Crosstalk between lesional and resident vascular macrophages in atherosclerosis

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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 were one of the first groups to comprehensively characterise 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 peer review; Dib *et al.* manuscript in preparation) (**Figure 1**). We also showed that vascular resident 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 peer review), 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**). However, how intimal and adventitial macrophages interactively orchestrate atherogenesis is currently unknown. Identification and understanding of the molecular mechanism of the macrophage interaction between two arterial niches 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 vascular resident macrophages lose their homeostatic functions during atherosclerosis and mediate inflammation by enhancing the recruitment and activation of inflammatory macrophages to the vessel wall. We seek to identify the molecular mechanism that regulates the crosstalk between two macrophage compartments to identify new potential therapeutic targets for alleviating atherogenesis (**Figure 3**).

Aims and Description of work

Aim 1: Identification of the molecular targets underlying the macrophage interaction using the scRNA-seq data of human and murine atherosclerotic tissues. Our group retains one of the most 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 atherogenesis 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 in human and mouse healthy and atherosclerotic tissues. Imaging approaches will help us define neighbouring cells in two arterial niches and discover potential cellular cross-talks that mediate atherogenesis 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 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 cocultures, we will measure the impact of the diseased tissue on the resident macrophages and their subsequent crosstalk to inflammatory macrophages 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 two vascular macrophage niches. For instance, mouse strains carrying the target gene will be crossed with *Cd11c*-Cre or CLR-Cre mouse lines to delete the molecule of interest in specific recruited or resident myeloid subsets and examine its effects on atherosclerosis and metabolism at large.

Deliverable 3: *In vivo* validation of macrophage subsets interactions 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).

Supervisor's recent relevant publications:

1. Park, I., Goddard, M., Cole, J., Zanin, N., Lyytikäinen, L.P., 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 peer review).
2. Cole, J.E., Park, I., Ahern, D.J., Kassiteridi, C., Danso Abeam, D., Goddard, M.E., Green, P., Maffia, P., and **Monaco,** (2018). Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity. Cardiovasc Res *114*, 1360-1371.
3. Seneviratne, A.N., Edsfeldt, A.O., Cole, J.E., Kassiteridi, C., Swart, M., Park, I., Green, P., Khoyratty, T.E., Saliba, D.G., Goddard, M.E., Sansom, S.N., Goncalves, I., Krams, R., **Udalova, I., Monaco, C**. (2017). Interferon Regulatory Factor 5 Controls Necrotic Core Formation in Atherosclerotic Lesions by Impairing Efferocytosis. Circulation.
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. Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. Nat Med. 2015 Jun;21(6):610-8. doi: 10.1038/nm.3829. Epub 2015 May 4.
5. Weiss M, Byrne AJ, Blazek K, Saliba DG, Pease JE, Perocheau D, Feldmann M, **Udalova IA**. IRF5 controls both acute and chronic inflammation. Proc Natl Acad Sci U S A. 2015 Sep 1;112(35):11001-6. doi: 10.1073/pnas.1506254112. Epub 2015 Aug 17.

Appendix. Pilot data and workflow

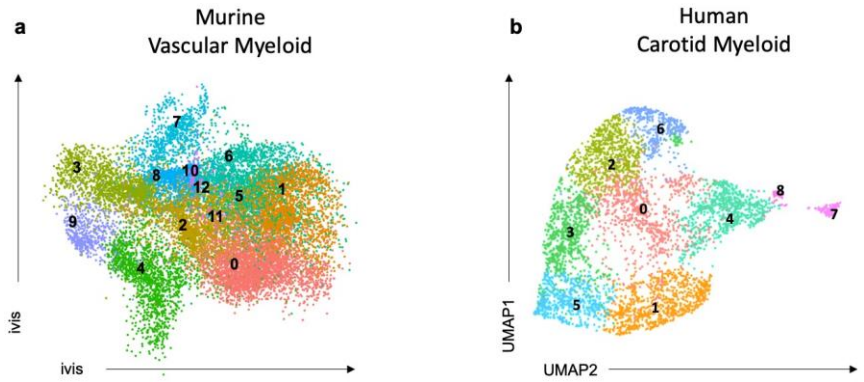

Figure 1. The myeloid landscape of murine and human atherosclerotic tissues by single cell RNA sequencing.

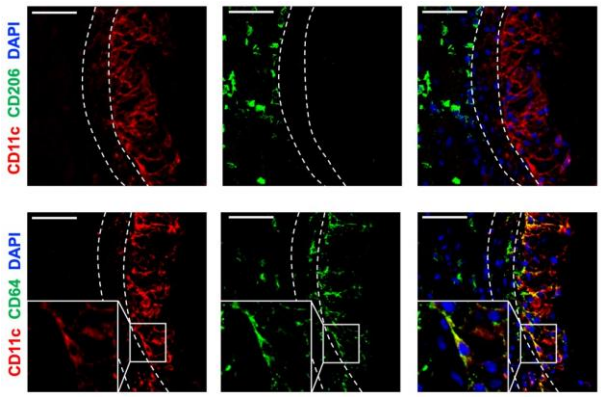

Figure 2. The atherosclerotic aorta comprises two distinct macrophage compartments.

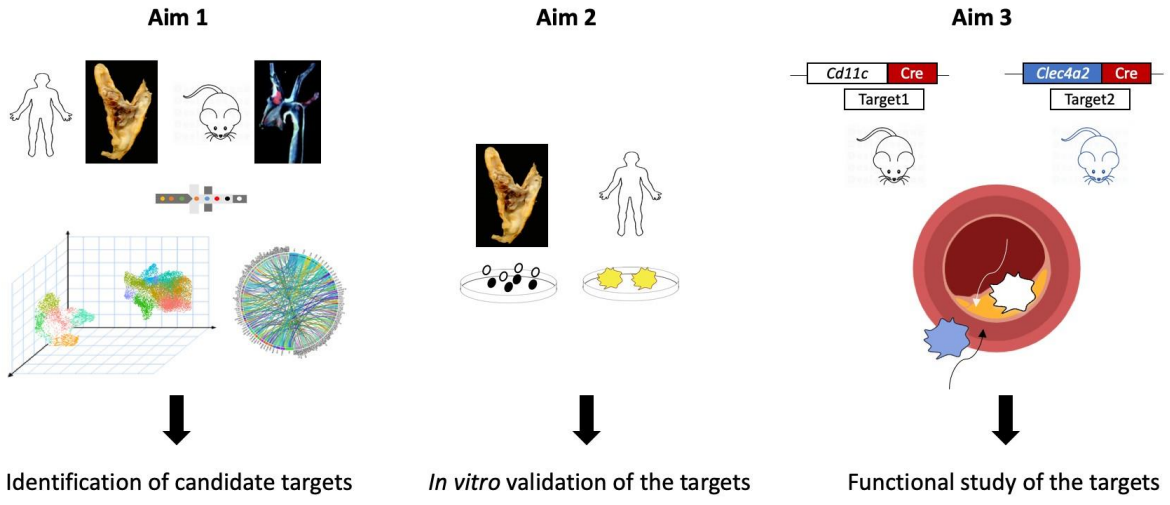

Figure 3. Workflow for identification and validation of the molecular mechanism of the macrophage interaction in atherosclerosis.