RELAZIONE DI DOTTORATO (anno 2005/06)

Role of the Receptor like Protein Tyrosine Phosphatase zeta (RPTPz): initial *in vivo* characterisation.

For its survival and its internal biological processes, the cell must rich an equilibrium between phosphorilation and dephosphorilation. Protein Tyrosine Phosphatases (PTP) play an active role in this equilibrium, and a part of this family, the subgroup of the Receptor like Protein Tyrosine Phosphatases (RPTP) act as a PTP as well as a receptor.

In the nervous system, RPTP play a role in axon formation and guidance, glial cells migration during neural development, and in neural migration and regeneration, neuron/glia interactions, glia differentiation and survival and myelin formation in adult.

RPTPz, member of the RPTP subgroup, is a transmembane protein with its external part made of a Carbonic Anhydrase like domain (CAH), a Fibronectin type 3 domain (FN3), and a part called Spacer that can be short or long due to alternate RNA splicing. Its internal part is made of 2 tyrosine phosphatase domains. The internal and external parts are linked to each other thanks to a single transmembrane domain.

RPTPz is widely express in all the nervous system (central and peripheral) in neurons and glial cells (except microglia).

As a receptor and a phosphatase, RPTPz has different ligands and substrates. One of the main substrate is phosphorilated βcatenin, when RPTPz dephosphorilate βcatenin the cell migration is stopped. But, when one of the possible ligand, the growth factor Pleiotrophin18 (PTN18) binds RPTPz, the phosphatase is inhibited and βcatenin remains phosphorilated. Other possible ligands are matrix proteins (e.g. Tenascin) or cell adhesion molecule (e.g. Contactin).

Previous studies have been published on RPTPz KO mice by Harroch et al. Harroch has deleted the CAH like domain of RPTPz in mice and observed that:

- The nerve ultrastructure of KO mice is less compact, the myelin lamellae tend to separate, be fragmented and deformed.
- During an Experimental Autoimmune Encephalomyelitis (EAE= animal model for

multiple sclerosis), KO mice show a greater susceptibility to the disease and an impaired recovery from the disease whereas control mice recover.

●Spinal cord of KO EAE mice shows, in histological staining, an intensive degeneration compared with control mice.

Based on available literature, RPTPz localisation and its implication in recovery from the EAE disease, my working hypothesis was that RPTPz may play a key role in myelination/remyelination process and nerve integrity.

To assess this hypothesis, in house RPTPz KO were generated by replacing the genome sequence from exon 3 to exon 9 by Lac Z gene. This sequence corresponds to the sequence encoding for CAH and FN3 domains. KO mice were then used first for behavioural phenotyping in order to obtain baseline values and assess *in vivo* deficiencies due to the KO.

Another subgroup of mice was challenged in diseased conditions (EAE) to assess the impact of RPTPz KO in conditions related to demyelination.

RESULTS

LacZ staining

The LacZ staining of heterozygotes mice shows a staining in all the nervous system with preponderance in the cortex, hippocampus, and cerebellum.

Analyses are ongoing for identification of cellular type by Immunocytochemistry of LacZ and specific cellular markers (neurons, glial cells, etc).

Behavioural phenotyping

For the behavioural phenotyping, -/- and +/+ littermate male mice were tested for exploratory behaviour and strength (grip strength test, openfield, and accelerated rotarod), memory (Y maze), nociception (tailclip, hotplate, Hargreaves, VonFrey), and electrophysiological readouts at two different ages (10-18 weeks and 40-48 weeks).

RPTPz KO mice show no significant differences compared to wild type littermates in strength (grip test), exploratory behaviour and anxiety (open field, nr of entries in Y maze) and mechanical nociception (tail clip).

No differences were detected in nerve conduction velocity measured by in vivo electrophysiology.

Accelerated rotarod, test for motor coordination and balance, show no differences in the learning process between the 2 genotype. However, KO mice seem to be worst performers showing a significant impairment in motor activity and coordination at both considered ages.

Together with the LacZ staining in the cerebellum, these data suggest a role for RPTPz in motor coordination.

In the Y maze spontaneous alternation test, working memory evaluated as correct alternation, was significantly affected in KO mice at the younger age considered. This defect in working memory and the presence of LacZ staining in hippocampal neurons may suggest of role of RPTPz in memory process.

