# NEUROINFLAMATORY RESPONSE OF HUMAN DIFFERENTIATED MICROGLIA FROM HEALTHY AND ALZHEIMER DISEASE PATIENT

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## BACKGROUND

Microglia are the main immune cells of the central nervous system (CNS), and it is well known that these cells play an important role in neuroinflammation and are involved in many neurodegenerative diseases as Alzheimer disease (AD). Human iPSC derived microglia from healthy individuals and AD patients provide new tools to better study microglial response in AD. Characterization of the inflammatory response of microglia (MG) in mono and co-culture with neurons and astrocytes is needed to better understand and mimic the disease. Especially Matrigel based cultivation systems support development of 3D structures coming closer to in vivo situations.

Here we studied the in vitro response of healthy and AD microglia to anti- and pro-inflammatory agents with and without the presence of neurons and astrocytes providing results with translational value.

## MATERIAL & METHODS

Differentiated Human derived Microglia from healthy and AD patient (APOE4/4 carrier) were used. For co-culture differentiated human derived GlutaNeurons and Astrocytes were added. The density of microglia in all the culture conditions were the same. Monoculture of microglia were seeded in PDL coated plates. For co-culture, the GlutaNeurons were seeded on a Matrigel formed matrix and on top Astrocytes and Microglia were seeded in a 2:1:1 ratio. At day in vitro 7, the cells were stimulated with pro-inflammatory agents LPS (100 ng/ml), Aβ1-42 monomeric and pre-aggregated (10 µM) and anti-inflammatory agent Dexamethasone (10 µM) for 24 h. Cytokine release (TNF-α, IL-6, IL-8 and IL-1β) was measured in the supernatant of the cells.

## RESULTS - Inflammation response on Microglia Monoculture

LPS induced inflammation is significantly higher in AD microglia compared to healthy microglia in monoculture. Addition of pre-aggregated Abeta1-42 leads to increased release of cytokines than Abeta1-42 in a monomeric state. Most important there is also a significant different response to Abeta1-42 in healthy and AD microglia.

