Effects of variable beta-amyloid (Ab1-42) fragment size on expression of pro-inflammatory, anti-inflammatory, and autophagy genes in human microglia cells.

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It has been observed that Amyloid-Beta 1-42 (Aβ1-42) aggregates into large neurotoxic insoluble fibrils. This spontaneous aggregation of the Aβ1-42 is believed to be critical to the development of late-stage Alzheimer's disease (AD). While investigations have characterized the kinetics of aggregation, the effect of fragment size on microglial clearance and neuroinflammation remains elusive. Therefore, the aim of this study is to successfully synthesize different Aβ1-42 fragments and examine the effect of fragment size on inflammation and phagocytosis as related to Alzheimer's disease in human microglial cells.

Methods

Amyloid-Beta1-42 was purchased from Bachem (#4014447) in a lyophilized form and was prepared according to Bartolini et al. (2007) with minor modifications. The size of the prepared Aβ1-42 samples were measured using transmission electron microscopy. To examine the expression profiling of pro-inflammatory, anti-inflammatory, and autophagy genes, commercially available human microglia cells (HMC3, ATCC) and non-commercially available C20 human microglia cells were incubated with 1 uM Aβ1-42 of varying fragment sizes. Cells underwent

1. an MTT assay of cytotoxicity to optimize Aβ1-42 dosage,
2. polarization with 10 ng/mL of lipopolysaccharide & 20 ng/mL of human interferon-gamma for pro-inflammatory activation and 20ng/mL of human interleukin-4 for anti-inflammatory activation and
3. treatment with amyloid-beta for various time points.

Phagocytosis was measured using

1. flow cytometry analysis of FITC- and ThT-labeled Aβ1-42,
2. phagocytosis assays utilizing spectrophotometry, and

3. use of confocal microscopy to observe fluorescently labeled Aβ1-42 peptides within the microglial parenchyma.

Results

The Aβ1-42 fragments spanned from single monomeric fibrils to large 4 mm aggregates. Treatment of time-dependent Aβ1-42 fragments to the microglial cells promoted the expression of pro-inflammatory genes in microglia. The expression of pro-inflammatory genes was significantly increased in response to monomeric amyloid-beta, gene expression was further increased with increasing fragment size. The expression of anti-inflammatory genes was variable among fragment size. Phagocytosis was not disturbed in either fragment sizes, contrary to some literature reporting.

Conclusion

Amyloid-beta oligomerization has a detrimental role in the activation of microglia and related inflammatory genes. Our results illustrate the role of Aβ1-42 oligomerization as a causative agent of neuroinflammation by eliciting the polarization of microglial cells towards pro-inflammatory phenotype while attenuating the expression of anti-inflammatory gene commonly. Considering that the peptides are phagocytosed to an equal degree, our future research will be focusing on the role of autophagy, a likely player in the dysfunctional processes of removal of Aβ1-42.