

**1er TALLER INTERNACIONAL “ENFERMEDADES AUTOINMUNES Y EL CEREBRO:  
PATOGÉNESIS Y DESARROLLO DE FÁRMACOS” / 1ST INTERNATIONAL WORKSHOP  
"AUTOIMMUNE DISEASES AND THE BRAIN: PATHOGENESIS AND DRUG DEVELOPMENT"**

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**PO-36: IMÁGENES INTRAVITALES DE LA AUTOINMUNIDAD EN EL SISTEMA NERVIOSO CENTRAL / INTRAVITAL IMAGING OF AUTOIMMUNITY IN THE CENTRAL NERVOUS SYSTEM**

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**Introduction:** Multiple Sclerosis (MS) is an autoimmune disease which is characterized by infiltration of immune cells into the central nervous system (CNS). However, it is not clear the cellular and molecular mechanism to induce such CNS inflammation. **Materials and Methods:** By using Experimental Autoimmune encephalomyelitis (EAE) as animal model of MS, together with intravital imaging by two-photon microscopy, the infiltration of fluorescently labelling encephalitogenic T cells are imaged in living animals. In addition, we introduced fluorescence protein-based activation sensors which allow detecting T cell activation in vivo. **Results:** Intravital imaging visualized infiltration into the through the blood-brain barrier (BBB). According to our observation, T cells arrive at blood vessels in spinal cord leptomeninges before onset of clinical EAE. After T cells crawled on intraluminal surface, they pass through BBB and penetrate into the CNS, where they interact with local antigen presenting cells. The interaction leads the elevated intracellular calcium in T cells and translocation of NFAT from cytosol to nucleus, which indicate the activation of T cells. The model can be used to develop therapeutic treatments. The infusion of anti-VLA4 blocking antibody diminished intraluminal crawling within a few minutes, whereas intrathecal injection of anti-MHC class II antibody or inhibitor of intracellular calcium signaling diminished T cell activation. These treatments efficiently ameliorate the clinical EAE. **Conclusion:** The intravital imaging visualized infiltration and activation of encephalitogenic T cells in vivo, which are the critical steps to induce CNS inflammation observed in EAE and MS. The results significantly deepened our understanding of mechanisms of EAE/MS and open new therapeutic target.

**PO-37: IMÁGENES INTRAVITALES DE LA SEÑALIZACIÓN POR CALCIO EN CÉLULAS T ENCEFALITOGÉNICAS / INTRAVITAL IMAGING OF CALCIUM SIGNALING IN ENCEPHALITOTENIC T CELLS**

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**Introduction:** Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the CNS. Experimental autoimmune encephalitis (EAE), a rodent model for Multiple Sclerosis, is induced by adoptive transfer of encephalitogenic T cells and elicits pathological features that resemble MS. The transferred T cells migrate through the body and interact with different environments. In this study, we aim to visualize T-cell stimulation *in vivo*. **Methods:** Combining a T-cell mediated rat model of EAE with intravital two-photon microscopy and two different fluorescence protein-based activation sensors, we can track the activation status of encephalitogenic T cells in different tissues on their journey into the CNS and in their target organ. **Results:** *In vitro* activated T cells first accumulate in secondary lymphatic tissues (e.g. the spleen) where, in the absence of antigen, they engage in transient contact with stroma cells indicated by short-lived calcium spikes. Subsequently, the T cells leave the spleen for the CNS. They roll and crawl along the luminal surface of the leptomeningeal blood vessels without displaying calcium activity or NFAT translocation, a measure of full T-cell activation. Having crossed the blood-brain barrier, the T cells scan the leptomeningeal space for autoantigen-presenting cells (APCs). Sustained MHC class II dependent contacts result in long-lasting calcium activity and NFAT translocation, whereby the capacity to activate T cells varies between individual APCs. **Conclusion:** The results show that autoreactive T cells experience various stimulations from their environment before entering the CNS. These checkpoints constitute potential therapeutic targets for the treatment of MS.