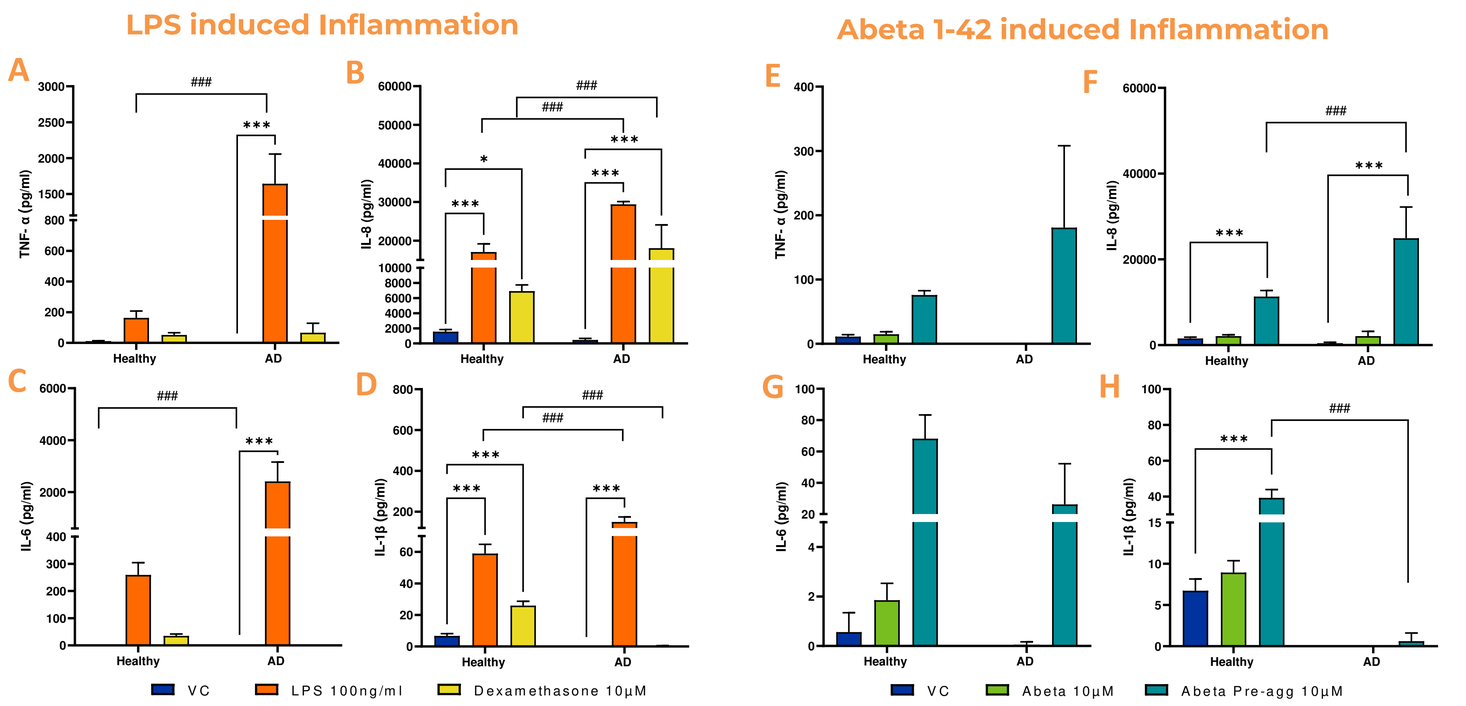



**Figure 1: Quantification of TNF-α, IL-6, IL-8 and IL-1β levels in the supernatant of LPS and Abeta1-42 stimulated Healthy and AD Microglia.** Differentiated Human derived Microglia were treated with Vehicle (VC), 100 ng/ml LPS, 10 µM Dexamethasone where TNF-α (A), IL-8 (B), IL-6 (C) and IL-1β (D) levels were analyzed. Differentiated Human derived Microglia treated with Vehicle (VC), 10 µM Monomeric Abeta1-42 and 10 µM of Pre-aggregated Abeta1-42 and TNF-α (E), IL-8 (F), IL-6 (G) and IL-1β (H) levels were measured. Data are presented as bar graphs with mean + SEM (n=6 per group). Two-way ANOVA, followed by Bonferroni's multiple comparison *post hoc test* compared to VC . **p < 0.01, ***p <0.001 and Healthy vs AD ###p<0.001.

## RESULTS - Inflammation response on Di-culture and Tri-culture

The response of different co-culture systems to pro-inflammatory stimuli in comparison to microglia monoculture is highly significant. Again, also a difference between healthy and AD microglia was observed.

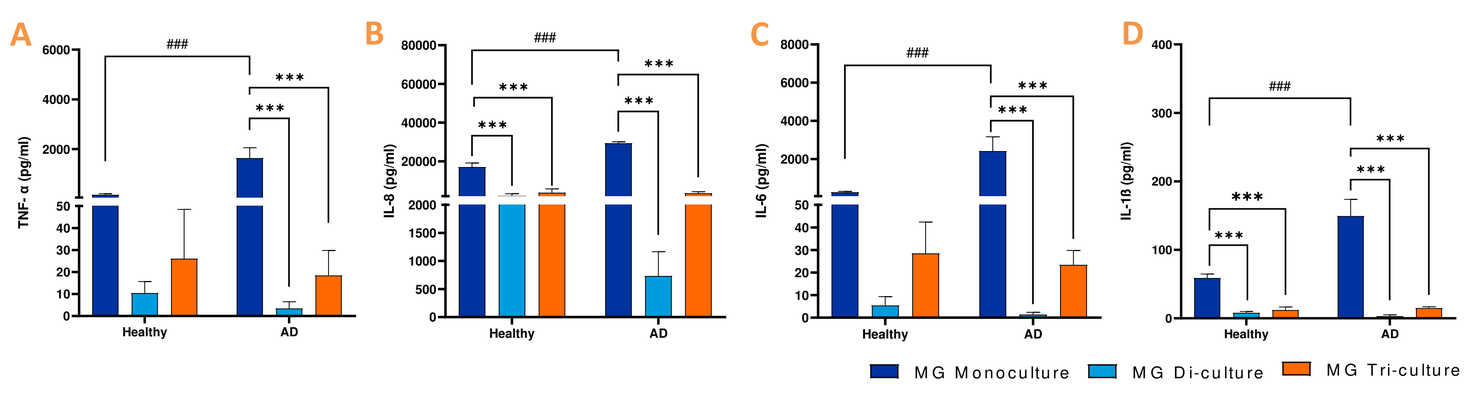
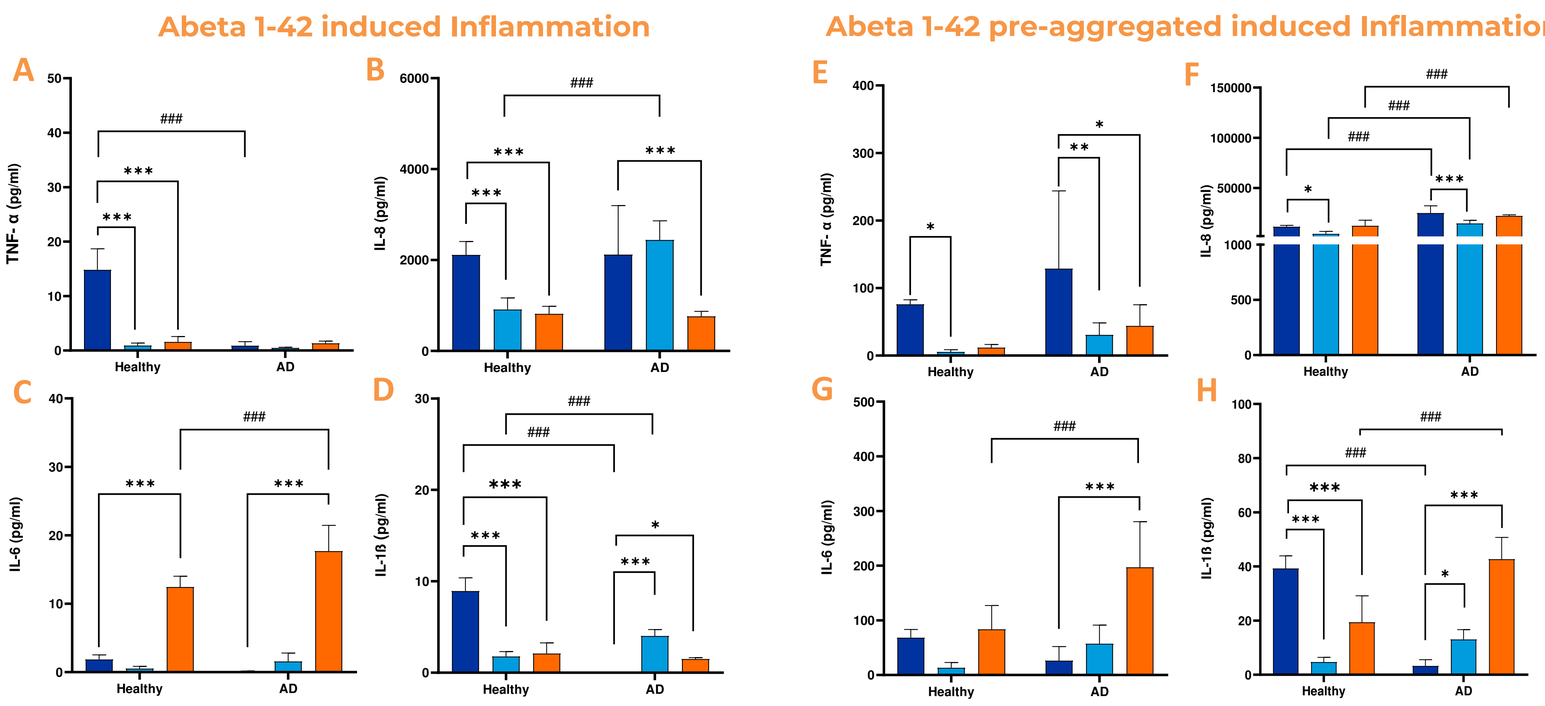