Concerning pain readouts, KO mice present a significant hyposensitivity to thermal (hotplate, Hargreaves) and tactile (Von Frey) stimuli at both ages considered, suggesting a potential role of RPTPz in nociception.

Disease conditions: Long term EAE

A long term (80 days) EAE was performed on -/- female mice and +/+ littermate. The protocol chosen for the induction of the disease was similar to the one used by Harroch et al (2002).

At the end of the experiment, plantar Hargreaves test was performed on non-paralysed forelimbs then electrophysiological readouts collected. Mice were then sacrificed and brain and spinal cord taken for detailed analysis.

During the long term EAE, no significant differences in the overall course of the disease (time onset, maximal clinical score, and mortality) were present. No recovery was observed in +/+ or -/- mice.

Since EAE mice were female and presented hind-limbs paralysed by the disease, the plantar Hargreaves test was performed on forelimbs and one group of healthy female for each genotype was included as control for both behaviour and electrophysiology recording.

In the Hargreaves test, -/- healthy mice show a significant hyposensitivity compared to +/+ confirming the results obtain during the behavioural phenotyping in male hind-limbs.

EAE induced hypoalgesia in +/+ mice but not in -/- which show no difference in thermal sensitivity between healthy and EAE animals.

During the in vivo electrophysiology in EAE and healthy control mice, different parameters were recorded that give information on nerve conduction velocity as well as myelin and axonal integrity (e.g.: Peripheral Conduction Time, Central Conduction Time, and Amplitude).

Peripheral conduction time (PCT), was not different between +/+ and -/- healthy animals (results in line with those obtained on male mice during the behavioural phenotyping).

No differences were recorded between healthy and EAE animals. These results are not surprising, considering that EAE is almost exclusively a central nervous system disease. Even with the KO for RPTPz, the peripheral nerve conduction is not affected.

Central conduction time (CCT) as index of demyelination was not different between +/+ and -/- healthy animals (confirming behavioural phenotyping results in male).

EAE induced a delay in CCT in +/+ animals (clue for a demyelinating process) while -/- mice presented no differences between healthy and EAE disease animals.

Amplitude of the nerve signal as index of axonal loss was a worsened in EAE condition in both +/+ and -/- mice, revealing the axonal loss in long term EAE and suggesting that the functional deficit in RPTPz KO mice may be due to an axonal pathology rather than a demyelinating dysfunction.

Detailed analyses on spinal cord of EAE diseased +/+ and -/- mice are ongoing.

In conclusion, EAE seems to not induce a significant difference in the course of the disease in terms of thermal sensitivity and CCT (contrary to +/+ mice) in RPTPz KO. The worsening in amplitude in -/- mice shows that RPTPz may be involved in axonal pathology.

References:

Harroch et al. (2000). Molecular and Cellular Biology 20(20):7706-7715.

Harroch et al. (2002). Nature Genetics **32**(3):411-414.

elenco dei seminari frequentati:

- Apomorphine-induced prepulse inhibition-disruption, an animal model for sensory-motor gated deficiency (schizophrenic behaviour). Held by Dr Adage Tiziana. (Serono).
- TNBS - induced colitis model in mice, On-going validation. Held by Dr Ardissone Vittoria (Serono).
- MRI quantification of knee osteoarthritis. Held by Dr Ladel Christoph (Serono).
- Erythropoietin and its non-erythropoietic derivatives in EAE and peripheral neuropathies. Held by Dr Roberto Bianchi (" Mario Negri" Institute for Pharmacological Research).
- MMP inhibitors and Minocycline derivatives in EAE model. Held by Valeria Muzio (Serono).
- How the brain repairs itself: new therapeutic strategies in inflammatory and degenerative CNS disorders. Held by Gianvito Martino (DIBIT - San Raffaele Hospital).

elenco dei corsi frequentati:

- Animal models for the study of neurological diseases: histological, molecular, and biochemical hallmarks. DiMI training Barcelona 26-30th June 2006.

elenco dei congressi frequentati:

- Neurodegenerative Diseases: Molecular Mechanisms in a Functional Genomics Framework. Max Delbrück Center for Molecular Medicine (MDC), Berlin, September 6-9, 2006

I didn’t present my work this year during these congress.