**PO-38: PERFIL DE CÉLULAS INDIVIDUALES DE PACIENTES CON ESCLEROSIS MÚLTIPLE TEMPRANA Y ESTABLECIDA / SINGLE-CELL PROFILING OF PATIENTS WITH EARLY AND ESTABLISHED MULTIPLE SCLEROSIS**

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**Introduction:** Multiple sclerosis (MS) is a disabling disease of the central nervous system (CNS) that is mainly affecting young women. It is characterized by destruction of CNS tissue, neuronal loss and axonal damage. Inflammatory features of MS include lymphocyte accumulations in the CNS and cerebrospinal fluid (CSF). In particular, MS brain lesions are marked by perivascular and parenchymal infiltrates of immune cells such as T- and B-cells, with activated CD8<sup>+</sup> T- cells being the dominating population. However, the function of these infiltrating cells in the tissue and the triggers for their invasion are still enigmatic. The CSF is considered a "window to the brain" as there is an overlap of lymphocytes from the brain parenchyma and the CSF. Here we used CSF from early and established MS patients to investigate whole transcriptome profiles of lymphocytes. **Materials and Methods:** We compared gene-expression patterns of CSF cells by single-cell RNA sequencing (scRNAseq). To this end, we combined scRNAseq with flow cytometry-based index-sorting, which allowed us to cope with the limited cell number of CSF samples. **Results:** Of note, even four of the patients with presumably preclinical MS had oligoclonal bands (OCBs), which is a hallmark of CSF in patients with established MS. By scRNAseq, we identified clonally expanded CD8<sup>+</sup> T cells, plasmablasts and - to a lesser extent - CD4<sup>+</sup> T cells. In contrast to non-expanded T cells, clonally expanded T cells showed characteristics of activation. Expanded plasmablast clones were detected only in MS patients with OCBs. **Conclusion:** Our data provide evidence for very early concomitant activation of three components of the adaptive immune system in MS, with a notable contribution of clonally expanded CD8<sup>+</sup> T cells.

**PO-39: AUTOINMUNIDAD CONTRA LA GLICOPROTEÍNA MIELÍNICA DE OLIGODENDROCITO (MOG): CÉLULAS B ESPECÍFICAS PARA MOG EN LA SANGRE DE PACIENTES CON DESMIELINIZACIÓN INFLAMATORIA / AUTOIMMUNITY AGAINST MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG): MOG-SPECIFIC B-CELLS IN BLOOD OF PATIENTS WITH INFLAMMATORY DEMYELINATION**

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**Introduction:** Autoantibodies (Abs) to the myelin oligodendrocyte glycoprotein (MOG) are found in the blood of some patients with inflammatory demyelination. The source of these antibodies is not yet completely elucidated. **Materials and Methods:** Patients with Abs to MOG were identified by cell-based assay. We differentiated blood derived B cells from patients with Abs to MOG and from healthy donors to Abs-producing plasmablasts. Therefore, we used for stimulation of peripheral blood mononuclear cells (PBMCs) the Toll-like receptor (TLR) 7/8 ligand R848 and interleukin-2 (IL-2) and tested the reactivity of the *in vitro* produced Abs to MOG-transfected cells. IgG levels were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** We analyzed the presence of MOG-specific B cells in the blood of anti-MOG positive patients (n = 20) and healthy controls (n = 26). The majority of patients harbor MOG-specific B cells in the blood. Rarely, MOG-specific B cells can also be found in healthy donors. MOG-Ab production was significantly higher in patients compared to the control cohort; whereas, the IgG production did not significantly differ between the two groups. Further, we could not detect a correlation between MOG-Ab levels in serum of patients and cell culture supernatants of stimulated PBMCs indicating different sources of MOG-Abs. **Conclusion:** Anti-MOG positive patients greatly differ in the amount of MOG-specific B cells in their blood. The determination of MOG-specific B cells might allow to stratify patients for optimal therapy.

