

SIM-A9 Microglia Exhibit Glucose Sensitive Responses To Oleic Acid And LPS

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Introduction

Dysregulated microglial immune responses in the CNS contribute to neurological disease. Microglia, heterogeneous CNS-resident immune cells drive inflammation in acute (trauma, ischemia) and chronic (Alzheimer’s, Parkinson’s, ALS, MS) conditions¹. Microglial immune function is coupled to metabolic state, with activation triggering metabolic reprogramming through modulation of metabolic pathways to a more glycolysis dominated state which supports proinflammatory responses. Nutrient availability further modulates these processes²

Under euglycaemic conditions, glucose concentrations in the human brain have been shown to range between 1.1 to 2.5mM ^{3,4}, which is in a similar order of magnitude to that measured in the rodent brain, 1-2mM⁵. Despite this, most in vitro immunometabolic studies of microglia use high glucose containing medium i.e. DMEM/F12 containing 17.5 mM glucose, a concentration substantially higher than physiological brain glucose levels.

Aim

The murine SIM-A9 microglial cell line, an immunoresponsive *invitro* model, was used to assess adaptive and immunometabolic responses to varying glucose and oleic acid (OA) concentrations following stimulation by LPS. The aim being to try to provide a physiologically relevant microenvironment, enhancing the relevance of the experimental conditions and providing insight into how microglial immune activity may be influenced by exogenous substrate availability.

