Quantitative Analysis of Immune Cell Migration and Spatial Processes in Health and Disease

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1 Brief Summary

The study of the immune system has benefited enormously from advanced intra-vital microscopy techniques that allow the visualization of immune cells in real time within their physiological tissue setting [4]. Analysis of such spatio-temporal data is conceptually challenging and computationally intensive. The parallel progress in analytic and computational approaches to correct for artefacts [1, 7] and to simulate migrating cells in various environments [8, 2] have markedly improved our ability to properly interpret such imaging data. Nevertheless, connecting the complex choreography of populations of immune cells to their coordinated function remains an exciting area of active research. Our meeting brought together both experimentally and theoretically oriented researchers with diverse backgrounds ranging from immunology and cell biology on the experimental side to mathematics, physics, and computer science on the theoretical side. The meeting built upon a previous successful BIRS meeting on a similar topic

Talks covered novel biological discoveries, emerging experimental techniques, and advances in simulation and modelling. The workshop led to new collaborations between the involved researchers.

2 Topics Covered

2.1 Migration and spatial processes in maintaining immune system steady state

Immune cells migrate through and between tissues even when there is no ongoing immune response. Such cellular movement is necessary in gathering signals during development (for instance for T cells in the thymus [10], or hematopoietic stem cells in the bone marrow), in mediating immune tolerance (as in the suppression of autoimmunity by regulatory T cells), in providing immune cells with trophic signals needed for their survival (such as cytokines or other receptor-ligand interactions), or in facilitating the detection of pathogen invasion (or malignant cells) by strategic localization of immune cell populations, or by their optimized search strategies [6]. Mathematical modelling is critical to understanding how the sometimes strikingly different movement patterns adopted by immune cells in these different circumstances aids in the execution of cellular functions. For example, like foraging mammals [11], immune cells are thought to migrate according to a Levy walk in some circumstances [5], and according to a persistent random walk in others [9, 3]. A further important subject in this area is multi-scale modelling to integrate data on between-organ migration with data on within-organ migration. Mature mathematical modelling approaches from other fields, such as

mathematical ecology and single-particle tracking, can be applied to immunology, and one major goal of our workshop was to exchange the latest results in these thriving areas to understand immune cell migration.

2.2 The role of tissue structure in site-specific immunity

The extracellular matrix within tissues provides the structural backbone of organs, and can substantially impact immune cell migration both by facilitating or restricting cell motility. In several tissues the topological properties of the tissue stroma directs migratory trajectories by acting as a primary substrate for cellular motion. In other instances, heterogeneities in tissue structure establish distinct microenvironments and substantially alter which immune cells come into contact with each other. Interactions of immune cells with stromal cells can regulate wound healing to restore tissue integrity or, alternatively, lead to chronic damage and fibrosis. Yet, for the past decade, most mathematical models of immune cell migration have not explicitly represented the interactions between cells and their environment. Instead most models treat cells as freely moving particles. One aim of our workshop was to chart a way forward for the field to create models that better reflect the importance of the tissue environment. This will likely require more complex modelling strategies (e.g., the Cellular Potts Model), and novel mathematical and/or computational techniques will have to be developed to make the modeling of large-scale systems (e.g., dense tissues) feasible. At the same time, it is crucial that mathematicians properly appreciate the key characteristics of the tissue environments in which cells migrate.

2.3 The impact of individual cell behaviour on immune response robustness in infection and cancer

Collectively, the complex behaviour of individual immune cells can generate either a robust immune response clearing the pathogen/tumour, or can result in impaired immunity enabling pathogen/tumour persistence. The use of fluorescently-tagged pathogens or tumour cells, together with longer-term imaging of sites of infection or tumour growth are beginning to reveal mechanisms by which pathogens or tumour can inhibit effective immune responses, through impacting cell migration, or even taking advantage of immune cell motility to enable spread to other locations. A growing body of clinical evidence suggests that migration-related factors play an important role in determining which patients do, or do not, respond to current immunomodulatory therapies. This is a crucial issue in the field of cancer immunotherapy, as several novel treatment options have been appearing in recent years (with sometimes impressive results). However, most of these immunotherapies only benefit a subset of patients, and it is currently not possible to predict in which patients the treatment will be beneficial. Additionally, this is putting a considerable burden on healthcare systems in many countries due to the high cost of immunotherapy. Thus, there is an urgent need for predictive biomarkers. Although the study of cancer growth or infectious disease spread is a mature field, the aspect of predicting responses to immunomodulating therapies has only recently become relevant, and this field is in still in its relative infancy. We therefore invited several researchers from immuno-oncology to the meeting in order to spark new collaborations in this area.