**PO-40: GRANDES EXPECTATIVAS EN UNA NUEVA TERAPIA PARA LA ESCLEROSIS MÚLTIPLE Y EL ICTUS CEREBRAL / GREATER EXPECTATIONS ON NOVELTHERAPY FOR MULTIPLE SCLEROSIS AND STROKE**

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**Introduction:** Remyelination restores myelin through the proliferation, migration and maturation of oligodendrocyte progenitor cells. We have evaluated the remyelinating/demyelinating (R/D) effect induced by Phycocyanobilin (PCB) in animal models of Multiple Sclerosis (MS) and stroke (I/R). Furthermore, we tested the clinical and molecular effect of the combination PCB and beta IFN in MS animal model. **Materials and Methods:** Experimental Autoimmune Encephalomyelitis (EAE) was induced in C57BL6 mice with MOG peptide and three doses of PCB (0,2;1 and 5 mg/kg) by oral route were assessed. Beta IFN was administered at 5000 U/ animal subcutaneously every second day. Total RNA from brain was extracted and the expression of genes: LINGO1, Notch-1, CXCL12 and MAL were evaluated by Real-time-quantitative-PCR. Additionally, in I/R animal model induced by endotelin-1 in Wistar rats, a total dose of PCB (0,2 mg/kg) was administrated. Immunocytochemical techniques were performed using anti-CNPase, anti-MBP and anti-neurofilaments antibodies. **Results:** There was a PCB effect demonstrated by a regulation of the genes linked to R/D processes. The combination reduced the clinical score. The ratio CXCL12/LINGO1 was upregulated in the early therapeutic schedule. Moreover, an immune-labeling was observed in oligodendrocytes and in white matter, using an anti-CNPase and anti-neurofilaments in I/R model. The preservation of the myelin sheath and mitochondria was also observed using an anti-MBP in PCB treated animals. **Conclusion:** The results obtained here are in line with previous outcomes of a clinical and molecular study regarding the effect of PCB in MS and I/R models.



**PO-41: ¿ES LA NEUROEPO UN POSIBLE NEUROPROTECTOR? / IS NEUROEPO A POSSIBLE NEUROPROTECTANT?**

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**Introducción:** Durante los finales de la década del pasado siglo y en el presente, un considerable número de investigaciones no clínica han avalado las propiedades neuroprotectoras de la eritropoyetina (EPO). La obtención de una EPOhr hiposialilada en una formulación nasal abrió el camino para el desarrollo del producto del NeuroEPO con estas expectativas. **Materiales y Métodos:** Estudios toxicológicos en tres especies de animales con diferentes dosis, de eficacia farmacológica en diferentes modelos de animales transgénicos y no transgénicos en enfermedades tales como; Infarto cerebral, ataxia y Alzheimer. Finalmente, los ensayos clínicos en voluntarios sanos y ataxia SCA-2. **Resultados:** Se acumulan evidencias sobre la capacidad de NeuroEPO para atravesar la mucosa nasal y llegar al cerebro, garantizando niveles suficientes para ejercer su efecto farmacológico. La toxicología mostró la seguridad del producto en todas las dosis estudiadas. Los estudios de eficacia en diversos modelos brindaron información sobre el efecto protector de la NeuroEPO, al disminuir el daño tisular cerebral, en la isquemia cerebral, mejorando la función motora, sensorial, el estado neurológico y disminuir la mortalidad. En el estudio clínico en voluntario sano corroboró la seguridad encontrada en animales, no manifestándose efectos adversos moderados y graves en la población estudiada. En pacientes con SCA-2, el tratamiento con NeuroEPO produjo mejoría clínica significativa asociada al alivio de los síntomas motores y otros síntomas como los movimientos oculares antisacádicos. **Conclusiones:** Se demuestra el efecto neuroprotector de la NeuroEPO por lo cual tiene potencialidades para ser evaluado en el tratamiento de otras enfermedades neurodegenerativas.



