Travel report on the working visit to the Institute of Biology of the Eötvös Loränd University in Budapest, Hungary

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From 1 February until 7 February 2016, I visited the Complement Research group of Dr. Mihály Józsi at the Department of Immunology of the Institute of Biology of the Eötvös Loränd University in Budapest, Hungary.

Their research aims to elucidate the role of the recently discovered factor H-related (FHR) proteins in the regulation of the complement system. Dr. Mihály Józsi is a well-known expert within the complement field and has discovered one of the FHR proteins, FHR-4A. The six FHR proteins are closely related to the crucial complement regulator factor H (FH), which in principle protects host cells from unwarranted complement activation. Currently, the FHR proteins are hypothesized to act as an additional step in the regulation of complement by competing with FH for certain surfaces and thereby fine-tuning when and where FH regulates complement activation.

However, despite several efforts, the complement research field has not been able to quantify either of the six FHR proteins due to the lack of specific reagents. This is one of the main objectives of my PhD-project and to achieve this we have generated over 50 monoclonal antibodies (mAbs) directed against FH and the FHR proteins. Recently we have completed the set-up of an assay to quantify FHR-3 and we are currently focussing on FHR-4A and its splice variant FHR-4B. FHR-4B consists entirely of 5 domains also found in FHR-4A, thus antibodies directed against FHR-4B will always be able to also recognize FHR-4A. Moreover, FHR-4A contains 4 additional domains which are highly identical to the 5 domains shared with FHR-4B. This leaves little room to obtain antibodies that can distinguish between FHR-4A and FHR-4B.

The aim of the working visit was to identify which of our 13 anti-FHR-4A mAbs were able to distinguish FHR-4A from FHR-4B. For this we used recombinant FHR-4A, FHR-4B as well as fragments of FHR-4A which Dr. Józsi has generated in his lab. By determining to which FHR-4A fragment(s) each of the mAbs was binding, we would be able to deduce to which domain the mAbs would be directed. In turn, that would allow us to select a combination of mAbs that could be used to set-up a FHR-4A specific assay. With the help and expertise of Dr. Józsi, I was able to complete these experiments within a few days and we determined the binding domain of the mAbs. As expected, some mAbs recognized both FHR-4A and FHR-4B of which most had two binding sites in FHR-4A, due to the high sequence identity between the domains. We also identified mAbs that are specific for FHR-4A, which now allows us to set up FHR-4A specific assays.
After completing these initial experiments we continued with studying FHR-4A and FHR-4B in functional assays and we found that several mAbs affected some of the functions of FHR-4A. We are currently pursuing these interesting findings to further elucidate the physiological role of the FHR proteins, which we will combine with the physiological levels to be published in one manuscript.

During the working visit I learned a lot on the FHR-4A and FHR-4B proteins and new assays to study their function within the complement system. The results obtained allow me to continue with developing an assay for FHR-4A. Furthermore, this working visit has initiated a valuable collaboration between the group of Dr. Mihály Józsi and our own complement group led by Dr. Diana Wouters. I am confident new and important findings regarding the regulation of FH by the FHR proteins will come out of this collaboration.

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