LEUKOCYTE DYNAMICS

Group Leader
Leo Carlin

Fellow of the Royal Microscopical Society

Research Scientists
Gemma Cairns[1]
Frédéric Fercoq[1]
Amanda McFarlane
Ximena Raffo Iraolagoitia
Qing Sun[2]

Graduate Students
Marco De Donatis
Ryan Devlin[3]
Désirée Zerbst[4]
Julia Lee[5]

[1]Breast Cancer Now
[2]CRUK Programme award
[3]TRACC programme; co-supervised with Ed Roberts
[4]Medical Research Scotland; co-supervised with Vignir Helgason
[5]TRACC programme; co-supervised with Sarah Walmsely (Edinburgh) and Jo Birch (SCS)

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 primarily 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 produce factors that 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, yet.

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. 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 (see Figure 1). 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. Neutrophils 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, a feature previously thought to only apply to other myeloid cells. 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 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. Neutrophils can be regulated by – and can regulate the function of – other immune cells, so an important goal is to look at a number of different cell types simultaneously to glean more information about the way that they interact and to uncover potential pathways to modify.

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. We continue to collaborate with several groups here at the Institute to investigate this in state-of-the-art pre-clinical models. We were excited to contribute to a major collaboration (Leo Carlin as co-senior author with Tom Bird, Owen Sansom, and Derek Mann) that addressed the role of neutrophils in the response of hepatocellular carcinoma on a non-alcoholic steatohepatitis background (NASH-HCC) to immunotherapy. In this context neutrophils were thought to be tumour promoting via their suppression of anti-tumour immunity. We found that by targeting the major neutrophil chemokine receptor CXCR2, we could make our models of NASH-HCC vulnerable to immune checkpoint inhibitors. Intriguingly, there were more, rather than fewer neutrophils in the tumours, but they had changed phenotype, suggesting the possibility of reprogramming neutrophil activity in the tumour microenvironment (Leslie, Mackey, Jamieson *et al.*, Gut 2022). We were excited to see the opening of a clinical trial based on this combination (CUBIC) and also received funding via a CRUK programme grant renewal to continue this highly productive collaboration.

Publications listed on page 103

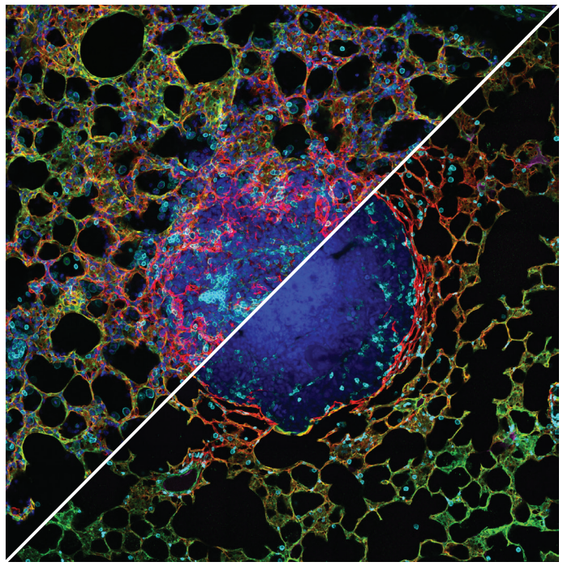

Figure 1
Two different types of vascularisation in mouse lung tumours grown from intravenous tumour cell line injection (experimental metastasis model). Images fused down centre line shown for comparison. **Left**, *Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Pdx1-Cre* (KPC) cell line with vessel co-optive pattern. **Right**, renal cortical adenocarcinoma (RENCA) cells with a pushing / angiogenic pattern. Both samples were stained to reveal nuclei (left, DAPI; right, Sytox Blue; BLUE), vasculature (CD31; GREEN, ICAM1 (RED) and leukocytes (CD45 ; CYAN). The image also illustrates clear differences in the pattern of leukocyte infiltration to the two similar sized tumours. Images were aquired by Marco De Donatis using Zeiss 880 (BAIR) in spectral detection mode.