Project title:

A spatial and functional map of immune responses in MASLD, Met-ALD and AILD: linking immune cells and fibrosis

Supervision:

Primary Supervisor: Prof Paul Klenerman (Liver Immunology, NDM, Oxford)

Co-Supervisors: Dr Emma Culver (Consultant Hepatologist, OUH/NDM Oxford) and Prof Ye Oo (Liver Immunology) Birmingham

GSK Supervisor: Stephen Atkinson (GSK Liver lead)

Collaborators:

Lucy Garner (Postdoc - Liver transcriptomics). Aneesha Bandari (Postdoc - Spatial biology).

Dr Jeremy Cobbold (Clinical Lead in MASLD)

Project Summary:

Context and challenges: Steatotic liver disease is defined by accumulation of fat in the liver and may be part of a systemic metabolic dysregulation (MASLD) often coupled with alcohol induced injury (Met-ALD), which are global health problems. Autoimmune liver disease (AILD) is defined as a chronic immune-mediated liver injury, with a growing recognition that steatosis impacts around one-third of patients. Steatosis is linked to liver inflammation and subsequently to the development of liver fibrosis and liver failure. The immunologic pathways that link hepatic dysfunction to fibrosis in metabolic and autoimmune disorders are poorly understood. Unconventional T cells and activated B cells have been shown to be dysregulated in MASLD and AILD, and may contribute to progressive fibrosis. Treatments to target these dysregulated immune pathways may aid fibrosis regression.

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

Aims:

1. To define the intrahepatic milieu in MASLD- driven and AILD-driven fibrosis as it progresses ex vivo, 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 T regulatory and MAIT cells) and B cell subsets (such as B regulatory).

2. To define the intrahepatic milieu in MASLD, Met-ALD- and AILD- fibrosis regression in response to therapy ex vivio, and compare the altered immune states in paired samples

3. To further define the in vitro secretome of unconventional T cells (MAIT cells) and B cells (B regulatory) of relevance to fibrosis and its resolution.

   Human MAIT cells have been shown to make both VEGF A and B, as well as secrete Vimentin – this unusual biology will be further explored. B cell subsets have been shown to drive fibrosis, via autoreactive antibodies, presenting autoantigens to t cells, and cytokine/chemokine interactions,

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), metabolic and alcohol related liver disease (MetALD) and autoimmune liver disease with/without steatosis (AILD).
- Mechanistic similarities and differences between different drivers of steatohepatitis (metabolic/alcohol/autoimmune) 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:

This programme of work is based on samples obtained through the TGLU biobank (liver biopsy and liver FNA samples), and Birmingham Biobank (liver tissue resection and transplant specimens), which has permissions to allow for data and sample sharing with industrial partners. TGLU Biobank Project for Cirrhosis and AILD (EL Culver) and Birmingham biobank (Ye Ooo). An MTA is already in place between Birmingham and Oxford for blood/tissue sample sharing.

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, with experience in the fields of viral hepatitis, early metabolic and cholestatic liver diseases. Data analysis and bioinformatics support will be provided by post-doctoral fellows - Lucy Garner and Aneesha Bandari (Postdoc - Spatial biology and Liver transcriptomics). The PhD fellow will be trained in both techniques and their analysis, with a focus on detailed evaluation of B and T cell niche and function. The in vitro studies for T cells are established in the Klenerman lab and B cells in the Y.Oo lab supported by AILD work from Culver, and these will be further pursued to address the specific roles of each mediator.

Research environment:

The PhD student will have the opportunity to do both wet and dry lab work and can commit up to 20% of their time for clinical development relevant to the project (metabolic liver and autoimmune liver clinics, supported by Culver and Cobbold). The clinical department and the liver immunology labs are in close proximity and work in parallel to allow for sample acquisition and processing, wet lab and transcriptomic experiments, and analysis. There are weekly lab meetings, monthly PhD meetings, and clear milestones against which to assess progress.

Proposal for time spent during PhD program

1st year: Defining metabolic/met-ALD and AILD cohorts. Acquiring the appropriate histology blocks via OcRHe and Birmingham Biobank. Design panels for and complete spatial transcriptomic runs.

2nd year: Develop analysis in the spatial domain, focusing on specific disease subgroups, more severe disease phenotypes, and paired samples pre-post treatment. Develop the in vitro study focused initially on the vimentin/VEGFA/B findings and B cell-fibrosis pathways, but exploring new findings emerging from yrs 1 and 2.

3rd year: Provide a description of key mediators linking B-T cell responses with fibrosis, as well as some mechanistic interrogation of these. Write up of project and presentation of data at National congresses.

Working closely aligned with GSK – Oxford exploratory interests during the PhD.

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,et al .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

The Role of B Cells and B Cell Therapies in Immune-Mediated Liver Diseases.

Cargill T, Culver EL. Front Immunol. 2021 Apr 14;12:661196. doi: 10.3389/fimmu.2021.661196. PMID: 33936097; PMCID: PMC8079753.

B cells in autoimmune hepatitis: bystanders or central players?

Schultheiß C, Steinmann S, Lohse AW, Binder M. Semin Immunopathol. 2022 Jul;44(4):411-427. doi: 10.1007/s00281-022-00937-5. Epub 2022 Apr 29. PMID: 35488094; PMCID: PMC9256567.

B lymphocytes directly contribute to tissue fibrosis in patients with IgG$_4$-related disease.

Della-Torre E, Rigamonti E, Perugino C, Baghai-Sain S, Sun N, Kaneko N, Maehara T, Rovati L, Ponzoni M, Milani R, Lanzillotta M, Mahajan V, Mattoo H, Molineris I, Deshpande V, Stone JH, Falconi M, Manfredi AA, Pillai S. J Allergy Clin Immunol. 2020 Mar;145(3):968-981.e14. doi: 10.1016/j.jaci.2019.07.004. Epub 2019 Jul 15. PMID: 31319101; PMCID: PMC6960365.