ISN-CAEN Research Report (December round) – 2016

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Host Supervisor: Dr. Klaus-Armin Nave – Max-Planck Institute of Experimental Medicine, Goettingen – Germany

Proposed project: Axon-glia interactions and demyelination

Research background: Leprosy is a chronic infectious disorder of the peripheral nerve caused by the infection of Schwann cells by the intracellular pathogen *Mycobacterium leprae* (M. leprae). The mechanism behind the pathology in Leprosy neuropathy is loss of axonal/glia interactions followed by dysfunction. In addition to that, nerve damage caused by M. leprae is characterized by demyelination, axonal loss and severe inflammation. Because there are no rodent models susceptibly to experimental infection, relatively little is known about the pathogenesis of Leprosy disease. Moreover, because biopsy of human nerves is not feasible in most circumstances, *in vitro* systems, such as primary Schwann cells/macrophages cultures as well as neurons-Schwann cells co-cultures are commonly used to mimic *in vivo* situations.

Schwann cells are deeply involved in a wide range of axonal behavior including the maintenance of axonal integrity. A number of reports have identified reciprocal communications between Schwann cells and axons and, more important, perturbing energy metabolism in Schwann cells directly leads to axonal loss and myelin abnormalities. Our group recently demonstrated a profound energy dysfunction in M. leprae-infected Schwann cells; we observed high levels of glucose uptake followed by its deviation towards the phosphate pentose pathway (PPP). Also, these metabolic changes resulted in a marked reduction in lactate generation and mitochondrial damage. When we blocked G6PD activity, the rate-limiting enzyme of the PPP, we successfully reduced M. leprae viability in infected Schwann cells.

Working hypothesis: Although M. leprae induces demyelination after contacting

myelin-producing Schwann cells, we largely unknown the fate of these myelin ovoids in infected cells. To explore that, myelinating Schwann cell-neuron co-culture is a very attractive model to interrogate the fate of myelin after M. leprae infection. In addition, teased fibers from peripheral nerve offers a reliable and quick method to obtain myelinated segments *in vitro*.

Methods and obtained data:

1) Purified sensory neurons and glial cells were obtained after enzymatic dissociation of dorsal root ganglia harvested from E14.5 mouse embryos. Cells were plated on collagen-treated coverslips and, after 2 weeks in myelinating medium (MEM + FBS 10% + NGF 50 ng/mL + ascorbic acid 50 ug/mL), we could observe several myelinated segments (Figure 1 and 2).

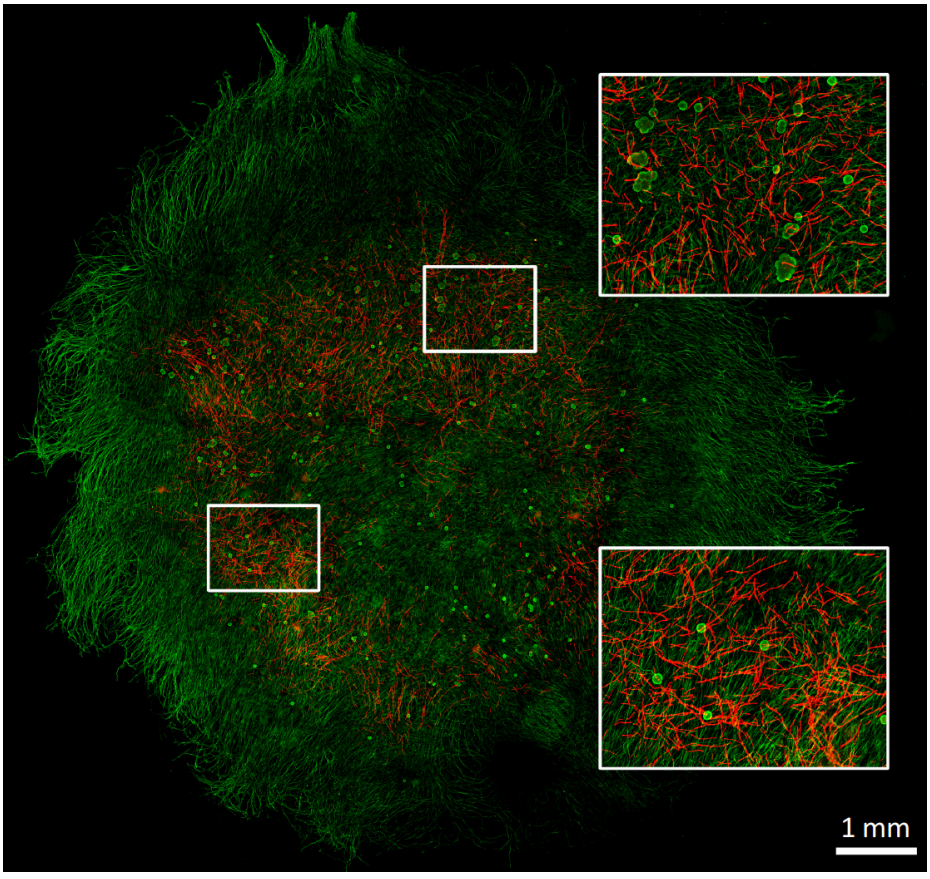

Figure 1: Myelinating co-culture of embryonic mouse sensory neurons and Schwann cells, that had myelinated for 2 weeks, showing several neurites (green - TUJ1) and associated myelin segments (red – MBP).