3 Presentation Highlights

1. Migration and spatial processes in maintaining immune system steady state

The meeting was kicked off by an extended, overarching presentation by **Matthew Krummel** who touched upon several aspects of how immune cells search and transfer information to each other. Over the past years, his lab has been exploring such questions using a variety of experimental systems at different scales, ranging from immune cell migration to molecule cluster organization at the cell membrane. Krummel's lab has employed both experimental and modeling techniques, and has written an influential review on the topic of search in the immune systems [6], and so his talk was a great kick off for the week.

Several talks focues on different aspects of **immune cell migration**: **Judy Cannon** showed T cell migration experiments and data in both lymph nodes and lung; this was related to **Ajitha Thanabalasuriar**'s presentation, which unravelled pulmonary immune responses to infection using intravital imaging. By contrast, Heather Melichar's talk focused on T cell dynamics during their education in the thymus, where suitable

T cells are selected that do not react strongly to the body's own proteins. The relevance of migration for disease control was highlighted by **Judith Mandl**'s talk, who showed how cell-shattering in a knock-out mouse can lead to a damaging bias of the T cell responses. **Janine Coombs**' talk highlighted the importance of migration in another important immune cell type, the natural killer (NK) cells whereas **Irina Grigorova** focused on B cells. **Audrey Gerard**'s presentation showed how T cells form an ecosystem in which they can co-exist and regulate each other. **Scott Mueller**'s talk joined several of these threads by discussing his lab's work on tracking the dynamics of immune responses in various tissues, both lymphoid and non-lymphoid.

Another theme of the meeting was the influence of the surrounding **tissue** on shaping the behaviour of local immune cells. We saw relevant examples of this in **Joshua Schiffer**'s talk of herpes simplex virus infection in the skin and **Takaharu Okada**'s presentation on atopic dermatitis and itching, and important auto-immune disease. **Christoph Konradt**'s talk zoomed in on immune niches in the intravascular compartment. The talks by **Mark Miller**, **Rob de Boer**, and **Mario Novkovic** all discussed the critical role of the central stromal structure in lymphatic tissue, the fibroblastic reticular cell (FRC) network. This topic generated intense discussion given that several of the attendees had worked on it both from the experimental and the mathematical side.

The extreme diversity and complexity of immunological processes in different tissue compartments is daunting from a mathematical modelling perspective. However, several talks during the meeting also highlighted the feasibility of extracting general mechanisms and insight despite this complexity. **Melanie Moses'** talk reminded us of scale invariance in biology and principles that hold across systems as diverse as ants and robots. **Grant Lythe**'s presentation emphasized the role played by randomness in cell positioning and movement, which is a core feature found in many immunological processes. Along the same line of identifying fundamental overarching principles in this seemingly confusing field, the organizers had also invited several speakers from **cell biology**. **Anna Labernadie** showed an impressively detailed *in vitro* experimental model for interrogating the migration of invading tumor cells at unprecedented resolution. **Ana-Maria Lennon** shared a wealth of insights on how dendritic cells respond to various quintessential environmental cues. On the modeling side, **Raphael Voituriez**, **Brian Camley** and **Johannes Textor** all discussed various mathematical and computational approaches that represent different aspects of moving cells; interestingly, the different models often reach similar conclusions in parallel.

From the **tumor immunology** perspective, **Bettina Weigelin** showed exciting data from both *in vitro* and *in vivo* experiments that highlighted how T cells co-operate to kill tumours, and she also shared intriguing unpublished data on the influence of temperature on tumor cells. Related to this, **Joost Beltman** discussed the use of mathematical and simulation models to estimate the killing efficacy of intratumoral T cells. **Morag Park** showed how analyzing the tumor microenvironments provides potentially useful clues for predicting patients response categories upon treatment with immunotherapy.

The meeting also featured several presentations that introduced novel **experimental techniques and modeling frameworks** to the community. **Anmar Khadra** presented an in-depth approach to interrogate protein-protein interactions at focal adhesion sites. **Jun Abe** showed an impressive real-time data acquisition and processing platform for intravital imaging. **Alex Huang** showed nice examples of micro-fabricated device engineering to systematically study the biophysical properties of migrating cells in more realistic flows. **Majid Abedi** introduced a novel computational model for 3D tissues that is based on molecular dynamics (MD) approaches and can therefore be implemented in powerful existing MD software packages.

