Inflammatory residual risk in CVD

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Residual risk in cardiometabolic disease: Almost all patients with or at high risk of atherosclerotic cardiovascular disease (ASCVD) are treated with statin therapy, in order to lower LDL cholesterol (LDL-C). Overwhelming evidence indicates a causal role for LDL-C in ASCVD, and for LDL-C lowering to reduced risk. The recent ODESSEY outcome trial demonstrates the effectiveness of very low LDL-C levels by statin and alirocumab after acute coronary syndrome which results in a substantial reduction in MACE. However, a significant proportion of ASCVD risk, termed 'residual risk' is not removed by LDL-C lowering, even to the very low levels (less than 1 mM or 40 mg/dl) achieved in ODESSEY and similar trials. Therefore, the relative contributions of other determinants of residual cardiovascular risk may have shifted. In most instances anti-inflammatory intervention in clinical trials is given to cohorts with substantial disease burden, to reduce subsequent risk, *not as a preventative measure*.

Trained immunity in innate immune cells: Immunological memory was considered an exclusive hallmark of the adaptive immune response. However, it is increasingly clear that innate cells can retain memory of previous exposure to an activating stimulus. This innate trained immunity was initially shown to act through mature myeloid cells. These cells exposed to an activating stimulus, are reprogramed such that on subsequent re-exposure they react more or less strongly than initially. A hallmark of the trained immune response is bioenergetic re-programming towards Warburg-type metabolism. Additionally, in murine models, early intermittent elevated hyperlipidaemia aggregates atherosclerotic plaque development by altering the composition of arterial macrophages, while a separate study has shown that oscillating elevated cholesterol accelerates atherosclerosis by reprogramming neutrophil progenitors in an IL-1b dependent manner. However, the long-term effects of high cholesterol on HSC and myeloid cell metabolism and function especially after lipid lowering is largely unknown.

Hematopoietic stem cells in trained immunity: Mature myeloid cells, such as monocytes and DCs, have a half-life of only 5–7 days in both mice and humans and so were thought incapable of long-term memory. It is now clear that trained immunity can occur in bone marrow progenitor cells (central trained immunity), as well as in blood monocytes and tissue macrophages (peripheral trained immunity). The advent of single cell sequencing and phenotyping of immune cells has revealed significant heterogeneity in the populations of myeloid cells within the blood and healthy tissues; but and the repertoire and number of immune cells in diseased tissues tend to be more complex. **Therefore, identifying pathways that are irreversibly altered by physiological perturbations relevant to cardiometabolic will be critical to identifying new target pathways to treat residual risk.**

Central hypothesis: We hypothesise that there are long-term consequences of exposure to high cholesterol levels which are non-reversible following normalisation of lipid levels. These consequences are due to irreversible epigenetic and metabolic changes which leads to activation of the innate immune system. We propose that determining the mechanisms underlying the long-term immunometabolic consequences of cholesterol induced trained immunity (CITI) will be clinically impactful in targeting the residual risk associated with ASCVD and cardiometabolic disease.

Work package one: How does exposure to high cholesterol alter circulating immune cells composition and function after LDL-C lowering?

Rationale: Preliminary data demonstrates that elevated cholesterol induces long term non-reversible changes to HSC number and immune cell composition in the bone marrow and circulation, in murine

models of hypercholesterolaemia. In this work package blood samples from patients with hypercholesterolaemia will be recruited through Oxford Biobank and used to translate our *in vivo* findings to clinically relevant cohorts.

Objective 1a: Generation of a single cell metabolic immune cells atlas for patients with before and after lipid lowering

Patients with familial hypercholesterolaemia who are naïve to lipid lowering intervention will be recruited. PBMC will be isolated and cryopreserved for further analysis. Recruited patients will also be recalled for a second visit after a minimum of 3 months of lipid lowering intervention.

Aim 1: Two custom CyTOF panels have been developed by collaborator Prof Claudio Monaco, Kennedy Institute of Rheumatology, University of Oxford (COMBAT and CATALYST; adapted from Ahern et al., Cell 2023) of 46 antibodies conjugated to heavy metals will be used to stain Cytodelics stabilised blood to generate an immune cell ATLAS. Immune cell metabolism is linked to their function. We hypothesis this metabolic plasticity is lost in myeloid cells that have come from a high cholesterol environment, and which are not reversible following lipid lowering. To assess immune cell metabolism scMEP CyTOF (adapted from Hartman *et al.,* Nature Biotechnology 2021) will be used to capture cellular metabolic state at single cell resolution.

Aim 2: Single cell multiome will be conducted to understand how the chromatin is altered in response to prolonged high cholesterol and if the changes are reversible after lipid lowering.

Work package two: Development of a human inducible pluripotent stem cell (hiPSC) bone marrow organoid model of cholesterol induced to identify to identify casual genes

Rationale: All preliminary data suggests that there are long-term non-reversible changes to HSC *in vivo*, however due to the rarity of these cells it makes functional studies on these very difficult. To overcome this challenge, we will use an iPSC derived bone marrow organoid model, this will allow for analysis of not only HSC, and the stromal cells which support HSC maintenance but the daughter hematopoietic cells in one contained system.

Objective 2a: Establish a model of cholesterol-induced trained immunity (CITI) in human iPSC derived bone marrow organoids.

Aim 1: Using a recently developed human inducible pluripotent stem cell (hiPSC) bone marrow organoid model developed by collaborator Prof Beth Psaila, MRC WIMM, University of Oxford to model cholesterol induced trained immunity (Khan et al. Cancer Discovery 2023; Olijnik et al. Nature Protocols 2024).

Objective 2b: Using a genome wide CRISPR screen to identify a gene to phenotype relationship in the development of CITI.

Aim 1: To date very few genome-wide screen CRISPR screens have been conducted in iPSC derived organoid models due to the complexity of design and scale of cells and organoids needed. A genome-wide pooled CRISPR screen will be undertaken in the CITI bone marrow organoid model developed in (Objective 2a) and will be used generate a large dataset of genes that have functional relevance to the development CITI.

Supervisor's recent relevant publications

Dr Gareth Purvis

Purvis GSD. et al, (2024) OxPhos in adipose tissue macrophages regulated by BTK enhances their M2-like phenotype and confers a systemic immunometabolic benefit in obesity. Diabetes.

Purvis GSD. et al, (2024) Ly6Chi Monocytes Are Metabolically Reprogrammed in the Blood during Inflammatory Stimulation and Require Intact OxPhos for Chemotaxis and Monocyte to Macrophage Differentiation. Cells

Rumianek AN. et al, (2022) A Human CD68 Promoter-Driven Inducible Cre-Recombinase Mouse Line Allows Specific Targeting of Tissue Resident Macrophages. Front Immunol.

Purvis GSD. et al, (2021). Bruton's tyrosine kinase (BTK) regulates myeloid cell recruitment during acute inflammation. Br J Pharmacol.

Prof Keith Channon

Kufazvinei TTJ. et al, (2024) Emerging opportunities to target inflammation: myocardial infarction and type 2 diabetes. Cardiovasc Res.

Chuaiphichai S. et al, (2023). Endothelial cell-specific roles for tetrahydrobiopterin in myocardial function, cardiac hypertrophy, and response to myocardial ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol.

Chuaiphichai S. et al, (2023). Endothelial cell vasodilator dysfunction mediates progressive pregnancy-induced hypertension in endothelial cell tetrahydrobiopterin deficient mice. Vascul Pharmacol.

Iqbal AJ. et al, (2014). Human CD68 promoter GFP transgenic mice allow analysis of monocyte to macrophage differentiation in vivo. Blood.