Project title: Prediction of T cell communication and differentiation dynamics by quantitative mathematical modeling

Project number: J29/2017

Executive Summary

The mammalian immune response depends on the interaction and collaboration of many highly individual cells. While ineffective responses to pathogens cause immediate risks for the whole organism, the same is true for overreactions of the immune system, which can induce directly lethal septic shocks as well as chronic inflammatory diseases such as rheumatoid arthritis. While research has provided an enormous body of knowledge about the regulatory mechanisms behind chronic inflammation, it is difficult to assess and quantify the contribution of each individual process with current biological methods. In particular, what are the critical components and conditions triggering a change towards chronic inflammation, despite the multi-faceted mechanisms that promote immune tolerance? In this project, we developed and applied mathematical modelling and data analysis techniques to investigate and quantify such regulation of immune responses. First, we arrived at a mathematical representation of immune cell proliferation, communication and decision-making allowing for both efficient data-based model simulations and conceptual analysis of simplified models. Second, we developed a highly efficient, user-friendly 3D simulation environment for analysis of immune cell communication in spatially confined lymphoid organs. Third, we initialized several collaboration projects with experimental groups at the DRFZ, in which we provided our expertise in quantitative methods for ongoing research projects. Overall, the methods for dissecting immune-cell communication networks developed in this Leibniz-funded research group provide the building blocks for systematic, data-based in silico perturbation studies that may help rationalize the personalized therapies of the future.

1. Achievement of objectives and milestones

Overall, the project was carried out as projected in the application, although some modifications in the distribution of labor and scheduling of project tasks was necessary due to budgetary restrictions (see section 2). Below, I will briefly describe activities and achievements in the respective work-packages.

Work-package 1 (ph1 and KT): Empirical assessment of T cell differentiation kinetics and spatial localization

As anticipated, this work-package was initiated directly after launching the project in January 2018. In the first six months, analysis of data-set 1 was carried out by a student internee, Sebastian Serve, together with KT. That resulted in preliminary scans of the kinetic microarray data set, that is **MS 1.1**, including exploratory analysis of the full data-set (e.g. principle component analysis) as well as preliminary analysis of the kinetics of highly relevant genes for Th cell differentiation, such as gata3. Further analysis of this data-sets included in-depth statistical profiling of gene-expression kinetics, kinetic cluster analysis and gene-classification with respect to biological function and kinetic profiles, and eventually lead to the first genuine research paper of the group (**Burt et al., Front Immunol 2022**).

Work package 2 (ph1 and KT): Response-time modeling of cell-to-cell communication in T cell populations.

In this work-package, there was a slight change of plans for scientific reasons: We soon realized that some adaptations to the original response-time modeling framework would be necessary for effective mathematical description of immune-cell dynamics. In particular, a data-based implementation of proliferation, apoptosis and decision-making was lacking, and was not completely straight-forward given that we sought to stick to the principle of mathematical descriptions that can be directly translated to measurable quantities. Therefore, this work-package was initiated by ph1 together with KT directly upon hiring of the PhD-student (Philipp Burt) in May 2018. Further, **MS 2.1** was reformulated: The most important short-term goal was now to derive a mathematical framework allowing to incorporate the critical ingredients of immune-cell dynamics, namely proliferation, cell-cell communication and cell differentiation, in a data-driven manner. Indeed, we found a solution to this problem within the first year of the project. That effort was followed by detailed analyses of small conceptual models with respect to biologically relevant scenarios, and culminated in exploring a scenario of T-cell decision-making in acute and chronic inflammation. This subproject was in the end the most successful one of the whole project and lead to our recent key paper (**Burt and Thurley, Sci Adv 2023**).

Another projected part of work-package 2 was spatial extension of the response-time modeling framework. Although scheduled for a later phase of the project (**MS2.3** in years 3 and 4), we were able to start with preparations for that work, since a Bsc student (Lukas Kiwitz) joined the group in June 2019. Together with KT, he designed a finite-element simulation framework for spatio-temporal, cytokine-mediated immune cell communication (Figure 3). That framework allows simulations of models such as previously published (Thurley et al., PLoS Comp Biol 2015), but with a much more convenient, python-based user interface and state-of-the-art finite-element based numerics. Therefore, simulations can be run on a well-equipped standard personal computer and can be adapted to specific biological situations. In the following, we used that modeling framework for deriving a deeper understanding of the implications of spatially distributed cytokine signaling, e.g. in terms of spatially inhomogeneous cytokine concentrations and cell-state transitions, and arrived at a preprint and recently to a full

manuscript (**Brunner et al. 2022,** https://doi.org/10.1101/2022.03.17.484722**; Brunner et al., submitted**).

Work package 3 (KT): Elucidating plasticity, memory and variability in immune cell differentiation

This work-package was carried out as intense collaboration project together with the Löhning group at the DRFZ. In previous work, that group observed an astonishing stability of Th-cell subsets over weeks and months, in particular for T-bet expressing so-called Th1 cells. We wondered how such stable gene-expression patterns can be obtained by transcriptional and/or epigenetic regulation, and KT formulated a conceptual model to analyze a set of plausible scenarios (**MS 3.1**). Those models suggest that the observed long-term stability in gene expression is best achieved by an epigenetic mechanism that was subsequently investigated in more detail by our collaborators. A publication of the results from this ambitious collaboration project is close to submission.

