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LEUKOCYTE DYNAMICS

Group Leader

Leo Carlin

Fellow of the Royal Microscopical Society

Research Scientists

Demi Brownlie
Gemma Cairns
Marco De Donatis
Ximena Raffo-Iraolagoitia
Mashidur Rana[1]
Qing Sun[2]

Graduate Students

Lindsey Arnott
Ryan Devlin[3]
Désirée Zerbst[4]

[1]MRC project grant with Newcastle
[2]Joint CRUK Programme Award with Newcastle
[3]TRACC programme; co-supervised with Ed Roberts, awarded PhD in 2024
[4]Medical Research Scotland; co-supervised with Vignir Helgason, awarded PhD in 2024

The immune system can exert both anti- and pro-tumour activity, therefore, understanding the role of immune cells in the cancer microenvironment is of critical importance. Our lab uses cutting-edge light microscopy and other techniques to investigate the spatiotemporal dynamics of immune cells in cancer.

The immune system has been implicated in almost every stage of cancer development, from initiation and growth, to dormancy, invasion, and metastasis. As the immune system co-evolved with microbes to protect against infection and as cancer cells are mutated host cells, the role of immunity in cancer is complicated. Even though immune cells can kill cancer cells and stabilise the primary tumour to help prevent its spread, they can also suppress anti-cancer immunity and benefit tumour growth and dissemination. The immune compartment of cancer is composed of the resident immune cells of the tissue and leukocytes that infiltrate from the circulation. The development of the cancer immune environment is inherently dynamic, and the processes that regulate immune cell recruitment and function are not well understood. Recent success in directing and strengthening the immune system's anti-cancer functions (e.g. immune checkpoint inhibition and CAR-T cells) highlight the potential for new therapies that can come from a better understanding of how immune cells are (dys) regulated. However, these strategies do not work for all cancers or all patients.

Specialised vasculature and leukocyte dynamics

Our group has a particular interest in the lung and the liver, both as sites of primary tumour development and as targets of metastasis. The extensive capillary network of the lung is unusual in several ways. Alveolar capillaries are of exceptionally small diameter (~5µm) and are in such close proximity to external mucosa which they share a basement membrane with the epithelium. In contrast to other organs, pulmonary capillaries are thought to be a major site of leukocyte extravasation, with markedly different mechanisms to the general paradigm of leukocyte recruitment.

Tumours in the lungs and liver interact with the vasculature in markedly different ways to those in other organs. For example, some tumours grow into and co-opt the existing microvasculature whereas others replace or push the vasculature and other tissue structures out of the way, generating their own neovasculature. This affects the way that immune cells access the different tumours. The liver is also a highly specialised immune environment consisting of a network of specialised blood vessels with a huge surface area. The liver's importance in homeostasis makes particular requirements for the way that immunity must function in this organ. Localisation and regulation of leukocytes within the pulmonary capillaries and liver sinusoids is not fully described or well understood.

The work of several groups has suggested that neutrophils are important in onco-immunology, and a high neutrophil-to-lymphocyte ratio is associated with poorer prognosis in many advanced cancers. Myeloid cells in general are crucial in many anti-microbial and tissue damage reactions and play a key role in initiating the host immune response to infection. Emerging data suggest that they are exquisitely sensitive to their microenvironment. In addition to potent effector mechanisms, including phagocytosis, degranulation and the recently described process of NETosis, neutrophils can contribute to the inflammatory milieu in a number of ways. Neutrophils can produce and consume chemokines, cytokines and growth factors and can modify the extracellular matrix. Additionally, the accumulation of apoptotic neutrophils and their subsequent clearance by macrophages is thought to directly contribute to anti-inflammatory programmes at the end of acute inflammatory responses. **Taken together, these features mean neutrophils have the potential to both antagonise and promote tumours depending on context** (McFarlane *et al.*, 2021, *J.Clin.Invest.*), and recent work has demonstrated that neutrophils actually benefit cancer spread in the process of lung and liver metastasis. Because of this diversity of actions and importance in the host defence, we need more mechanistic detail in order to interact with neutrophils in a way that would inhibit cancer but not leave the patient at risk of serious infection. Myeloid cells can be regulated by – and can regulate the function of – other immune cells, so an important goal is to look at several different cell types simultaneously to glean more information about the way that they interact and to uncover potential pathways to modify.