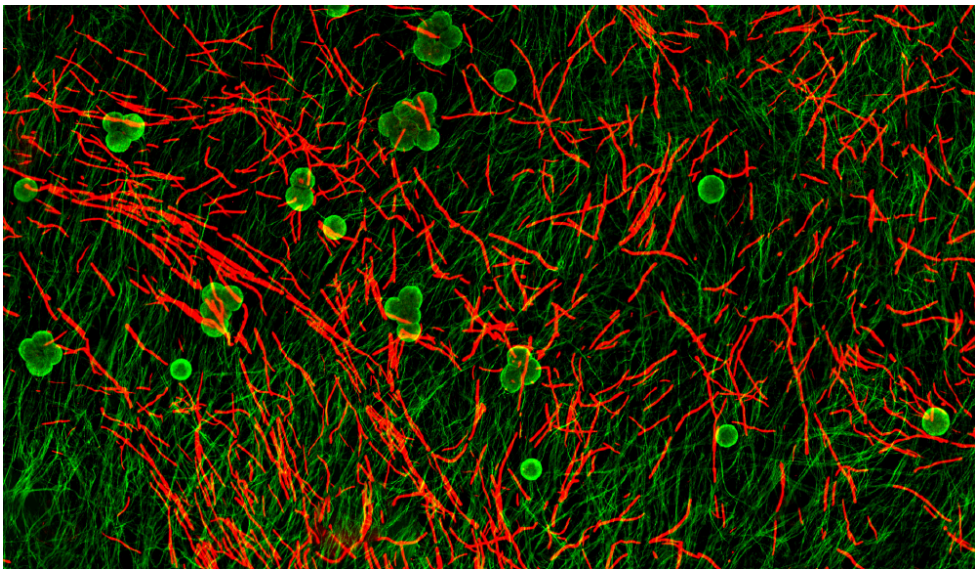

Figure 2: High magnification of a myelinated co-culture stained for MBP (red – myelin) and Tuj1(green - neuronal cell bodies) showing the high density of myelinated fibers.

2) For the teasing fiber preparation, sciatic nerve segments were harvested from adult mice and placed on ice-cooled PBS Ca^{+2} free. The surrounding tissue were carefully removed and the nerve fibers were teased apart in glass slides. Then, the tissue was fixed and processed for immunolabeling against the myelin marker MBP (Figure 3).

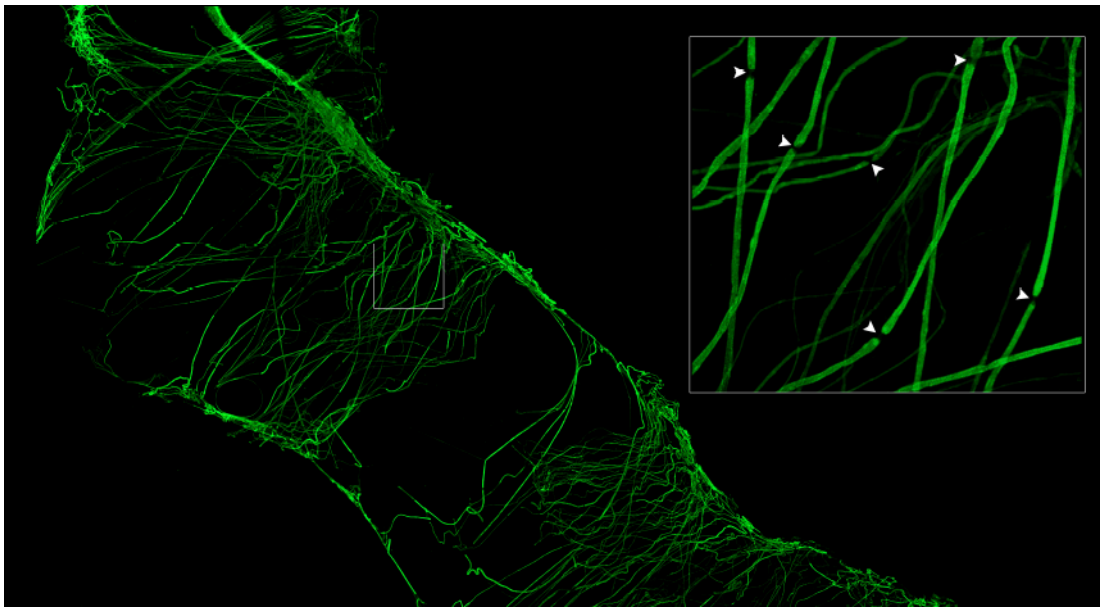

Figure 3: Teased fiber preparation from mouse sciatic nerve showing several individual fibers (green – MBP) and their nodal regions (arrowheads).

