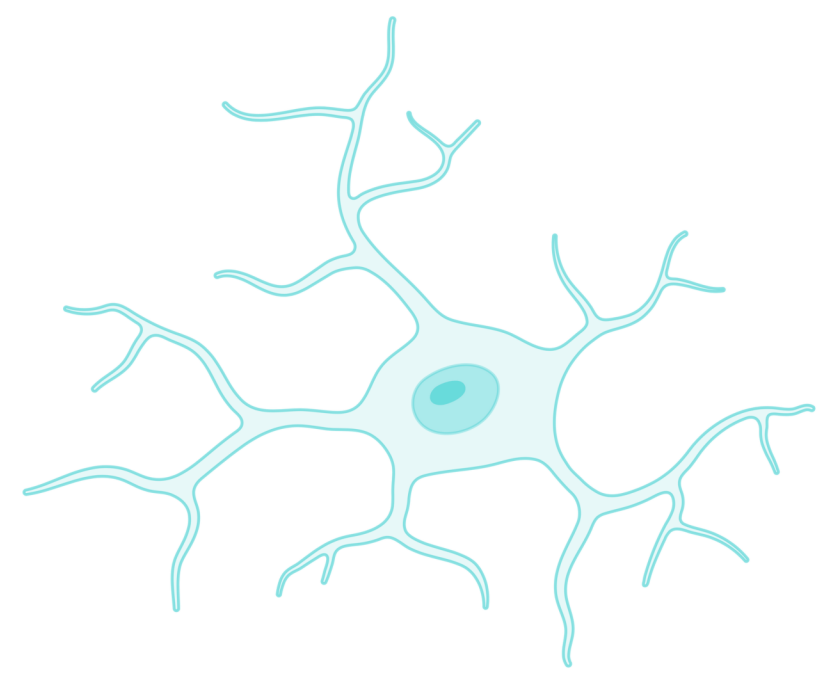


Unraveling neuroinflammation: Sphingolipids as key mediators of inflammatory responses in activated microglia

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BACKGROUND



Microglial cells are myeloid cells located within the central nervous system. Despite their common lineage with monocyte-derived macrophages, microglia maintain distinct molecular characteristics and develop specialized functions, crucial for **brain health and disease**.

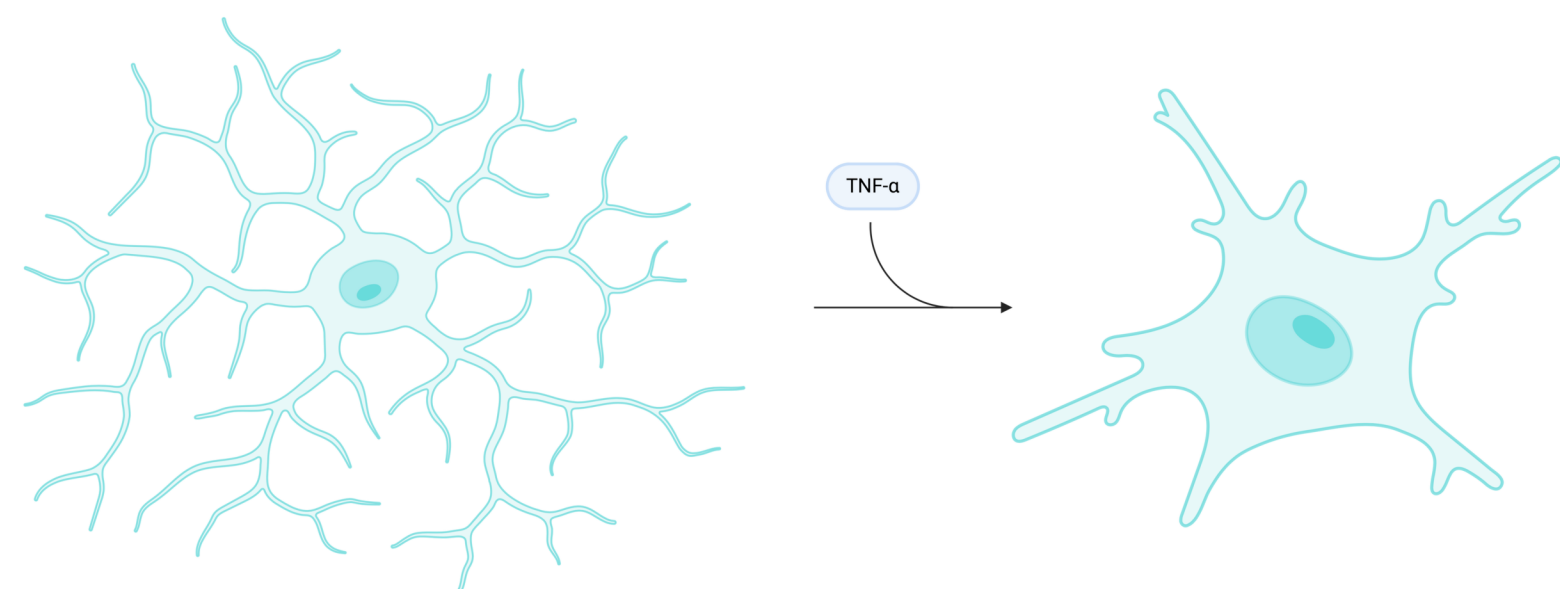
The prolonged activation of microglia can be associated with neuroinflammation, contributing to the progression of neurological disorders. Activated microglia regulates lipid metabolism to meet increased bioenergetic demands and produce secondary messengers.

