Deciphering the biology of resident vascular macrophages in atherosclerosis

Oxford supervisors: Professor Claudia Monaco[1] and Professor Irina Udalova[1]

Novo Nordisk supervisor: Dr Alexey Epanchintsev[2]

Departments: 1. Kennedy Institute of Rheumatology
2. Novo Nordisk Research Centre Oxford (NNRCO)

Project outline

Background and pilot data: Atherosclerosis is a chronic inflammatory disease which is the underlying cause of cardiovascular mortality. Macrophages are considered integral to the pathogenesis of atherosclerosis; however, they have not been therapeutically targeted due to their complex nature. We comprehensively characterised the vascular immune atlas in human and murine atherosclerosis using single cell RNA sequencing (scRNA-seq) and mass cytometry (Cole *et al*. 2018; Park *et al.* under revision; Dib *et al.* manuscript in preparation) (**Figure 1**). We also showed that resident vascular macrophages that reside in the adventitia exert a protective role during atherosclerosis in mice via an inhibitory C-type lectin receptor (Park *et al.* under revision), whereas IRF5-dependent CD11c+ intimal macrophages are endowed with pro-atherogenic functions such as necrotic core formation (Seneviratne *et al.* 2017). These two distinct macrophage subpopulations are conserved in human and mouse, and are located in two different compartments in the microvasculature (**Figure 2**). Fate mapping studies revealed that monocytes reconstitute the majority of vascular macrophages during atherosclerosis, however, the underlying mechanism as to how monocytes differentiate into “protective” resident macrophages is poorly understood. We aim to investigate the molecular mechanism of resident vascular macrophages in protection against atherosclerosis by identifying targets using scRNA-seq data of human and murine atherosclerotic immune cells and testing the candidates on human induced pluripotent stem cell (iPS)-derived macrophages and *in vivo* mouse models. Identification and understanding of the molecular mechanism of resident vascular macrophages would present a novel therapeutic target for treatment of atherosclerosis.

Hypothesis and goals: Our data showed that the atherosclerotic artery consists of two distinct macrophage compartments, which differently contribute to atherogenesis. On the basis of our previous studies and pilot omics data, we hypothesise that resident vascular macrophages exert homeostatic functions during atherosclerosis by alleviating inflammation and enhancing reverse cholesterol efflux in the vessel wall. We seek to investigate the molecular mechanism that regulates athero-protective properties in resident vascular macrophages to identify new potential therapeutic targets for alleviating atherogenesis (**Figure 3**).

Aims and description of work:

Aim 1: Identification of the molecular targets driving athero-protective properties in vascular macrophage subsets using the scRNA-seq data of human and murine atherosclerotic tissues. Our group retains the comprehensive immune landscape data set of human carotid and aortic tissues and murine atherosclerotic aortas using scRNA-seq and mass cytometry. In this project, we aim to refine, implement and interpret these data sets to identify molecular targets that drive athero-protection in macrophage subpopulations using bioinformatics analyses including protein-ligand interactome analysis. We will also identify conserved mechanisms in macrophage subsets between human and mouse. These findings will be validated with *in situ* imaging approaches, which will allow the study of the spatial distribution of specialised macrophage populations in the large and medium-sized arteries of human and mouse in the healthy and atherosclerotic conditions. Imaging approaches will help us discover potential cellular cross-talks between macrophages and neighbouring cells within the vessel wall that mediate athero-protection through macrophage programming.

Deliverable 1: Identification of candidate targets associated with atherogenesis in human and mouse.

Aim 2: Validation of macrophage reprogramming using candidate targets. Key pathways of interest emerging from pilot analysis of the available scRNA-seq datasets include reverse cholesterol transport, NFkB signalling, cell migration, NLRP3 inflammasome and oxygen sensing. Selected pathways will be validated for relevant gene expression in human and murine tissues of different disease states, and targets will be prioritised for further functional validation. Furthermore, 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 that are ontologically similar to tissue-resident macrophages. In these co-cultures, we will measure the impact of the diseased tissue on the properties of resident macrophages and their subsequent functional alteration using scRNA-seq and cell migration assays at NNRCO. Target validation will be performed using knockouts (CRISPR-Cas9) 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 macrophages.

Aim 3: Functional study of the cellular targets 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 vascular macrophages. We have access to a range of published and unique murine strains that will enable a detailed analysis of specific vascular macrophage niches. For instance, mouse strains carrying the target gene will be crossed with *Cd11c*-Cre or *Lyve1*-Cre mouse lines to delete the molecule of interest in specific intimal or adventitial macrophage subsets and examine its effects on atherosclerosis and metabolism at large.

Deliverable 3: *In vivo* validation of specific deletion or ablation of candidate targets in atherosclerosis.

Contributions: The Monaco lab will provide the global oversight of testing any hypothesis originating from their single cell data set, including identification (Deliverable 1), validation and functional testing *in vivo* and *in vitro* (Deliverable 2 and 3); the Udalova lab will contribute with expertise in genomics of inflammation *in vivo* and *in vitro* and molecular and functional characterisation of myeloid cells (Deliverable 2 and 3); NNRCO will support targeting in human cells including iPS-derived macrophages, CRISPR Cas 9-targeting and further RNA-seq and ATAC-seq approaches for mechanistic insights *in vivo* and *in vitro* (Deliverable 2).

