Insights from modeling metabolism and amoeboid cell motility in the immune system

Steijn, L. van

Chapter 6

Discussion

In this chapter, we will first summarize the results described in the previous chapters of this thesis. After that, we discuss future directions in the field of cell migration modeling.

6.1 Summarizing discussion

In Chapter 2, we presented ZebraGEM 2.0, an improved whole-genome scale metabolic reconstruction for zebrafish. Compared to the previous version of ZebraGEM [34], ZebraGEM 2.0 has been extended with the oxidative phosphorylation pathway, Gene-Protein-Reaction assocations and a more realistic biomass function. Due to the Gene-Protein-Reaction associations, it can now be used for knock-out studies, respiration experiments, and prediction of minimal feed, of which we have shown several examples. Furthermore, we analysed changes in metabolism upon *Mycobacterium marinum* infection by integrating gene expression data of control and *Mycobacterium marinum* infected zebrafish larvae. The model predicts a lowered growth rate and reduced histidine metabolism for the infected larvae. The biosignature of reduced histidine metabolism is also seen in other studies on human patients, mice and zebrafish [280, 281]. Overall, this improved model can be used to predict changes in zebrafish metabolism in other experimental conditions based on expression data, which can point out specific pathways, reactions or metabolites to further study experimentally. Moreover, the model can be used as a reference framework for interpreting omics data, for example, by showing RNAseq data on the metabolic network structure [282].

In Chapter 3, we studied the influence of cell-matrix adhesions on lymphocyte cell motility type. The type of motility is important for lymphocytes, as motility types differ in their effectiveness as immunosurveillance behaviour. In this chapter, we proposed an extension of the Act model [73] with cell-substrate adhesions to model lymphocyte motility. The model includes the adhesions between cell and extracellular matrix and contains four processes of adhesion dynamics: 1) *de novo* adhesion formation at the

actin-rich leading edge of the cell, 2) expansion of already existing adhesion patches, 3) spontaneous detachment of adhesions, and 4) adhesions breaking as the cell retracts. By increasing *de novo* adhesion formation, as well as the energy required to break adhesions, cell speed and cell persistence drop, and further increase results in pivoting behaviour, which is also observed in B-lymphocytes with sustained attachment areas [143]. However, the addition of these four processes fails to explain floating cells with no or few adhesions that are unable to migrate efficiently. Hence, we extended the model by including an extra feedback from the total adhesion area to the effectiveness of propulsion. Including this feedback, the model can display low motility at low total adhesion areas as well as stop-and-go motility types at sligthly higher *de novo* adhesion formation rates. Finally, we also saw that the ratio between the *de novo* adhesion formation and expansion of adhesion patches influences the spatial distribution of adhesions and the persistence of migration: cells with mainly small adhesion at the leading edge are more persistent than cells with a single or few larger adhesion patches near the cell center or rear. All in all, the behaviour captured by this model is very rich and is comparable to behaviours seen in different types of lymphocytes. Furthermore, the model show that parameter values regarding *de novo* adhesion formation, adhesion patch expansion and strength of the adhesion affect motility type. The molecular processes that underlie these parameters, such as which integrins are expressed and where they localize, or the strength of the matrix-integrin bond, could be studied experimentally to see if they result in the motility types predicted by the model. Such studies could deepen our understanding of how molecular defects in the interactions between immune cells and the ECM, e.g., in multiple myeloma [143] or inherited immune disease, eventually lead to altered immune cell migration and immunity defects.

In Chapter 4, we used the Cellular Potts model (CPM) to study why cells are efficient at performing topotaxis. Previous work on active Brownian particles (ABPs) has shown that part of the topotaxis effect can be explained by reduced cell persistence in denser pillar grids [75]. However, the active Brownian particle model cannot explain the extent to which *Dictyostelium discoideum* cells perform topotaxis. Using two different methods to model persistent cell motion, we fill in the gap between active Brownian particles and *Dictyostelium discoideum*. One method implements a persistent random walker model into the CPM and can be viewed as an ABP model with deformable volume. The other method, the Act model, phenomenologically models actin polymerization [73]. Both methods resulted in more efficient topotaxis than ABPs, so deformable volume makes cells more efficient at topotaxis. Furthermore, the actin-based method showed inhibition

of locomotion upon colliding with pillars, and reoriented in a different direction than the ABP-based method. This active reorientation leads to even more efficient topotaxis. We conclude that, for biological cells, cell volume and active reorientation enhance the persistence driven topotaxis already predicted by the ABP model. We can further use this model to explore more cell-steering grid properties, such as alternative pillar shapes or pillar adhesivity patterns, for applications in tissue engineering. Moreover, studying this model setup provides us with insight in cell motility in environment crowded by ECM and other cells, and, together with Chapter 3, allows us to explore how the cellular microenvironment can influence the direction and type of cell motility.