**PO-42: LA ERRADICACIÓN PROTEICA NATIVA INDUCIDA POR PÉPTIDOS DE LA CASCADA JNK ES NEUROPROTECTORA FRENTE A LA ISQUEMIA CEREBRAL / PEPTIDE-INDUCED NATIVE PROTEIN ERADICATION OF JNK CASCADE IS NEUROPROTECTIVE AGAINST CEREBRAL ISCHEMIA**

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**Introducción:** Las terapias para impedir los procesos involucrados en la isquemia cerebral y proteger al cerebro humano isquémico todavía no están disponibles en la clínica. El objetivo del presente estudio fue diseñar y evaluar péptidos que tuvieran actividad neuroprotectora mediante el uso de un método recientemente descrito en la literatura<sup>a</sup>. **Materiales y Métodos:** Tres péptidos con diferentes secuencias de unión a JNK fueron sintetizados. Los niveles de JNK fosforilada (pJNK) y la viabilidad celular se determinó luego del tratamiento in vitro de neuronas corticales primarias, mediante western blot y el ensayo de actividad LDH, respectivamente. Se realizó un ensayo de neuroprotección in vivo con el método de tinción con TTC en un modelo de isquemia cerebral focal inducida por el vasoconstrictor endotelina-1 (ET-1). **Resultados:** Dos péptidos lograron un potente efecto inhibitor de la cascada JNK al disminuir significativamente los niveles de pJNK de forma tiempo- y concentración-dependiente. Ambos protegieron a las neuronas primarias y a líneas neuronales (N2a y SH-SY5Y) frente al daño excitotóxico (glutamato o NMDA) y por estrés oxidativo (H<sub>2</sub>O<sub>2</sub>). En el modelo de isquemia cerebral inducido por ET-1, estos péptidos redujeron significativamente el volumen de infarto cerebral en comparación con los animales isquémicos tratados con el vehículo. **Conclusiones:** Los nuevos péptidos diseñados lograron inactivar la vía JNK en neuronas y proteger frente a la isquemia cerebral, lo cual abre oportunidades de desarrollo para una nueva estrategia terapéutica neuroprotectora frente al ictus isquémico.

<sup>a</sup>a) Fan X, Jin WY, Lu J, Wang J, Wang YT (2014). Rapid and reversible knockdown of endogenous proteins by peptide-directed lysosomal degradation. *Nature Neuroscience*, 17(3), 471–480; b) Fan X, Wang YT (2015). SNIPER Peptide-mediated degradation of endogenous proteins. *Current Protocols in Chemical Biology*, 7(1), 1–16.

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**PO-44: DETECCIÓN DE MOG-ABS EN PACIENTES: COMPARACIÓN DE MOG EXPUESTO EN CÉLULAS TRANSFECTADAS Y EN ESFERAS RECUBIERTAS POR LÍPIDOS / DETECTION OF MOG-ABS IN PATIENTS: COMPARISON OF MOG DISPLAYED IN TRANSFECTED CELLS AND LIPID COATED BEADS**

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**Introduction:** Antibodies against Myelin Oligodendrocyte Glycoprotein (MOG) can be found in a proportion of patients with inflammatory CNS diseases. Currently, the most reliable detection of autoantibodies against MOG is a cell-based assay, in which the full-length version of MOG (FL-MOG) is expressed in transfected cells. Alternatively, autoantibodies are detected by an ELISA assay, in which only the external domain of MOG (ED-MOG) is used. However, for unknown reasons, this technique fails to recognize most of the MOG+ patients. **Materials and Methods:** In this study, we systematically explore the underlying mechanism for the missing detection. We tested the influence of the fluid environment of the cell membrane on the antibody binding and detection. To create proximity to a membrane, we propose a new *in vitro* assay for MOG detection using silica beads coated with a supported lipid bilayer. **Results:** The newly developed technique is able to detect the monoclonal antibody against MOG (8-18C5), but largely fails in the detection of autoantibodies in serum of MOG+ patients. **Conclusion:** We explore differences in the structure between FL-MOG and ED-MOG that might cause the difference in detection. Here we present our latest progress: in contrast to cells expressing FL-MOG, also cells transfected with ED-MOG are less capable of detecting autoantibodies in the MOG+ patients. Strikingly, even highly MOG positive patients showed a strongly decreased detection rate.