Follow-up: Back to Brazil, I submitted the requirements for our Ethnical Committee for the Use of Experimental Animals to use pregnant female mice for the myelinating co-culture. While I am waiting their response, I started using the live teasing preps to interrogate the role of M. leprae in inducing myelin abnormalities. As preliminary data, I observed myelin breakdown phenotype close associated with M. leprae burdens (Figure 4).

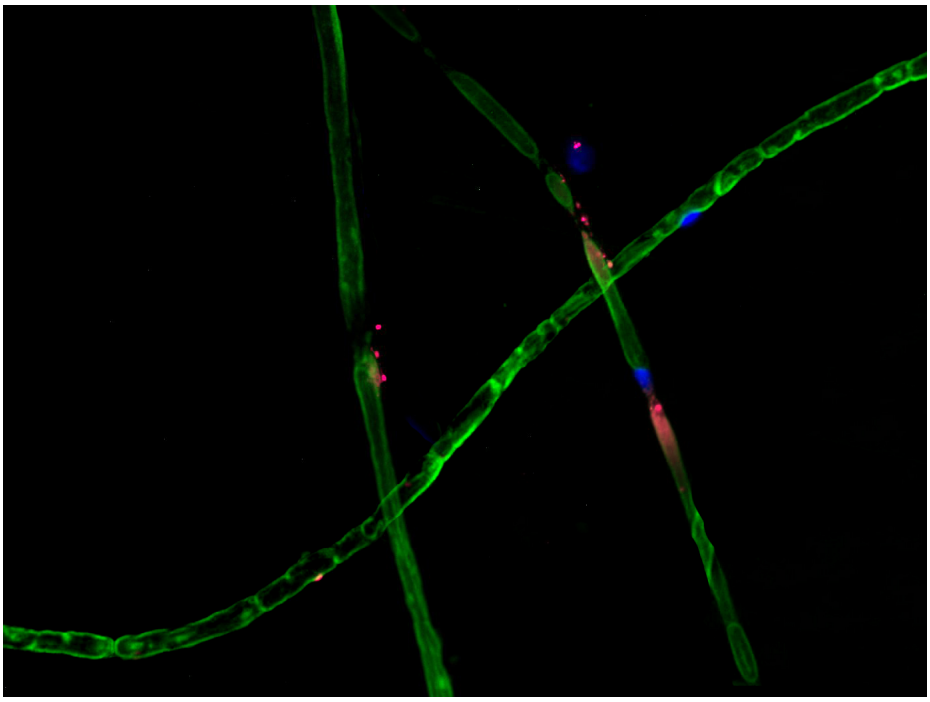

Figure 4: Myelinated teased fibers stained for MBP (green - myelin), showing signals of myelin degeneration closed associated with M. leprae (PKH red).

Host lab: The work in the Dr. Nave lab on axonal/glial interactions is highly cited and has led to new ideas concerning the importance of Schwann cells in peripheral neuropathies. By joining his internationally recognized laboratory, I had the opportunity to immerse myself in an environment with many students and professors who are all focused on dissecting the role of axonal-glia metabolic coupling and myelin abnormalities in disease. More important, this research lab visit allowed me to learn the induction of myelination in Schwann cells-neurons co-cultures and the preparation of live teasing fibers, which is a feasible and quick method to obtain myelinated fibers *in vitro*.

My goal in joining Dr. Nave's lab was to get advanced research training beyond that possible offered in my home country. This exchange program was fundamental to improve my lab skills with tools that will allow me to perform cutting-edge research in peripheral neuropathies. My career plan is run a laboratory focused on disorders of the peripheral nerve and I can confidentially say that this opportunity, sponsored by International Society for Neurochemistry, undoubtedly positively impacts on my quest for a permanent faculty position in Brazil.

I would first like to thank Dr. Nave for receiving me in the lab. I am gratefully indebted for his valuable comments on my research project and for the remarkable opportunity to receive his mentoring career advice. I am very confident that this lab visit will enable us to establish future collaborations in our common field of interest. I would also like to thank Dr. Thereza Kungl, who greatly assisted me in all experiments. This accomplishment would not have been possible without your help. Finally, my sincere acknowledges for the grant assistance provided by ISN, that was used to support my travelling, housing and meals expenses during my period in Goettingen.

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