Another major data-analysis effort was launched by the second PhD-student of the group in terms of a collaboration with Thomas Dörner at Charite-Berlin, where we analyzed a large transcriptomics data-set (>500 sequencing samples) on PBMC derived from SLE and Sjögren's patients under various conditions. In this project, which was partly done in collaboration with my new group members in Bonn, we performed detailed statistical analyses on disease-related transcriptomic differences in lymphocytes and obtained very interesting results especially with regard to cell-cell communication patterns, and we could recently publish the results (**Kwon et al., NPJ Syst Biol Appl, in press**). Moreover, several other modeling and data-analysis projects together with DRFZ collaborators in this framework lead to already published or submitted research papers (see Section 3).

2. Activities and obstacles

The project was largely carried out as projected. The main concern in the starting phase was a necessity to re-assess the distribution of labor because of the 10% budget cut, which effectively prevented hiring a second PhD-student (funding occurred to be insufficient for 2 times a 3-years position). We were able to fill that gap by two complementary strategies:

> 1st, we attracted in total 5 Msc students to do research projects and internships in the new group. This was achieved by taking part in teaching activities and by reaching out to potential students at various opportunities. Such activities made it possible to conceptualize and initiate work-flows and project ideas that would have been out of reach to be done by KT and ph1 alone.
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> 2nd, we acquired third-party funding, especially in the starting period of the project. That effort resulted in two DFG research grants (see below), so that we were (since August 2019) able to synergize with the DFG funded projects in a mutually beneficial way. In particular, the DFG project together with the Löhning group allowed to study specific data-driven models based on data on acute and chronic inflammation.

In addition to these difficulties in the start-up phase, the project was affected by the pandemic (2020/21), especially with regard to the planned international collaborations which largely had to be discontinued for the duration of the project.

Finally, the project concluded prematurely in 02/2021 due to me taking a new position in Bonn, with only a small part of the budget being continued until 12/2022. Nevertheless, most of the project goals have been reached (see Sections 1 and 3).

3. Results and successes

A first publication of the group was accomplished already in the starting phase (**Hammer et al., Nat Immunol 2018**), and contains a contribution to quantifying NK cell proliferation dynamics by mathematical modeling. Further publications together with of collaborators at the host institute followed later (**Cendon et al., EJI 2022; Burt et al., Cells 2022**), have been submitted (manuscripts with the Triantafyllopoulou and Hutloff groups) or are close to submission (Löhning group). The first genuine research articles of the group were also published recently (**Burt et al., Front Immunol 2022; Burt et al., Sci Adv 2023; Kwon et al., NPJ Syst Biol Appl., in press; Brunner et al., submitted**).

A high priority in the starting phase of the new group was acquisition of third party funding. We succeeded to acquire funding in terms of two DFG research grants that not only cover our modelling and data analysis efforts, but additionally also quantitative experiments performed in collaborating research groups: one project together with the Löhning group at DRFZ, and another project together with the Triantafyllopoulou group at DRFZ (within DFG priority program Innate Lymphoid Cells).

4. Equal opportunities, career development and internationalisation

The group hired 1 international PhD student (Gino Kwon, South Korea) out of 3, and hosted two international student internees (Voula Tassopoulou, Greece, and Pau Pascual, Spain). Integrating such international students into our science, and helping with their administrative tasks etc., was a priority in my group shared by all lab members. All job announcements contained the statement: “The DRFZ is an equal opportunity/affirmative action employer, committed to excellence through diversity, and strongly encourages applications and nominations of members of under-represented groups. Individuals with disabilities will be given priority, if qualified accordingly.” All members of the group participated in regular discussion clubs organized at the DRFZ, such as the weekly T-cell club and the monthly Computational Immunology Club that I co-organized myself. These clubs were an excellent format to exchange ideas and foster collaborations with experimental groups, and also were an opportunity to gain practice in scientific presentations and discussions. Furthermore, all group members attended international conferences.

5. Structures and collaboration

Collaborations were implemented and carried out as planned, with exception of the international collaborations that were severely hampered by the Covid-19 pandemic. Collaborations with the Romagnani, Radbruch, Chang and Löhning groups were highly successful and already lead to publications.

6. Quality assurance

Good scientific practice is a high priority in all my research activities. In weekly lab meetings, all lab members presented their work, and lively exchange of ideas and critical discussions among group members were encouraged. Group members also presented their work in regular discussion clubs in the DRFZ or the weekly meeting of the institute for theoretical biology where my group was co-opted (see above). Research data management was performed according to DFG guidelines, including regular backups of computer code and other research results on DRFZ-based data servers.

7. Additional resources

The DRFZ provided an amount of Eur 107 580,04 total co-funding.

8. Outlook

Further areas of active research in the Thurley group include data analysis and modeling contributions with regard to specific immunological questions, e.g. related to the onset of inflammation processes in viral infections and to cancer-immunotherapy. Moreover, we continue working on the conceptual and mathematical foundations of our modelling approaches regarding both data-based network analyses and 3D multi-scale model formulations.