After mathematical of modelling cell motility, we turn to analyzing cell tracks of leukocytes in *in vivo* zebrafish in Chapter 5. Here, we studied the role of TLR2 and MYD88, both part of the TLR-signalling cascade, on leukocyte migration upon tail wounding in zebrafish larvae. Neutrophils and macrophages of both *tlr2*$^{-/-}$ larvae and *myd88*$^{-/-}$ larvae were compared with those of wildtype siblings. There was no difference in number of leukocytes and leukocyte basal migration between unchallenged mutant and wildtype larvae. However, upon tail-wounding, both *tlr2*$^{-/-}$ and *myd88*$^{-/-}$ larvae showed less recruitment of neutrophils and macrophages at 2 to 6 hours post wounding than their wildtype siblings. We further analysed cell track data of cells distant from the wound to study how leukocyte migration is changed in the mutants. Besides analyzing the speed, net displacement and meandering index, we also analyzed the mean velocity towards the tail end and the mean squared displacement from which we derived persistence times. For distant neutrophils there was no difference in speed, but the directional movement toward the wound and persistence of motility were reduced in the mutants compared to wild type neutrophils. For macrophages, there was a similar difference in directionality, but on top of that, the *tlr2*$^{-/-}$ and *myd88*$^{-/-}$ macrophages had lowered speed. From this extensive cell track analysis, we conclude that TLR2 and MYD88 play a role in the directionality of leukocyte migration upon wounding.

6.2 Future work

6.2.1 Combining signals in cell migration

In this thesis, we came across a number of cues that guide cells or influence cell motility, as seen in Chapters 3 and 4. Cells *in vivo* encounter many of these cues at the same time. There have been some efforts in understanding combined cues. Li et al. used a 3D model of breast cancer cell migration

with interstitial fluid flow, autochemotaxis and ECM fibres to study how these cues are combined in cell motion [283]. They showed that the flow of self-secreted chemoattractant and the alignment of the ECM fibres with the fluid flow resulted in synergistic motility: cell displacement was higher when fluid flow and ECM alignment with fluid flow were increased together than the sum of cell displacement when only one of the two effects (increased fluid flow; increased ECM alignment with fluid flow) were applied. In an experimental topotaxis setup, the effects of chemotaxis and topotaxis on directed migration of *Dictyostelium discoideum* were studied [74]. Here, the sum of drift in the case of aligned chemotaxis and topotaxis and in the case of opposed chemotaxis and topotaxis is equal to twice the drift of topotaxis. Thus, the chemotactic and topotactic drifts can be added up vectorially. We still need more insight in how cues work together to be able to grasp cell motility *in vivo*. Model studies can aid in this endeavour by explicitly integrating multiple cues at the same time. An interesting aspect to study here is the interplay of the molecular machinery of cell locomotion and the different signalling cues.

6.2.2 A stroll in the cellular landscape

Aside from the chemical signaling in chemotaxis and haptotaxis, more studies now also focus on the structure of the environment. While cells move through a tissue, they encounter non-motile cells, ECM, interstitial fluid, which vary through different tissues; bone tissue is structured differently from lymph nodes. In seperate models from Hecht et al. and Tweedy et al. [284, 285] chemotaxing cells within a maze were studied and both models showed that the interplay between chemotaxis and the environmental structure can result in directional cues. In the model from Hecht et al., cells could get stuck in a dead end of a maze which was permeable for a chemoattractant. Secretion of a chemorepellant which could not penetrate through the maze walls could resolve this. In the model of Tweedy et al., cells rapidly consumed the chemoattractant, guiding the cells away from chemoattractant depleted dead ends. Both studies contribute to the questions that arise: to what extent is cell motility shaped by the structural organization of the environment? And how can we find out about that? In the example of synergy between autochemotaxis, fluid flow and aligned ECM fibres [283], the model was used to test the different combinations of fibre and flow alignment and showed that they have a synergistic effect on directed cell motility. To extract this information from *in vivo* or *in vitro* experiments is a lot harder. The lack of knowledge on the exact shape of the environment could then skew the perceived effectiveness of chemotaxis.