Methods

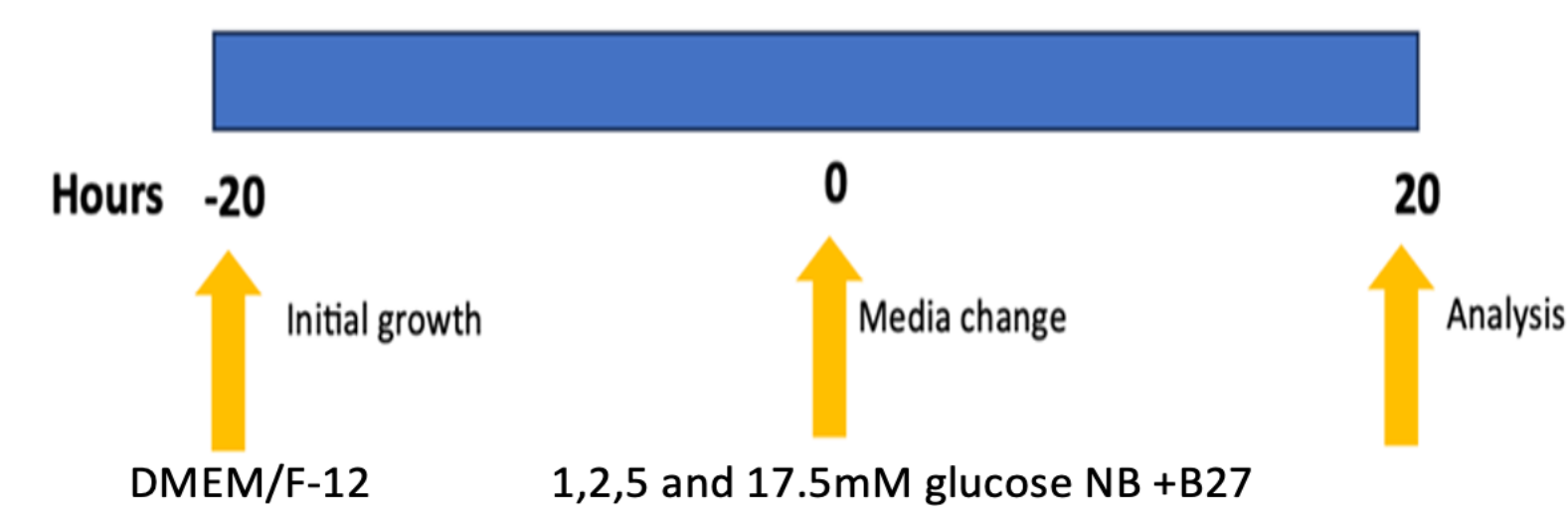


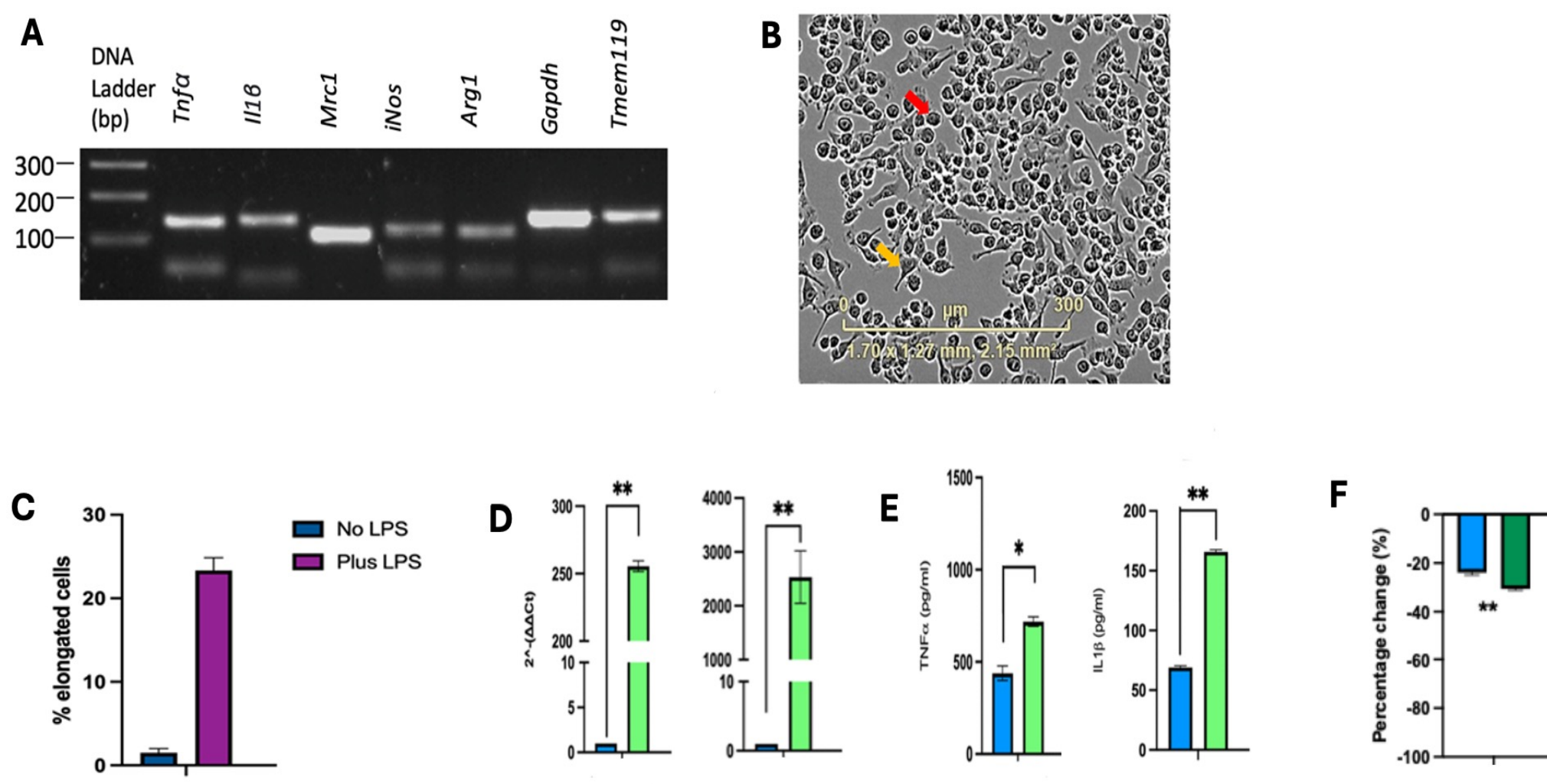
Diagram of cell culture model

- SIM-A9 cells maintained in DMEM/F-12 medium and after 20 hours, the medium was replaced with NeuroBasal+B27 (NB) medium containing 1, 2, 5 or 17.5mM glucose for 20 hours.
- Treatments included 2.5ng/ml LPS and 100µM albumin-bound OA.

- Confluency and morphology were monitored by IncucyteZOOM, Essen Bioscience.
- RT-PCR validated the cell line, and qRT-PCR (GAPDH-normalised) quantified $Tnf\alpha$, IL-1 β and $Pfkfb3$. Cytokine secretion (TNF α and IL-1 β) was measured by ELISA and media glucose concentrations were determined by luminescent assays (Promega, UK). Statistics were performed in GraphPad Prism, Prism 10.

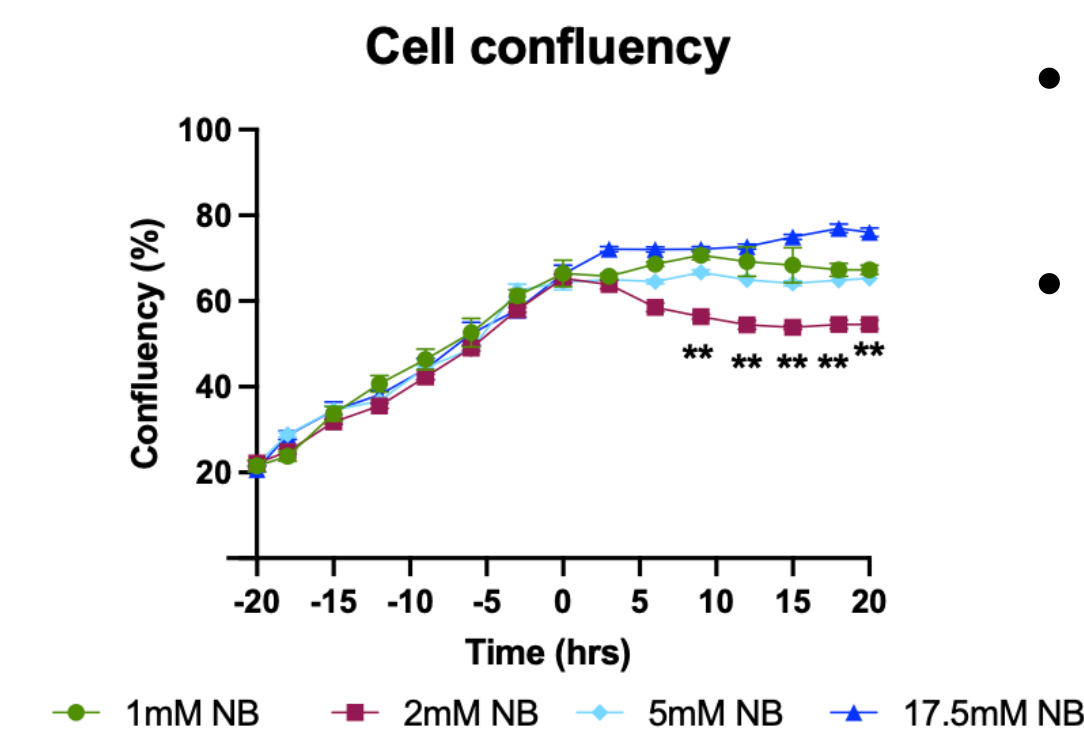
SIM-A9 cells exhibit microglial-like characteristics