**PO-45: ABUNDANTES CÉLULAS B REACTIVAS PARA LA ÁCIDO GLUTÁMICO DESCARBOXILASA (GAD) EN DESÓRDENES NEUROLÓGICOS ASOCIADOS A ANTICUERPOS ANTI-GAD / ABUNDANT GLUTAMIC ACID DECARBOXYLASE (GAD)-REACTIVE B CELLS IN GAD-ANTIBODY-ASSOCIATED NEUROLOGICAL DISORDERS**

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**Introduction:** High levels of antibodies against glutamic acid decarboxylase (GAD) are observed in serum and cerebrospinal fluid of patients with different neurological disorders, but GAD-antibody-producing cells in these disorders are largely unexplored. **Materials and Methods:** In patients with GAD-antibody associated neurological disorders (n=15) and healthy controls (n=13) blood-derived B cells were differentiated into antibody-producing cells and GAD-antibody and IgG-production was studied. Frequencies of GAD-reactive B cells were calculated by limiting dilution experiments as well as GAD-specific T cell-proliferation was compared to proliferation induced by measles virus antigen and tetanus toxoid. GAD-antibody production by bone marrow cells was analysed in one patient and three controls. In two patients GAD-antibody producing cells were studied longitudinally under immunosuppressive treatment. **Results:** We detect circulating GAD-reactive B cells in peripheral blood that readily differentiate into antibody-producing cells. These cells are highly elevated in most patients with GAD-antibody-associated disorders compared to controls. They mainly produce GAD65 antibodies of the IgG1 and IgG4 subclasses and are as abundant as B cells reactive for common recall antigens. Immunosuppressive treatment with azathioprine or mycophenolate-mofetil leads to a reduction of GAD-antibody producing cells in peripheral blood and GAD-antibody levels in blood. Bone marrow cells represent an additional source of GAD-antibodies. **Conclusion:** Circulating B cells and bone-marrow plasma cells are a source of GAD-antibodies. The identification of GAD-antibody-producing cells has implications for the selection of cell specific biologics. The strikingly high abundance of circulating GAD-reactive B cells indicates a strong dysregulation of self-tolerance in patients with GAD-antibody-associated disorders.

**PO-46: MENINGOENCEFALITIS ASÉPTICA RECURRENTE COMO MANIFESTACIÓN EN EL SNC DEL SÍNDROME ASOCIADO A CRIPIRINA EN UN PACIENTE ADULTO HETEROCIGÓTICO PARA LA VARIANTE Q703K EN EL EXÓN 3 DEL GEN NLRP3 / RECURRENT ASEPTIC MENINGOENCEPHALITIS AS CNS MANIFESTATION OF CRYOPYRIN-ASSOCIATED SYNDROME IN AN ADULT PATIENT HETEROZYGOUS FOR THE Q703K VARIANT IN EXON 3 OF THE NLRP3 GENE**

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**Introduction:** Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory disorder (AID) caused by mutations in the NLRP3 gene. The p.Q703K variant is a low-penetrance mutation and has rarely been described in patients with central nervous system (CNS) involvement. **Case report:** We report the case of a 27-year-old male presenting with recurrent episodes of sterile meningoencephalitis. Cerebral magnetic resonance imaging (MRI) showed large FLAIR-hyperintense lesions including the basal ganglia, thalamus, pons and mesencephalon. Examination of cerebrospinal fluid revealed granulocytic pleocytosis, elevation of total protein and blood-brain-barrier dysfunction. Extensive laboratory testing showed no evidence for infectious, rheumatological or other inflammatory CNS diseases. Steroid treatment led to prompt improvement of clinical symptoms and MRI changes but re-occurred after reduction of steroid therapy. Extensive molecular genetic testing (whole exome sequencing) for an AID only revealed heterozygosity for the Q703K variant in exon 3 of the NLRP3 gene. Diagnosis of CAPS was made and anti-IL-1-treatment started. Steroid therapy could be reduced and the patient remained stable under this therapy regimen. **Discussion:** This is an unusual case of a patient heterozygous for the Q703K variant with severe CNS manifestation, who responded to anti-IL-1 therapy. **Conclusion:** The described case illustrates that CAPS should be considered in adult patients with recurrent sterile meningoencephalitis.

