Targeting the transition to inflammatory lipid-associated macrophages in CVD

Oxford supervisors: Professor Claudia Monaco[1], Dr Lea Dib[1], Professor Ashok Handa[2]
Novo Nordisk supervisors: Dr Luke Payne[3], Dr Giorgio Caratti[3], Dr Charlotte Daly[3], Dr Alexey Epanchintsev[3]

Departments:
1. Kennedy Institute, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences
2. Nuffield Department of Surgical Sciences
3. Novo Nordisk Research Centre Oxford

Project outline

The immune system is integral to the pathogenesis of a broad spectrum of CVD[1]. Positive signals with canakinumab[2] and colchicine[3] strongly suggest a critical role for innate immunity in CVD. Repurposing methotrexate[4] and p38MAPK inhibitors[5] failed to reduce CVD events, indicating that mechanism-driven immunomodulatory strategy will be more effective than general immunosuppression. The challenge is to identify the key culprits of atherosclerosis-specific inflammation amongst the plethora of immune mediators while sparing host defence.

My lab and others have applied single cell (sc) RNA sequencing (RNAseq) and mass cytometry (CYTOF) to study immunological networks in human and mouse atherosclerosis[6-9]. Using single cell transcriptomics to profile approximately 22,000 CD45+live cells derived from human carotid endarterectomy specimens ("discovery cohort"), alongside bulk RNAseq and immunohistochemistry in the Carotid Plaque Imaging Project (CPIP) study ("validation cohort"), we revealed the existence of 2 distinct lipid-associated macrophage (LAM) populations in human plaques; the widely described TREM2hi LAMs with homeostatic and efficient lipid handling signature, and a yet unreported population, perilipin-2 and TREM1 (PLIN2hi/TREM1hi) LAMs, displaying simultaneously lipid, inflammatory and apoptotic gene signatures. In the CPIP cohort (n=115), the transcriptional and protein signature of inflammatory LAMs (iLAMs) is enriched in plaques from CPIP patients with carotid artery disease who recently experienced stroke compared to those who did not. Our data reveal the cellular basis of lipid-driven inflammation in human atherosclerosis and links it to plaque vulnerability to complications[10].

Hypothesis and Goal Using computational and functional analyses we indicated the existence of a **cellular transition from homeostatic (TREM2^{hi}) to iLAMs (PLIN2^{hi}/TREM1^{hi}) in human atherosclerosis** (Figure 1). We have refined a model system where human atheroma cells are used to condition culture medium that is able to induce the **PLIN2^{hi}/TREM1^{hi} LAM state in matched blood monocyte derived macrophages.** In this project we propose to identify therapeutic targets responsible for this transition using multi-omics platforms centered on human tissues and functional validation from murine models.

Aims and Description of work
Aim 1: Identification of the molecular targets underlying the pathogenic LAM transition using multi-omic data of human atherosclerotic tissues. Our group retains one of the most comprehensive

immune scRNAseq and mass cytometry datasets of human carotid and aortic tissues and murine atherosclerotic aortas using. In this project, we aim to refine, implement and interpret these datasets to identify molecular targets that drive atherogenesis using bioinformatics analyses including protein-ligand interactome analysis and targeted and untargeted proteomics. Examples of ligand-receptor interactions emerged from the scRNASeq dataset in Figure 2. These findings will be validated with *in situ* transcriptional and proteomic imaging approaches, allowing the study of the spatial distribution of specialised macrophage populations in atherosclerotic tissues. Imaging approaches will help us define Transitional LAM states and discover potential cellular cross-talks that mediate atherogenesis through macrophage programming.

Deliverable 1: Identification of candidate targets associated with LAM transition in human.

Aim 2: Validation of LAM reprogramming using candidate targets. We will investigate the function of the target genes by using *ex vivo* atheroma cell culture from human carotid endarterectomies combined with human induced pluripotent stem cell (iPS)-derived macrophages. The use of iPS-derived macrophages will allow target validation using knockouts, CRISPR-Cas9 activation or blockers as appropriate, and using established macrophage functional readouts, including inflammation, genomics and metabolism. **Deliverable 2: *In vitro* validation of target pathways in human LAMs.**

Aim 3: Functional study of the LAM transition using murine models of atherosclerosis. Functional studies on the candidate targets in macrophages will be performed using conditional genetic deletion, ablation approaches and fate mapping studies for ontogenetic analysis of LAMs. We have access to a range of published and unique murine strains that will enable a detailed analysis of LAM niches. For instance, mouse strains carrying the target gene will be crossed with *Cx3cr1*-Cre mouse lines to delete the molecule of interest in myeloid subsets or to trace their fate within lesions.

Deliverable 3: *In vivo* validation of LAM transition in atherosclerosis.

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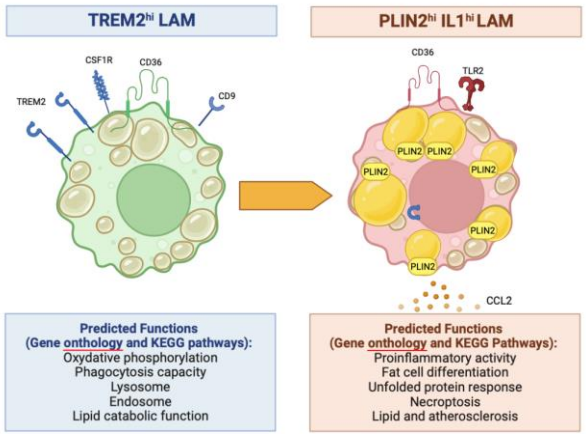

Figure 1. Characteristics of the two types of lipid-associated macrophages in human atherosclerosis.

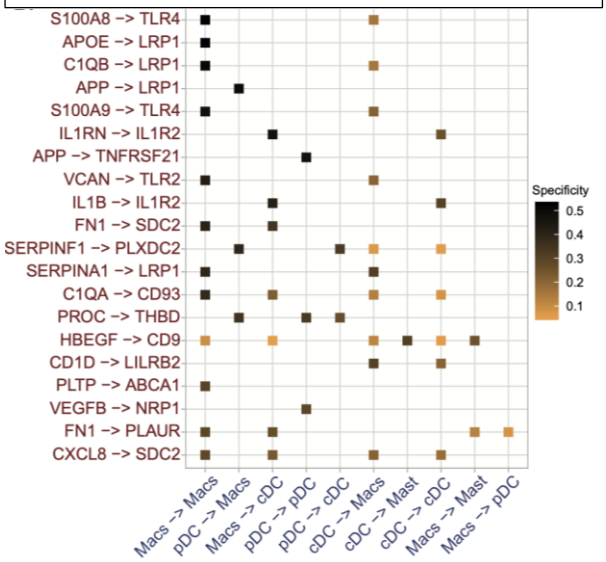

Figure 2. Small selection of ligand receptor interactions of relevance for macrophage interactions.

Supervisor's recent relevant publications
Prof Monaco

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Prof Handa

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