Supervisors' recent relevant publications:

1. Park I, Goddard M, Cole J, Zanin N, Lyytikäinen LP, Lehtimäki T, Andreakos A, Feldmann M, **Udalova I**, Drozdov I, **Monaco C.** C-type lectin receptor CLEC4A2 promotes resident macrophage homeostasis and protects against atherosclerosis (Manuscript under revision).
2. Cole JE, Park I, Ahern DJ, Kassiteridi C, Danso Abeam D, Goddard ME, Green P, Maffia P, **Monaco C** (2018). Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity. Cardiovasc Res *114*, 1360-1371. PMID: 29726984
3. Seneviratne AN, Edsfeldt AO, Cole JE, Kassiteridi C, Swart M, Park I, Green P, Khoyratty TE, Saliba DG, Goddard ME, Sansom SN, Goncalves I, Krams R, **Udalova I, Monaco C** (2017). Interferon Regulatory Factor 5 Controls Necrotic Core Formation in Atherosclerotic Lesions by Impairing Efferocytosis. Circulation 136(12):1140-1154. PMID: 28698173
4. Dalmas E, Toubal A, Alzaid F, Blazek K, Eames HL, Lebozec K, Pini M, Hainault I, Montastier E, Denis RG, Ancel P, Lacombe A, Ling Y, Allatif O, Cruciani-Guglielmacci C, André S, Viguerie N, Poitou C, Stich V, Torcivia A, Foufelle F, Luquet S, Aron-Wisnewsky J, Langin D, Clément K, **Udalova IA**, Venteclef N (2015). Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. Nat Med 21(6):610-8. PMID: 25939064
5. Weiss M, Byrne AJ, Blazek K, Saliba DG, Pease JE, Perocheau D, Feldmann M, **Udalova IA** (2015). IRF5 controls both acute and chronic inflammation. Proc Natl Acad Sci U S A 112(35):11001-6. PMID: 26283380

Appendix. Plot data and workflow

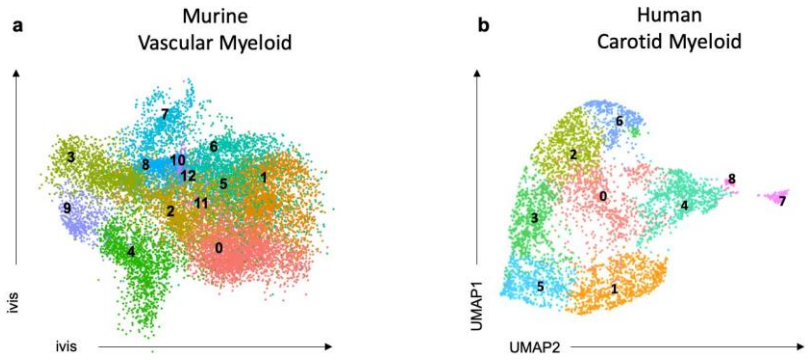

Figure 1. The myeloid landscape of murine and human atherosclerotic tissues by single cell RNA sequencing.

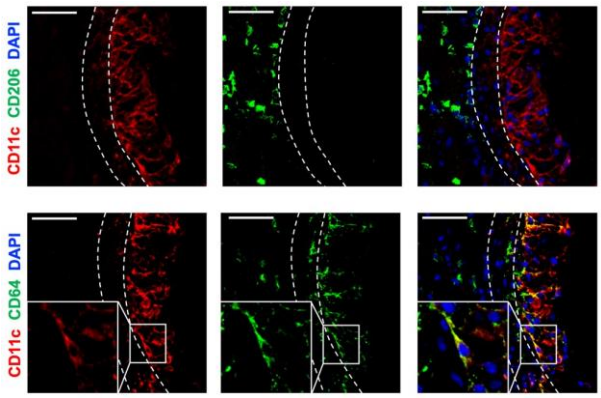

Figure 2. The atherosclerotic aorta comprises two distinct macrophage compartments.

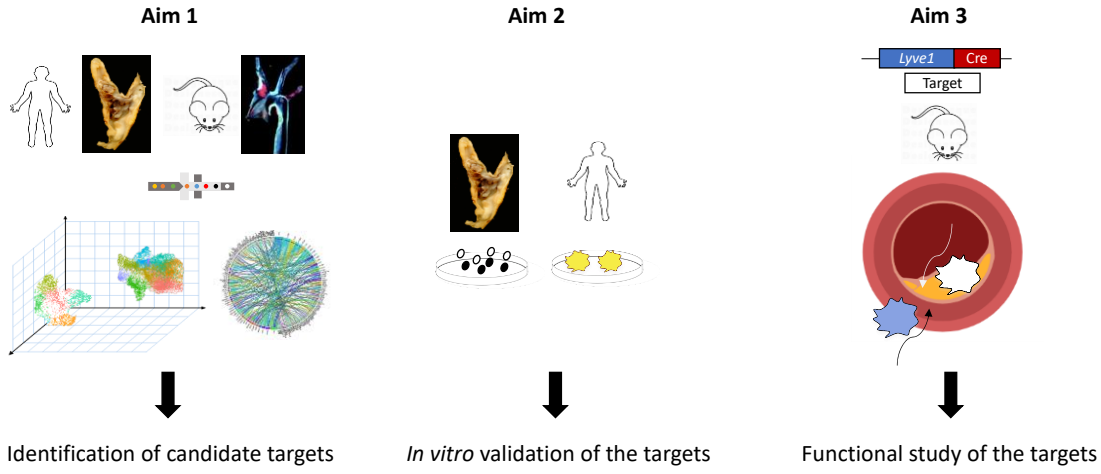

Figure 3. Workflow for identification and validation of the molecular mechanism of the macrophage interaction in atherosclerosis.