1. Lachmann HJ. Periodic fever syndromes. Best Practice & Research Clinical Rheumatology 2017
2. Levy R, Gérard L, Kuemmerle-Deschner J, et al. Phenotypic and genotypic characteristics of cryopyrin-associated periodic syndrome: a series of 136 patients from the Eurofever Registry. Annals of the Rheumatic Diseases 2015
3. Finetti M, Omenetti A, Federici S, Caorsi R, Gattorno M. Chronic Infantile Neurological Cutaneous and Articular (CINCA) syndrome: a review. Orphanet Journal of Rare Diseases 2016

**PO-89: DETECCIÓN Y PURIFICACIÓN DE AUTOANTICUERPOS / DETECTION AND PURIFICATION OF AUTOANTIBODIES**

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**Introduction:** For the detection of autoantibodies several assays exist. In some labs it is done via cell-based assay and doing immunofluorescence. In our lab we established cell-based assays and detecting it by flowcytometry. Furthermore, we also established assays to screen the patient samples (CSF, serum) by ELISAs. **Materials and Methods:** Constructs were cloned in pEGFP vector to report the transfection efficiency. For transmembrane anchored proteins the GFP is directly fused to the protein of interest (e.g. MOG). If the protein is displayed on the surface through a GPI anchor, we use the pEGFP vector with a T2A ribosome skipping sequence. This leads to the expression of two single proteins (GFP and protein of interest) out of one mRNA. Proteins tested as antigens in an ELISA are expressed by the eukaryotic HEK-EBNA system. Our proteins harbour a His-tag for purification as well as an Avi-tag for the site specific enzymatic biotinylation. With the biotin coupled to the antigen, it can also be bound to Streptavidin ELISA plate. **Results:** We could detect antibodies against Neurofascin in a cohort of CIDP patients by using the MaxiSorp ELISA. For the MOG protein, all assays (MaxiSorp-/Streptavidin-ELISA, cell-based assay) were carried out. In this study, it was seen that the cell-based assay is more beneficial and it doesn't always overlap ELISA results. **Conclusion:** Each screening for antigens must be established and validated with the different assays. For screening patients to Neurofascin an ELISA was used, whereas for the detection of MOG autoantibodies the flow cytometry cell-based assay works better.

PO-90: TECNOLOGÍAS DE VANGUARDIA PARA EXPLORAR LA HETEROGENEIDAD DEL SISTEMA INMUNE / STATE-OF-THE-ART TECHNOLOGIES TO EXPLORE IMMUNE CELL HETEROGENEITY

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**Introducción:** El sistema inmunológico está compuesto por una variedad de células que actúan de manera coordinada para proteger al organismo contra una multitud de infecciones y enfermedades. La gran variabilidad de patógenos y amenazas existentes se corresponde con la alta heterogeneidad del sistema inmunológico. **Materiales y Métodos:** El estudio de las células inmunitarias a nivel individual, la unidad fundamental de inmunidad, se ha transformado recientemente, de una imagen microscópica cualitativa, a un análisis transcriptómico cuantitativo casi completo. Este cambio ha sido impulsado por el rápido desarrollo de múltiples tecnologías basadas en el estudio de la expresión de genes a nivel de células individuales. **Resultados:** Estos nuevos avances han aumentado la detección de tipos celulares menos frecuentes y estados celulares transitorios o intermedios, lo cual nos permite realzar la individualidad de cada célula y amplía en gran medida la resolución de la actual clasificación de los diferentes tipos celulares y las trayectorias de diferenciación. **Conclusiones:** Aquí, discutimos el reciente avance y la aplicación de tecnologías de una sola célula, sus limitaciones y futuras aplicaciones para estudiar el sistema inmunológico.