Project title:

A spatial and functional map of immune responses in MASLD: linking T cells and fibrosis

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Summary:

Context and challenges: Metabolic liver disease – formerly NASH, now designated MASLD – is a substantial global health issue and also a growing one. Accumulation of fat in the liver as part of a systemic metabolic dysregulation (often coupled with alcohol induced injury) is linked to liver inflammation and subsequently to the development of liver fibrosis and liver failure. Although GLP1 agonist therapies are impacting this to some extent we are left with a large burden of liver injury and risk to manage and further interventions will be of benefit. However the immunologic pathways that link hepatic dysfunction to fibrosis are poorly understood. Unconventional T cells have been shown to be activated in MASLD and also in animal experiments to contribute to fibrosis.

Hypothesis: T cell mediated immunity – especially provided by unconventional T cells - plays a role in orchestrating the local inflammatory and fibrotic environment in the liver. Defining this mechanistically will provide new targets for intervention.

Aims: 1.Ex vivo - We aim to define the intrahepatic milieu in MASLD- driven fibrosis as it progresses using a combination of spatial transcriptomic techniques (imaging and sequencing based). We will focus on the localisation/niche and in situ functionality of key T cell subsets including MAIT cells. 2. In vitro – Human MAIT cells have been recently shown to make both VEGF A and B, as well as secrete Vimentin – this unusual biology will be further explored to define their full secretome of relevance to fibrosis and its resolution.

This programme of work is based on samples obtained through the TGLU GI illness biobank, which has permissions to allow for data and sample sharing with industrial partners.

Alignment with therapeutic area and key scientific theme(s):

The project aligns as follows:

- **Steatotic liver disease** to encompass metabolism associated steatotic liver disease (MASLD), alcohol related liver disease (ALD) and also MetALD.
- Mechanistic similarities and differences between alcohol and metabolically driven steatohepatitis and how these influence differing rates of disease progression.
- Predictors of and underlying mechanistic basis for fibrosis regression within the context of therapeutic intervention in advanced fibrotic liver disease.

Project delivery:

Spatial transcriptomic approaches are very data intense and new tools are being locally developed in concert with other GSK-aligned groups in Oxford to explore these. We currently use a mixture of Visium and Xenium approaches and the fellow will be trained in both techniques and their analysis, with a focus on detailed evaluation of T cell niche and function. The samples will be obtained through the TGLU biobank – biopsy approaches for MASLD are used clinically allowing for a wide sample base and we will also aim to address the impact of alcohol in collaboration with our clinical colleagues in the metabolic liver clinic, where such information can be collated. The in vitro studies are established in the lab and will be further pursued to address the specific roles of each mediator.

Research environment:

We are used to having fellows working between the liver immunology labs and the clinic and would find a good working blend (around 20% clinical time) to allow for sample and cohort acquisition, wet lab and transcriptomic experiments, and analysis. The exact numbers of samples and controls assessed by the spatial transcriptomic approaches will be predefined according to the budget and sample availability. The first year would likely be spent defining the best cohort and acquiring the appropriate blocks, with an initial spatial dataset obtained. The second year would develop further analysis in the spatial domain, focusing on specific patient subgroups according the 1st year results – especially addressing more severe disease phenotypes and the impact of alcohol, as well as other relevant diseases where similar mechanisms might be of importance to GSK. We would also aim to develop the in vitro study focused initially on the vimentin/VEGFA/B findings but exploring new findings emerging from yrs 1 and 2, and take this part forward into year 3 aiming to provide a description of key mediators linking T cell responses with fibrosis, as well as some mechanistic interrogation.

Relevant papers

Single-cell analysis of human **MAIT** cell transcriptional, functional and clonal diversity.

Garner LC, Amini A, FitzPatrick MEB, Lett MJ, Hess GF, Filipowicz Sinnreich M, Provine NM, Klenerman P.Nat Immunol. 2023 Sep;24(9):1565-1578. doi: 10.1038/s41590-023-01575-1. Epub 2023 Aug 14.PMID: 37580605

Stimulatory **MAIT** cell antigens reach the circulation and are efficiently metabolised and presented by human **liver** cells.

Lett MJ, Mehta H, Keogh A, Jaeger T, Jacquet M, Powell K, Meier MA, Fofana I, Melhem H, Vosbeck J, Cathomas G, Heigl A, Heim MH, Burri E, Mertz KD, Niess JH, Kollmar O, Zech CJ, Ivanek R, Duthaler U, Klenerman P, Stroka D, Filipowicz Sinnreich M.Gut. 2022 Dec;71(12):2526-2538. doi: 10.1136/gutjnl-2021-324478. Epub 2022 Jan 20.PMID: 35058274

Single-cell integration reveals metaplasia in inflammatory gut diseases.

Oliver AJ, Huang N, Bartolome-Casado R, Li R, Koplev S, Nilsen HR, Moy M, Cakir B, Polanski K, Gudiño V, Melón-Ardanaz E, Sumanaweera D, Dimitrov D, Milchsack LM, FitzPatrick MEB, Provine NM, Boccacino JM, Dann E, Predeus AV, To K, Prete M, Chapman JA, Masi AC, Stephenson E, Engelbert J, Lobentanzer S, Perera S, Richardson L, Kapuge R, Wilbrey-Clark A, Semprich CI, Ellams S, Tudor C, Joseph P, Garrido-Trigo A, Corraliza AM, Oliver TRW, Hook CE, James KR, Mahbubani KT, Saeb-Parsy K, Zilbauer M, Saez-Rodriguez J, Høivik ML, Bækkevold ES, Stewart CJ, Berrington JE, Meyer KB, Klenerman P, Salas A, Haniffa M, Jahnsen FL, Elmentaite R, Teichmann SA.Nature. 2024 Nov;635(8039):699-707. doi: 10.1038/s41586-024-07571-1. Epub 2024 Nov 20.PMID: 39567783

A longitudinal single-cell atlas of anti-tumour necrosis factor treatment in inflammatory bowel disease.

Thomas T, Friedrich M, Rich-Griffin C, Pohin M, Agarwal D, Pakpoor J, Lee C, Tandon R, Rendek A, Aschenbrenner D, Jainarayanan A, Voda A, Siu JHY, Sanches-Peres R, Nee E, Sathananthan D, Kotliar D, Todd P, Kiourlappou M, Gartner L, Ilott N, Issa F, Hester J, Turner J, Nayar S, Mackerodt J, Zhang F, Jonsson A, Brenner M, Raychaudhuri S, Kulicke R, Ramsdell D, Stransky N, Pagliarini R, Bielecki P, Spies N, Marsden B, Taylor S, Wagner A, Klenerman P, Walsh A, Coles M, Jostins-Dean L, Powrie FM, Filer A, Travis S, Uhlig HH, Dendrou CA, Buckley CD.Nat Immunol. 2024 Nov;25(11):2152-2165. doi: 10.1038/s41590-024-01994-8. Epub 2024 Oct 22.PMID: 39438660

Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic **liver** disease.

Riva A, Patel V, Kurioka A, Jeffery HC, Wright G, Tarff S, Shawcross D, Ryan JM, Evans A, Azarian S, Bajaj JS, Fagan A, Patel V, Mehta K, Lopez C, Simonova M, Katzarov K, Hadzhiolova T, Pavlova S, Wendon JA, Oo YH, Klenerman P, Williams R, Chokshi S.Gut. 2018 May;67(5):918-930. doi: 10.1136/gutjnl-2017-314458. Epub 2017 Nov 2.PMID: 29097439