To date, research has been primarily focused on their released proteins. However, microglia secrete and degrade a huge array of bioactive lipids, such as **sphingolipids**, which may promote inflammatory stimuli or cells crosstalk during neuroinflammation.

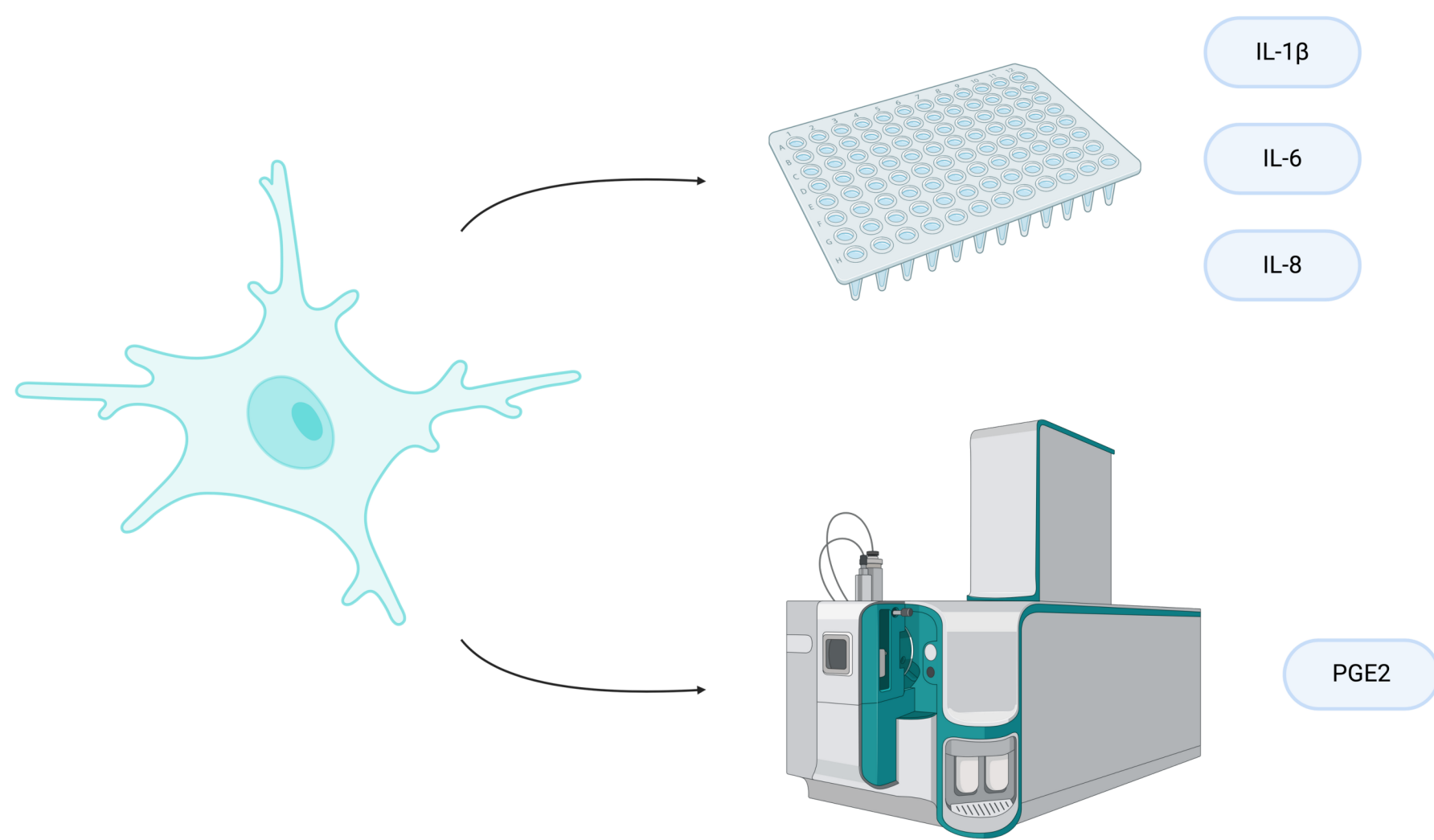
METHODS

The project aimed to elucidate, through liquid-chromatography coupled to mass spectrometry (LC-MS/MS), the sphingolipid metabolism and its alteration on immortalized **human microglial clone 3 cell line (HMC3)**, along with identifying if the secretome lipid component may be implied in cell-cell propagation of inflammation.

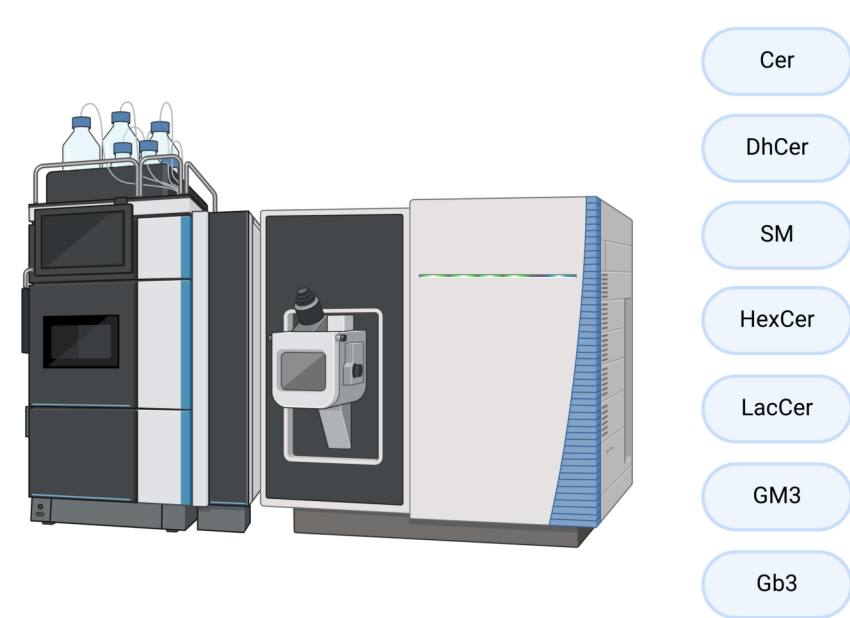
To achieve this goal, HMC3 cells were stimulated with 24-hours **tumor necrosis factor α (TNF α)** 5 ng/mL treatment.