- A.** SIM-A9 cells exhibit a microglial-like phenotype through $Tnf\alpha$, IL-1 β , iNOS, Mrc1, Arg1 and TREM 119 expression
- B.** Cells were predominantly amoeboid, with a subset displaying ramified processes, opposite to primary human microglia.



C-F. LPS stimulation promoted a more ramified morphology (C), increased cytokine expression (D) and secretion (E), and enhanced glucose uptake (F), demonstrating a functional inflammatory response.

Cell culture model in Neurobasal

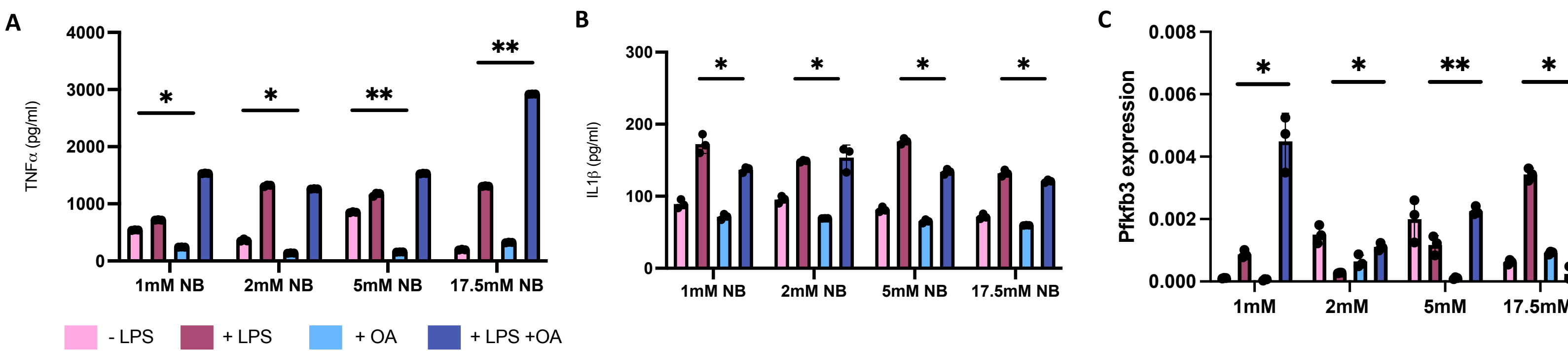


- Culture in NB medium provides a defined environment that minimises basal activation.
- Cell viability remained optimal across all glucose concentrations (96-98%) over 20 hrs in NB medium, however 2 mM glucose significantly reduced confluency, compared to other concentrations.

Discussion

Metabolic state strongly shapes immune responses. A physiologically higher glucose condition (5mM) drives a distinct inflammatory and metabolic phenotype compared to physiological glucose levels, promoting pro-inflammatory activation and a shift towards glycolysis. Glucose availability therefore defines overall metabolic capacity. Lipid exposure (oleic acid) can suppress glycolysis, but this effect is reversed in the presence of inflammatory stimuli such as LPS. Cytokine production is also metabolically selective, with TNF α being more glycolysis-dependent, while IL-1 β is relatively glycolysis-independent. Overall, these findings highlight that immune function is tightly regulated by the surrounding metabolic milieu.

Pro-inflammatory and metabolic profile



- Cytokine secretion (A-B) and $Pfkfb3$ (C) expression was glucose-dependent, with TNF α and $Pfkfb3$ showing the highest at 5mM.
- LPS increased TNF α and IL-1 β secretion and across all glucose conditions.
- OA alone reduced cytokine secretion and glycolysis marker, except at 17.5 mM glucose.
- OA + LPS produced cytokine-specific effects, enhancing TNF α but reducing or maintaining IL-1 β relative to LPS alone.
- OA + LPS further increased $Pfkfb3$ expression relative to LPS alone, except at 17.5mM.

References

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