

Supervisor's report on Matouš Vobořil's Diploma thesis:

Aire-expressing cells in immune peripheral tissues.

The study describes an investigation into the presence and basic characterization of cells which potentially impart the process of peripheral tolerance. This is a very hot topic in the field of "*Tolerance and autoimmunity*" which so far provided only a very limited amount of data, which is at this moment quite controversial. Notably, while a few studies identified Aire-expressing population of cells in the secondary lymphoid organs (SLOs) which function to eliminate autoreactive cells from the immune periphery, their origin, identity and precise phenotype are intensively debated. The work of Matouš significantly contributes to this effort. It not only provides basic characteristics of cells expressing *bona-fide* Aire protein but it also maps several lymphoid as well as non-lymphoid organs for their presence and thus significantly extends the search for a network of peripheral organs which could impose peripheral tolerance. This, in turn, provides an experimental framework for further studies in the field of tolerance and, in a long run, prevention of autoimmunity.

Matouš joined our research group in the fall 2012 as an undergraduate student. He quickly mastered many essential molecular, cellular and tissue culture techniques. He worked with several transgenic mouse models and excelled in work with FACS analysis, fluorescent microscopy and qRT-PCR analysis. Thus, after a short while, he was able to conduct relatively complex experiments independently. Experiments where he had to carefully isolate cells from many different peripheral tissues are very tedious, intensive and time consuming. Here, Matouš was able to demonstrate his strong commitment to this project, technical skills and growing experience. The novelty of his work is the identification of Aire expressing cells in non-lymphoid tissue, specifically the lungs. While Matouš will continue to work on related topics as the PhD student in my laboratory, major objectives of his Master thesis have been completely achieved and far exceeded my expectation.

The thesis is written in a standard format in English. The work is split into classical chapters with smooth and logical transitions between them. The Introduction, Literature review and Materials and Methods sections highlight the rationale of the study, describe its design and procedures as well as methods required for accurate analyses. The result and discussion sections present novel findings in a clear way and put them into the context of current knowledge of cellular basis of peripheral tolerance.

Conclusions and recommendation

In my opinion, Matouš Vobořil is a very diligent, accurate, and well organized student. His results provided the foundation for future studies towards understanding the cellular basis and fundamental processes guiding peripheral tolerance. Richness of experimental approaches, clear presentation of results and concise but dense discussion fully attest that Matouš is well prepared for his scientific carrier. Moreover, the fact that his thesis is written in English further strengthens his potential as a successful PhD student.

Based on the above review, I fully recommend Matouš Vobořil's thesis to be accepted as the fulfilment of the requirements for the degree of Master of Science.

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