**Figure 2: Quantification of TNF-α, IL-6, IL-8 and IL-1β levels in the supernatant of LPS stimulated in different culture conditions.** Monoculture of Microglia, Di-culture of Microglia and GlutaNeurons and Tri-culture of Microglia, GlutaNeurons and Astrocytes were treated with 100 ng/ml LPS. TNF-α (A), IL-8 (B), IL-6 (C) and IL-1β (D) levels were measured. Data are presented as bar graphs with mean + SEM (n=6 per group). Two-way ANOVA, followed by Bonferroni's multiple comparison *post hoc test* compared to Monoculture . **p < 0.01, ***p <0.001 and Healthy vs AD ###p<0.001.




The response of different co-culture systems to different states of Abeta1-42 is highly significant. A difference between healthy and AD microglia was observed at Di and Tri- culture models.

**Figure 3: Quantification of TNF-α, IL-6, IL-8 and IL-1β levels in the supernatant of Abeta1-42 stimulated at different culture conditions.** Monoculture of Microglia, Di-culture of Microglia and GlutaNeurons and Tri-culture of Microglia, GlutaNeurons and Astrocytes were treated with 10 µM Monomeric Abeta1-42. TNF-α (A), IL-8 (B), IL-6 (C) and IL-1β (D) levels were measured. 24h after treatment with 10 µM of Pre-aggregated Abeta1-42, TNF-α (E), IL-8 (F), IL-6 (G) and IL-1β (H) were measured. Data are presented as bar graphs with mean + SEM (n=6 per group). Two-way ANOVA, followed by Bonferroni's multiple comparison *post hoc test* compared to Monoculture . **p < 0.01, ***p <0.001 and Healthy vs AD ###p<0.001..

## CONCLUSION

Human iPSC-derived microglia are a valuable tool to study neuroinflammatory processes and alterations. The ability to use healthy and diseased cells opens a great opportunity to test the response of microglia-targeted treatments *in vitro*. Differences in cytokine release of healthy and AD microglia in monoculture has demonstrated that the AD microglia are more responsive to the tested pro-inflammatory stimuli than the healthy control microglia. However, the different response of cells in Di-culture and Tri-culture conditions compared to microglia in monoculture, underlines the importance of other cell types, like astrocytes and neurons, in neuro-inflammatory processes. These co-culture systems, including iPSC derived cells from AD patients better resemble the situation in the human brain.

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