4 Scientific Progress and Outcome

A concluding plenary discussion on the final day highlighted several emerging themes from the meeting, which we summarize below.

4.1 Interplay between cells and tissues

One central aspect that was highlighted is the central role of the tissue environment to determine cell behaviour. It also became clearer that this is a bi-directional interaction, in which tissue and cell imprint on each other, rather than only the tissue influencing the cell. An important question that was raised was to what extent it would be possible, based on modelling, to predict the behaviour of a cell once it reaches the target

tissue. It was pointed out that oftentimes, immunologists think of the tissue environment mainly in terms of chemokines and cytokines, but that the biomechanical and structural aspects deserve greater attention. It was also questioned how strong our direct evidence for the existence of chemokine gradients actually is, given that these are very difficult to measure within living tissue. Whereas we often think of, and model, chemokine gradients as stable and static, in reality they might exist only transiently. Finally, due to emerging imaging techniques, we will be able to move more and more towards the imaging of whole tissues versus only isolated structures, which will bring about novel insights and challenges for mathematical modelling.

4.2 Optimal search strategies

A recurring theme during the meeting has been the question of optimal search strategies. This is a controversial topic in many fields, and it also proved to be challenging in the field of T cell migration. Despite the fact that several previously published papers presume that T cell migration has been optimized by evolutionary pressure, the mathematical basis of some of these models – especially Lévy walks – has been questioned. It is often not clear to what extend a complex, "optimal" search strategy would really be necessary and robustly be selected for compared to a much simpler, "good enough" strategy. In reality, "optimization" could often just mean that evolution contributed to shaping a strategy, not necessarily parameter optimization in mathematical sense. It could be more relevant to think in terms of trade-offs that shape the migration strategy of a cell – such as exploration versus exploitation – instead of locating precise optima, which may be very model-dependent and noisy. In fact, it was even debated whether optimization of search strategies is at all a relevant question for T cells. There were also discussions about the sizes of effects when comparing cells in different conditions, when often too much focus is on statistical significance instead of biological relevance of a small difference in a migration parameter.

4.3 Interplay between modeling and experiments

Given the meeting's mixed audience, there was also intensive discussion on how collaborations between modellers and experimentalists should be conducted for maximum scientific benefit. Experimentalists appreciated the potential for models to generate new hypotheses or, importantly, rule out hypotheses that are inconsistent with the data. Everyone agreed that a crucial aspect is rapid iteration between models and experiment. However, experimentalists felt that modellers could do more to make clearer which kind of questions can, and cannot, be answered by what type of model, especially in the light of debates within the modelling field where different kinds of models are built and promoted and sometimes they come to different conclusions. In this regard, there is for instance no good consensus yet whether simple or holistic models are generally to be preferred. However, it was pointed out that much can be learned by starting from a simple model and stretching it until it "breaks".

Concrete strategies that were identified to improve future collaborations between modelers and experimentalists included:

- Communicate better with each other to make clear how they can be most useful for each other. For instance, modelers should make clearer to experimentalists what kind of data they really need to make progress.
- There should be a better appreciation that modelling is a non-trivial enterprise that has a real cost, even if there is a lower amount of physical resources needed compared to many experiments. Often there seems to be an assumption that models are "free", which seems to lead to overly optimistic expectations regarding what models can achieve and in what time.
- To the extent possible, modeling would greatly benefit from standardization of assays, especially *in vitro* assays. This would make it easier to integrate and compare data across studies and laboratories, accelerating scientific progress.
- Finally, any collaboration between modelers and experimentalists should have a scientific question at heart that both parties are genuinely interested in answering. Ideally, modelers and experimentalists should already work together in the design of the experiments and the models.

4.4 New collaborations established

According to the feedback we got from the participants, everyone very much enjoyed this productive and focused meeting and the many opportunities for scientific exchange and open and frank discussion. The meeting also led to new collaborations between the participants. For example, a discussion triggered during Johannes Textor's talk on Lévy walks led to a collaboration with Raphael Voituriez in which a critical issue in Lévy walk theory has been identified with potentially major implications for the field of optimal migration. This has led to a joint manuscript which is already submitted. Discussions triggered by Ana-Maria Lennon's talk led to a new collaboration between her and Judith Mandl's lab, in which the migration of individual T cells in specifically constructed micro-channel environments will be rigorously quantified for the first time, leading to new data of great value for modeling purposes. Discussions with Mario Novkovic led to the sharing of his data on the topology of the networks on Follicular Reticular Cells in lymph nodes. Several of the participants agreed to join forces to organize a further meeting on this topic area in the next few years.

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