Another example of where knowledge of the shape of the environment plays a role in interpretation of cell motility is a recent study on T cell motility in liver [286]. Cell tracking data showed that these T cells display superdiffusive behaviour, which is often associated with Lévy walks and optimal fouraging. However, the data lacked the infrequent large displacements, which are a key characteristic of Lévy walks. They hypothesized that T cells performed Brownian random walks, but that the channeled structure of the liver shaped their motion such that it became superdiffusive. To test this, they extracted the liver structure from imaging data and modeled the motion of Brownian walkers inside this structure. This sufficed to reproduce the superdiffusive behaviour, confirming their hypothesis that liver structure shapes cell motility.

A study combining modeling and imaging showed that the crowded environment of the lymph node plays a dominant role in T cell motion [72]. Both liver and lymph node show limited space for the cell to move in. This brings us to the point of the dimensionality of 3D environments. Obstacles in the form of cells, cell layers and ECM fibres can reduce the 3D space to 2D or 1D space for cells to move in. Some of this structure is immutable, such as mineralized ECM in bones, but cells can also alter parts of this structure; they can degrade or rearrange the ECM. Understanding the interplay between immutable and mutable structures in cell migration is useful for further understanding of immune cell patrolling as well as immune cell penetration in tissues such as tumours and granulomas.

6.2.3 Exchange between *in vivo* and *in vitro* motility parameters

The previous paragraphs point to a discrepancy between observed motility and the inherent motility. This also makes it hard to directly translate motility parameters between *in vivo* and *in vitro* data. Currently, Bayesian inference methods are being used to extract data on chemotaxis fields from cell track data from the lymph node [275]. However, the underlying model does not take the spatial structure of the lymph node into account. This could result in wrong estimates of the inferred chemotaxis fields or motility parameters.

Incorrect estimates often bring novel insights. In Chapter 4, we matched the CPM parameters such that our modeled cells had the same motility as *Dictyostelium* cells on a 2D substrate. The subsequent topotaxis experiments showed a discrepancy in speed between the *Dictoystelium* cells and the simulated cells. This teaches us that our model still lacks some elements that do play a role in the experiment. This points to further research on what

those elements are and, in the long run, contributes to our understanding of cell motion.

In Chapter 4, we used a simple hill-climbing algorithm to optimize two parameters to obtain experimental cell motion. In this case it was a straightforward answer to a straightforward question. However, the role of machine learning in this field is currently growing [287, 288]. When we want to increase the accuracy of inference methods, we must turn to more detailed models, which inevitably come with more parameters. Machine learning can aid in exploring the right areas of parameter space. Vice versa, computational modeling can also aid in quantifying the uncertainties in parameter estimations from *in vivo* or *in silico* data, by generating training and test data sets [288].

6.2.4 Patroling and more: other tasks of immune cells

So far, we have mainly discussed cell motility. However, cell migration is of course only a small aspect of the complex behaviour of cells. Leukocytes, such as neutrophils and macrophages play a role in clearing out pathogens, and hence, must also direct part of their energy to digestion of pathogens. Furthermore, they also relay and amplify their own recruitment by producing cytokine and chemokines, which also requires a portion of their energy. Hence, we can view the different tasks they have to fulfill from a metabolic viewpoint.

When we want to understand the possible trade-offs in immune cell motility, pathogen clearance and cell signaling, we can of course make use of multiscale models. Integrating metabolism in motility models such as the CPM or other agent-based modeling frameworks allows us test hypotheses on infection clearance on a tissue scale. Recent work by Graudenzi et al. combined the CPM and FBA framework by computing the growth rate for indivual cells in the CPM using FBA [289]. We can also think of combining constraint-based metabolic models with the CPM through the Hamiltonian of the CPM. We can compute the energy available for movement depending on the leukocyte state (migrating, phagocytizing, or signalling) and use that energy budget for energetically unfavourable moves, instead of the Boltzmann probability.

Advancements in single cell experimental techniques help in this approach. Sequencing data of isolated cells can function as a basis for the metabolic component of these models. Data from cells known to be in different states, such as infection state, or recruited/recruiting, would be of great value here.

Overall, this thesis presents a number of models that can be used as building blocks for multiscale modeling, where combining metabolism and cell migration models can give us further insight in how immune cells fight infections.