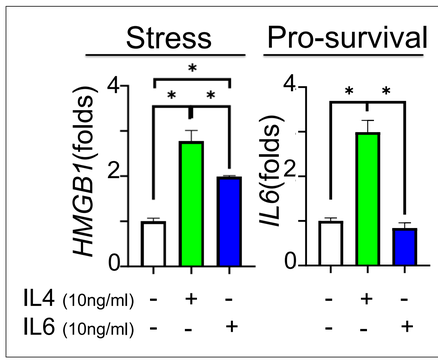

Figure 3: IL4 and IL6 trigger beneficial stress responses in human astrocytes that induce activation of Mar-1 synthesis. Expression of HMGB1, a stress alarmin was induced by the addition of 10ng/ml of IL4 and IL6 in astrocytes. Addition of IL6 did not induce its own expression but it increased the transcription of IL4 which is an activator of ALOX12, an enzyme involved in the synthesis of Mar-1. *p<0.001 . ANOVA plus Tukey's HSD.

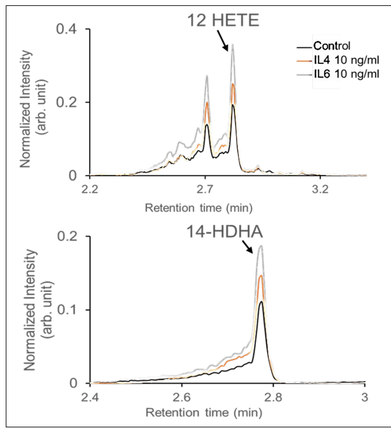

Figure 4: IL4 and IL6 enhance the activity of ALOX12 in human astrocytes in culture. Human astrocytes were incubated with 10ng/ml of IL4 or 10ng/ml of IL6 for 24 hours. Lipids were extracted from the medium with Waters™ Sep-Pak C18 cartridges and processed by LC-MS/MS. The chromatograms were normalized using the internal standard (Arachidonic Acid (AA) d8) and overlapped to appreciate the differences in the peak heights. 12-HETE is the product of lipoxygenation of ALOX12 taking as substrate arachidonic acid (AA) while 14-HDHA is the stabilization of peroxide in carbon 14 of the DHA (14-HpDHA), the product of the same enzyme when the substrate is DHA (Fig. 1).

Conclusions

•TNFα and IFNγ 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 HMGB1 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.

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 TNFα and INFγ.

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 4C. The cells were washed and incubated for one hour with secondary antibody conjugated with Alexa-fluor 555. DAPI (Thermo Fisher cat# D1306) 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, Whaltham, 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.**

References

Badanjak K, Fixemer S, Smajić S, Skupin A, Grünewald A. The Contribution of Microglia to Neuroinflammation in Parkinson's Disease. Int J Mol Sci. 2021 Apr 28;22(9):4676. PMCID: PMC8125756

Yin P, Wang X, Wang S, Wei Y, Feng J, Zhu M. Maresin 1 Improves Cognitive Decline and Ameliorates Inflammation in a Mouse Model of Alzheimer's Disease. Front Cell Neurosci. 2019;13:466. PMCID: PMC6803487

Escartin C, Galea E, Lakatos A, et al. Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci. 2021 Mar;24(3):312–325. PMCID: PMC8007081

Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta. 2015 Apr;1851(4):397–413. PMCID: PMC4324013

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017 Jan 26;541(7638):481–487. PMCID: PMC5404890

Acknowledgements

REU Summer Program, Genetics Summer Program, Neuroscience Center of Excellence SUN Program, PREP program, LSUHSC-NO ENDURE

This research project was supported by Award Number: DBI-2349224 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program