Reprogramming innate immune function in diabetes through metabolic intervention

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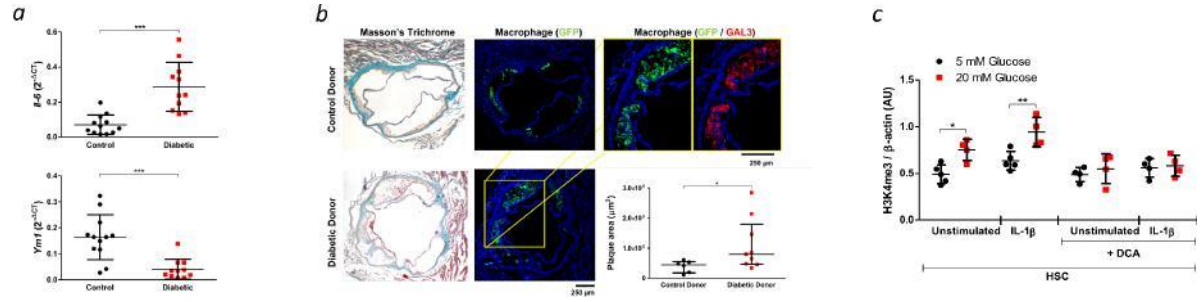
Project outline

Background

Recent trials have re-emphasized that improving glycemic control does not reduce *atherosclerotic complications* (e.g. acute MI) in type 2 diabetes. In addressing this therapeutic paradox, we have recently shown that hyperglycemia drives the development of 'trained' innate immunity in macrophages and their bone marrow precursors (panel *a*). This results in functional changes in bone marrow-derived macrophages that persist *even after full normalization of blood glucose* and which drive atherosclerosis *in LDLR$^{-/-}$ mice* (panel *b*). These effects are mediated through epigenetic modification and, importantly, are diminished by inhibiting glycolysis (panel *c*). Induction of trained immunity, was associated with H3K4me3 and H3K27ac epigenetic marks and > 1000 differential peaks in ATAC seq. The implications are that diabetes alters cellular metabolism in hyperglycaemia, resulting in fundamental and persistent reprogramming of the immune system at the level of the bone marrow. [*Figures taken from Edgar et al 2018, In revision*]



Hypothesis

In monocyte/macrophages, metabolic signals of starvation: **(1)** have anti inflammatory effects driven by (a) increased fatty acid oxidation and (b) receptor-mediated pathways (GPRR109a); **(2)** manifest as resistance to pro-inflammatory stimuli and to the development of trained immunity and **(3)** protect against atherosclerosis in diabetes.

Aims

Having identified trained immunity as a mechanism of so-called '*metabolic memory*' in diabetes, we aim to explore how this can be modified. We seek to identify targets for beneficial immuno-metabolic modulation that could counter the trained immunity phenotype and thereby to identify strategies for both prevention and treatment of vascular disease in diabetes.

Plan for proposed work

Glucose is homeostatically regulated at its lower level and therefore unlikely to signal itself. In starvation, β-hydroxybutyrate increases in human blood plasma and can be used as an energy source when blood glucose is low. We have shown[5] that activation of GPR109a (cognate ligand = β-hydroxybutyrate) has profound anti-inflammatory effects in both monocytes and adipocytes. This suggests at least two plausible ways that β-hydroxybutyrate could mediate anti-inflammatory / athero-protective effects, *independent of glycemic control.*

We will define the metabolic status of cells exposed to normal glucose and raised β-hydroxybutyrate using metabolomic assays and related informatics tools and integrate cellular energetic using the Seahorse bioanalyser. Inflammatory responses will be assessed under pro-inflammatory conditions and screening with qRT-PCR (for M1/M2 markers), progressing to RNA-seq. By combining ATAC seq, we will define how cellular metabolism leads to epigenetic modifications and associated functional changes in bone marrow-derived macrophages and their stem cell precursors. Equivalent approaches in hyperglycaemia implicated specific transcription factor pathways (e.g. RUNX1 and PU.1) based on patterns of open chromatin. We expect that starvation-simulation will result in particular chromatin modifications that will not merely be the converse of hyperglycaemia-related changes. We will use *in vitro* model systems to interrogate the effects of starvation-simulation on macrophage function and on the propensity to develop (and recover from) hyperglycaemia-induced trained immunity. Following identification of a putative atheroprotective cellular phenotype, we will induce this in bone marrow cells of CD68-GFP transgenic mice, to allow the effects on atherosclerosis to be determined *in vivo* by BM transplant to *LDLR*$^{-/-}$ mice. As in our previous work we will then examine human cells (in this case from blood) to assess the effects of the human relevant starvation intervention in diabetes (post-bariatric surgery) on immune cell function. The overall approach will be to make observations in cell systems, test causation in mice and corroborate relevance in human tissue.

Supervisor's recent relevant publications

1. Edgar L, Akbar N, Krausgruber T, Gallart-Ayala H, Jade Bailey, Corbin AL, Khoyratty TE, Chai JT, Alkhalil M, Rendeiro AF, Ziberna K, Arya R, Cahill T, Bock C, Lemieux ME, Riksen NP, Netea MG, Wheelock CE, Crabtree MJ, Udalova IA, Carnicer R, **Choudhury RP.** Hyperglycaemia-induced trained immunity in macrophages and their precursors promotes atherosclerosis. 2018, In Revision.
2. Chai JT, Ruparelia N, Goel A, Kyriakou T, Biasiolli L, Edgar L, Handa A, Farrall M, Watkins H, **Choudhury RP.** Differential Gene Expression in Macrophages From Human Atherosclerotic Plaques Shows Convergence on Pathways Implicated by Genome-Wide Association Study Risk Variants. *Arterioscler Thromb Vasc Biol*. 2018; 38:2718-2730.
3. Akbar N, Digby JE, Cahill TJ, Tavare AN, Corbin AL, Saluja S, Dawkins S, Edgar L, Rawlings N, Ziberna K, McNeill E; Oxford Acute Myocardial Infarction (OxAMI) Study, Johnson E, Aljabali AA, Dragovic RA, Rohling M, Belgard TG, Udalova IA, Greaves DR, Channon KM, Riley PR, Anthony DC, **Choudhury RP**. Endothelium-derived extracellular vesicles promote splenic monocyte mobilization in myocardial infarction. *JCI Insight*. 2017; 2.
4. Ruparelia N, Chai JT, Fisher EA, **Choudhury RP**. Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nature Review Cardiology*. 2017; 14:133-144.
5. Digby JE, Martinez F, Jefferson A, Ruparelia N, Chai J, Wamil M, Greaves DR, **Choudhury RP**. Anti-inflammatory effects of nicotinic acid in human monocytes are mediated by GPR109A dependent mechanisms. *Arterioscler Thromb Vasc Biol*. 2012; 32:669-76.