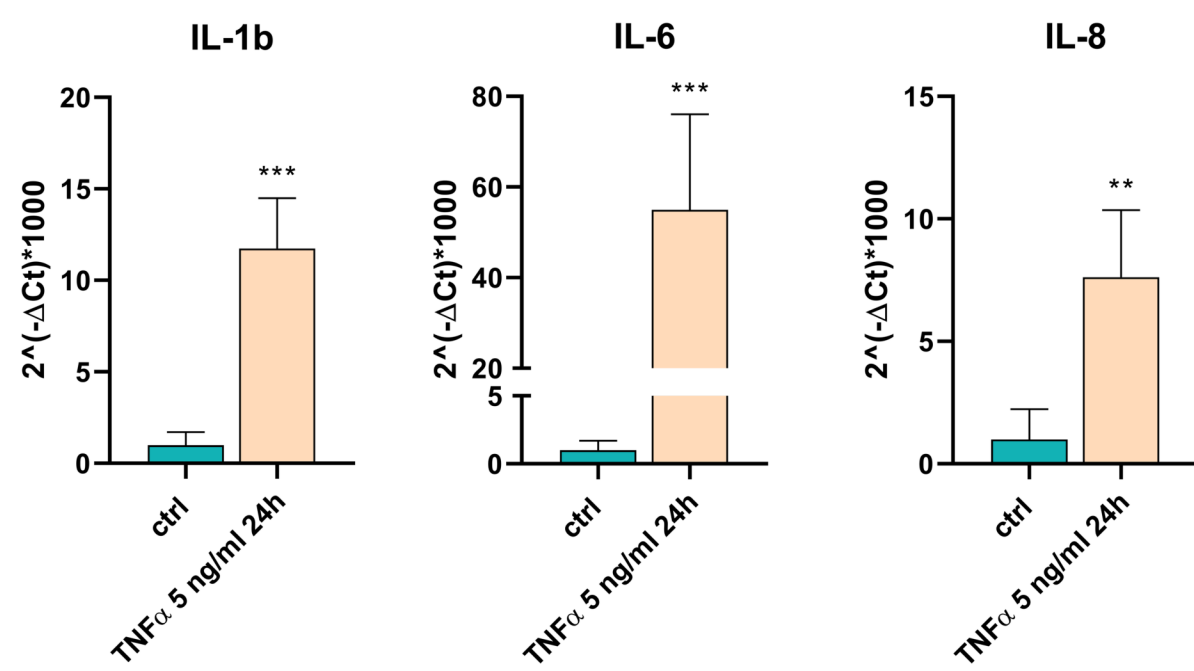
Inflammation, consequent to cell activation, has been assessed through **RT-PCR** for different interleukine markers (IL-1 β , IL-6, IL-8) and through **LC-MS/MS**, quantifying prostaglandine E2 (PGE2) levels.



Then, **sphingolipid amount** (Cer, DhCer, SM, HexCer, LacCer, Gb3 and GM3) has been quantified with **LC-MS/MS**, both on cells and supernatants.



The 24h inflammatory stimuli (TNF α , 5 ng/mL) produce a **visible increase in microglial cell activation**, as obtained from RT-PCR results for interleukine markers. In particular, IL-6 seems to be the most expressed gene.

