Project Details	
Project Code	MRCNMH26Ex Guo
Title	Capturing primitive haematogenesis and microglia function ex vivo using human stem cell-based models
Research Theme	NMH
Project Type	Wet lab
Summary	This project aims to uncover how early human immune cells, especially microglia (the brain's immune cells), form during development. Since studying human embryos directly is not possible, you will use a recently established stem cell system that mimics early embryonic conditions to generate these cells in the lab. By defining the signals guiding their development, you will create reliable methods to produce microglia and test their functions. This research will enhance our understanding of the initial stages of blood formation, as well as the brain's immune system, and enable the development of patient-specific microglia models for neurodegenerative diseases.
Description	**Background and Aim** During early embryonic development, the extra-embryonic yolk sac is proposed to give rise to primitive erythroid cells that nourish the embryo, as well as macrophages that persist in adult tissues, including microglia in the brain. However, where and how these primitive hematopoietic cells are formed in humans remains unknown mainly due to restricted access to embryonic tissues. We have recently established a novel approach enabling reliable differentiation of human naïve pluripotent stem cells (PSCs) to extra-embryonic yolk sac cells by mimicking the embryonic signalling environment (PMC8189436; DOI: 10.1016/j.stem.2024.05.003). Furthermore, we demonstrated that these extra-embryonic yolk sac cells can differentiate into hematopoietic lineages. This stem cell-based approach provides a unique opportunity to study human primitive haematopoiesis and microglia differentiation in defined ex vivo conditions. This project aims to elucidate the signalling environments that regulate cell fate specification from the extra-embryonic yolk sac towards microglia and to assemble a robust and scalable protocol for producing functional microglia for disease modelling and drug screening. **Objectives** Ob1. Using fluorescence lineage reporter cell lines and flow cytometry analysis for surface marker expression, you will examine the temporal requirements of signalling environments that regulate key stages of primitive haematopoiesis and microglia differentiation. You will be actively involved in the initial selection of signalling agonists and antagonists for testing under close supervision, and you will perform the assays independently following initial period of technical training. Ob2. You will optimise these signalling conditions to establish a robust approach for differentiating human naïve PSCs (both ESCs and iPSCs) into microglia. You will design the prototype differentiation protocol as well as plan and optimise the protocol. Ob3. Through time-series single-cell RNA sequencing, you will characterise gene expression signatures of differentiating cultures and compare them with their embryonic counterparts to map differentiation trajectories and to validate the identities of the differentiated cells. You

	will plan and collect samples for sequencing. You will implement data analysis pipelines for inferring gene expression signatures and trajectories. You will acquire hands-on training from a data scientist in the team. Ob4. In collaboration with co-supervisor Dr Thomas Piers, you will perform functional assays on the derived microglia (e.g. phagocytosis, activation state assays, and cytokine secretion assays) and compare transcriptomics with open source datasets derived from primary human microglia). You will be actively engaged in the design of these assays and perform the experiment independently. Ob5. In collaboration with Dr Thomas Piers and (UoE) and Dr Daniel Whitcomb (UoB), you will generate microglia from patient-derived induced pluripotent stem cells (iPSCs) carrying mutations associated with neurodegenerative diseases and investigate their function using neuron co-culture as well as in 3D organoids to model disease-relevant phenotypes. You will drive the work of generating patient microglia and will perform the neuron co-culture assays. **Impact** This project will establish a platform to study human primitive haematopoiesis and microglia development in vitro, addressing fundamental gaps in our understanding of early human immune ontogeny. It will enable scalable generation of microglia for disease modelling and drug discovery, contributing directly to MRC priorities in themes of regenerative medicine, neurodegeneration, and translational stem cell research.
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