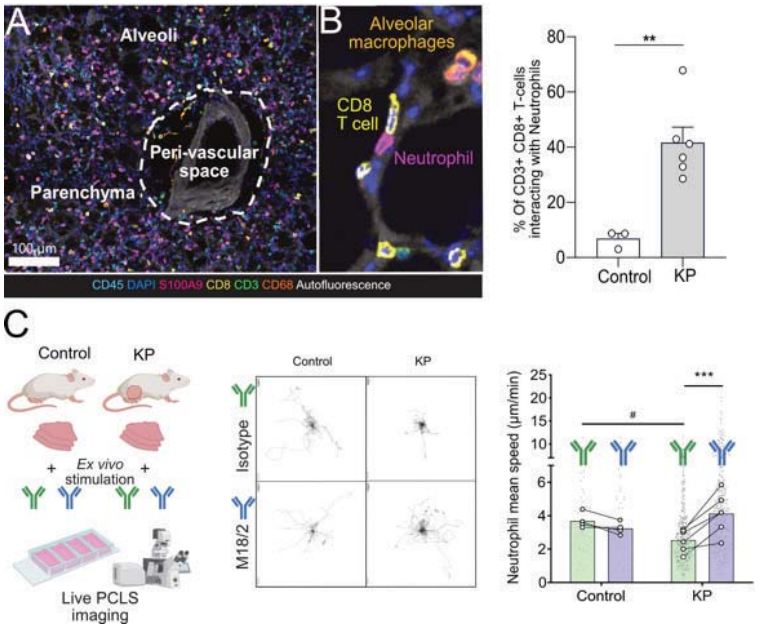

Figure 1. Neutrophil dynamics in the metastatic breast cancer pulmonary pre-metastatic niche. **A.** 3D multiplexed immunofluorescence of precision cut lung slice from a mammary (KP) tumour bearing mouse revealing abundant S100A9$^+$ neutrophils. **B.** CD8$^+$ T cells and neutrophils are commonly colocated. **C.** live imaging of ex vivo lungs from KP mice reveals slowed neutrophil motility that can be reversed by an antibody (M18/2) that activates β_2 integrin (an important immune cell adhesion molecule). Data reproduced from Fercoq, Cairns *et al.*, 2024, *bioRxiv*.

This year we preprinted work in which we probed the location and motility of neutrophils within the lungs of mice bearing spontaneously lung metastatic mammary tumours at a stage before overt lung metastasis could be observed. Using imaging, we found that the pulmonary capillaries were packed with neutrophils in mammary tumour bearing mice (Figure 1A). CD8+ T cells were in close contact with the neutrophils (Figure 1B). Live imaging revealed that the neutrophils moved more slowly than their counterparts in the lungs of healthy mice (Figure 1C). We are working on the hypothesis that this aberrant neutrophil intravascular motility, which we have evidence disrupts blood flow through the pulmonary capillaries, may protect seeding tumour cells and allow them to produce metastases (Fercoq *et al.*, 2024, *bioRxiv*). We are currently revising this work for publication and were pleased to receive interest and insightful comments from Simon Cleary on the "PreLights" Company of Biologists promising pre-prints website (***https://prelights.biologists.com/highlights/neutrophil-slowing-obstructs-the-capillaries-of-the-pre-metastatic-lung-in-breast-cancer/***).

In summary, by looking across multiple, relevant, cancer models, we aim to do three things: 1) uncover general mechanisms by which immune cells and their regulation contribute to the cancer microenvironment; 2) uncover cancers with the strongest or most manipulable interaction with particular immune cells; 3) monitor how treatment with immuno- and chemotherapeutic agents affects leukocyte localisation to develop better treatment schedules and combinations.

This year it was a pleasure to see Dr Marco De Donatis, our former PhD student who joined us for an additional year as post-doc to complete experiments, move on to an exciting post in immunotherapy at UCL, and two of our PhD students, Dr Desiree Zerbst and Dr Ryan Devlin be awarded their PhDs. We were also delighted that Dr Ximena Raffo-Iraolagoitia was promoted to Associate Scientist, well done and well deserved all! On a personal note, I was honoured that this team and the BAIR's excellent work was recognised by my own promotion to Senior Staff Scientist and the core-funding for the Leukocyte Dynamics Group research renewed. Thanks to all our colleagues, and the panel that contributed to this.

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