

LEUKOCYTE DYNAMICS



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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 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 with pathogens 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. tumour infiltrating lymphocyte (TIL) therapy and immune checkpoint inhibition) 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.

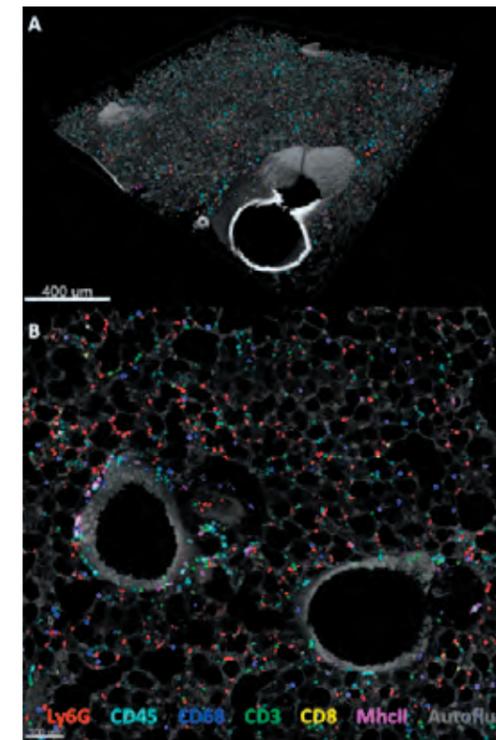
Specialised vasculature and leukocyte dynamics

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 that 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. 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 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, and recent work has demonstrated that neutrophils actually benefit cancer spread in the process of lung metastasis. Because of this diversity of actions and importance in the host defence, we need more mechanistic detail in order to interact with

Figure 1
Imaging the (pre-) metastatic niche in the lung. **A.** rotated and **B.** top down projections of 3D multiplexed immunofluorescence to identify immune cells in the lung from a mouse bearing a primary mammary tumour.



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. Recently we have extended a cleared or live 3/4D tissue spectral unmixing workflow (see example image from Fred Fercoq in collaboration with the Roberts, Blyth and Coffelt groups) to image 6+ cellular markers *in situ*. We are actively applying this technique, which preserves important localisation data lost in many other techniques, to better understand the “who, when and where” of the immune (pre-)metastatic niche in the lung and liver.

As you would expect this has been a challenging year for our group. However, the team and our colleagues both in the Institute and further afield have shown great resilience and have made the most of any and all resources available to continue to make progress in addressing our central question: How do the immunological mechanisms that regulate neutrophil retention in the lung and liver (including their site of developmental origin) influence cancer, metastasis and immunotherapy?

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