Final NENS report - Violetta Refolo

Duration: 3 months

Home Lab: Assoc. Prof. Nadia Stefanova, Neurodegenerative Research Lab, Innsbruck Medical University

Host Lab: Assoc. Prof. Marina Romero-Ramos, CNS Disease Modeling group, Dep. of Biomedicine, Aarhus University

Getting a NENS stipend was a great opportunity for me, as it gave me the chance to visit Prof. Romero-Ramos´ lab at the Aarhus University for 3 months (1st March 2015 – 30th May 2015); in this period I could learn how to profile the morphology of microglial cells, which represents an important part of my project, dealing with the profiling of microglial activation as a therapeutic target in MSA transgenic mice.

The principle aim of this project is to characterize the evolution of microglial activation with regard to Multiple System Atrophy, detecting markers for the different microglial activation stages and correlating the collected data to human pathology.

To date, it is well known that microglia, the resident immune cells of the CNS, play a key role in MSA pathogenesis, in particular in the neuroinflammatory processes involved in the disease. However, which kind of effect they have on the pathological process is still not completely clear. It has been proposed that these cells could exert a classic pro-inflammatory response (M1), resulting in the expression of pro-inflammatory cytokines and enhanced microbicidal capacity, and an alternative anti-inflammatory one (M2), characterized by scavenging of debris and tissue remodelling and repair (Perego et al., 2011; Boche et al., 2013). Furthermore, microglial cells have been recently subdivided in four distinct activation profiles, with different morphology (Sanchez-Guajardo et al., 2010), by the CNS Disease Modeling group in Aarhus. According to this method, the morphology of each Iba1 positive cell (microglia) is scored during the stereological analysis according to Sanchez-Guajardo et al. (2010). The profiling and classification of the four activation stages of these cells (A, B, C, D) is achieved according to the length and thickness of cell processes, the characteristics of cell body and the appearance of the nucleus.

Respectively, Type A cells possess a round dense nucleus, no visible cytoplasm, and long thin processes with few small branching; Type B cells have a dense nucleus surrounded by a visible thin cytoplasm, processes are thin and very elongated, with many branches of less defined edges; Type C cells are characterized by enlarged, less defined nucleus surrounded by elongated and irregular body with shorter redefined processes of varying thickness and little branching; Type D cells have a big cell body merging with the processes, which are few, thick and short; the nucleus is not always distinguishable and occupies most of the cell body, which is merging with the processes.

As part of my project, in Prof. Romero- Ramos´ lab I have performed Iba1 immunohistochemistry on free floating sections of wild type and transgenic PLP mice (for both groups, 2, 5 and 15 months old animals), in order to detect microglial cells; after that I have stereologically analysed Substantia Nigra with the optical fractionator, defining the subtype (A, B, C or D) of each microglial cell counted. I have also performed Immunohistochemistry for other markers, such as MHC II, to get a better insight in the overall immune response in these animals.

Preliminary data show increased neuroinflammation in 5 and 15 months old PLP mice (prevalence of B type microglia, and presence of C and D type cells), if compared with younger transgenic and WT mice. In the 5 months old PLP animals, many clusters of microglia are also detectable; at a preliminary observation, they seem to be more abundant than in the 15 months old ones. Definitive data will be available after analysis of the remaining samples for each group.

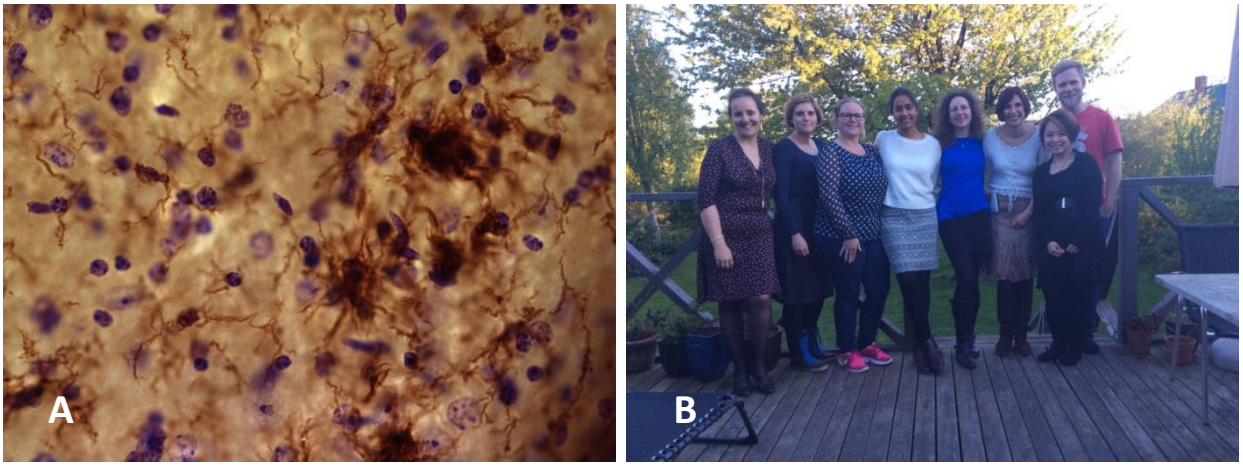

Fig. 1 Activated microglia in MSA model (A); Prof. Romero-Ramos´ group and me (B)

Learning this method has surely been of great importance for me, representing a fundamental step in the development of my project. Furthermore, I will have the possibility to transfer this knowledge to my colleagues at my home institution.