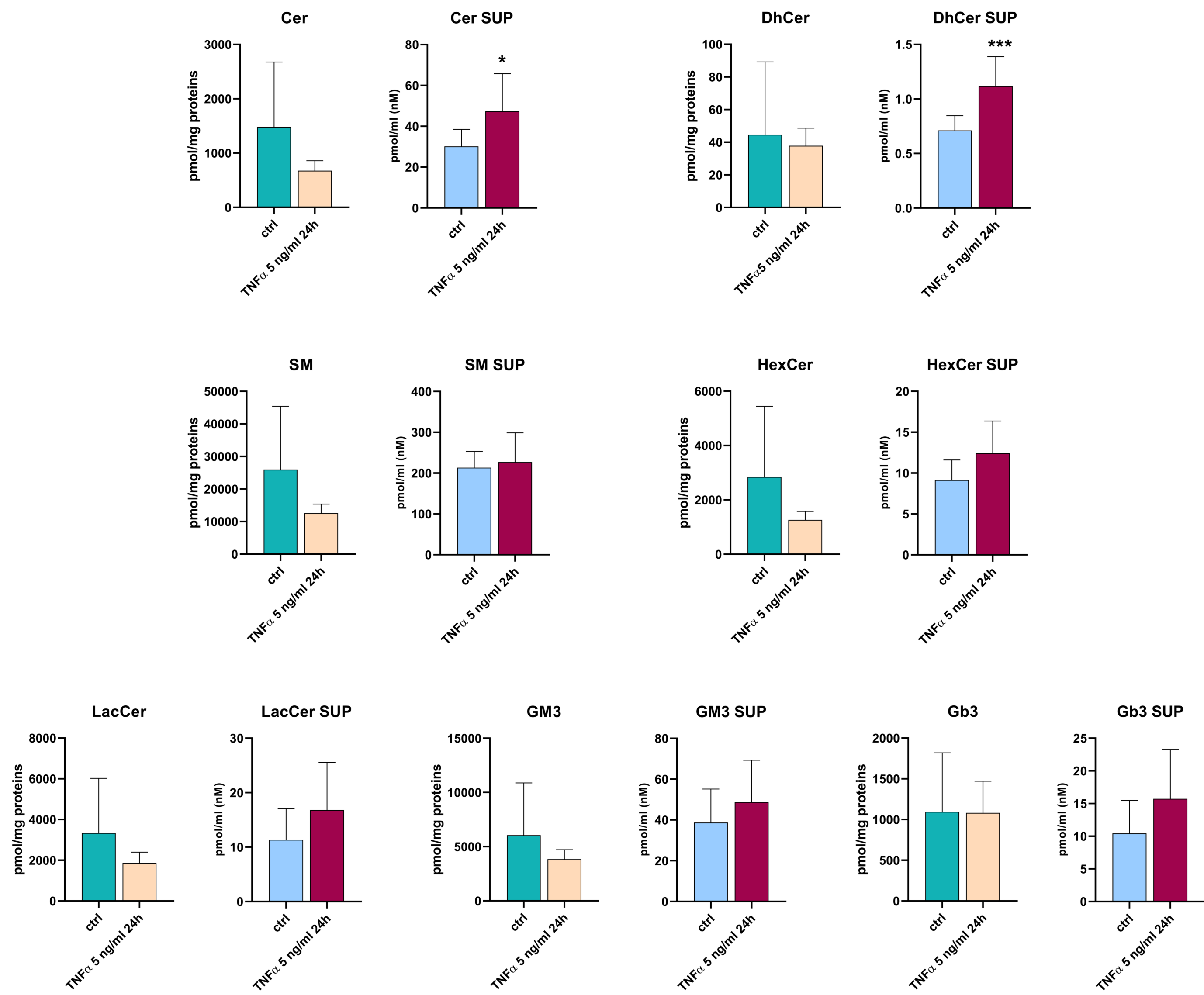


Prostaglandin E2 levels did not agree with those obtained by RT-PCR, probably due to poor instrumental sensitivity. Further studies using a different analytical technique will be necessary.

Moreover, the inflammatory stimuli produce a distinctive increase in the de-novo sphingolipids biosynthesis.

This can be particularly visible in the raise of the early intermediates of the biosynthesis, which are the dihydroceramides (DhCer) and ceramides (Cer). On the other hand, no significant change is visible in complex sphingolipids and glycosphingolipids.

Of particular interest, however, is **the increase in bioactive and proinflammatory ceramides in the cellular supernatant**.



RESULTS

CONCLUSIONS

Microglial cells play a significant role in the central nervous system, and their activation holds pivotal importance in various pathological conditions.

Although these are only preliminary data, our findings indicate the potential for microglial cells to **propagate inflammatory signals via sphingolipids**, possibly releasing them into the cellular supernatant to surrounding cells through, potentially, a paracrine mechanism.

Further analyses will be performed to confirm these results and to investigate how phytocannabinoids might impact on both sphingolipid, endocannabinoids and eicosanoid levels in this condition.