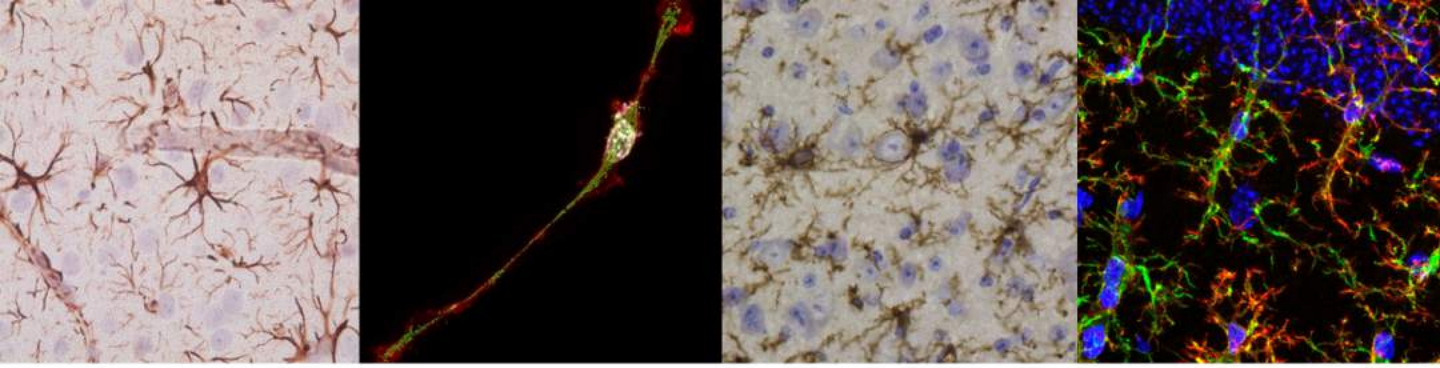

X Reunión de la Red Glial Española

4 de septiembre de 2019

Palacio de Congresos e Exposicións de Galicia
Santiago de Compostela

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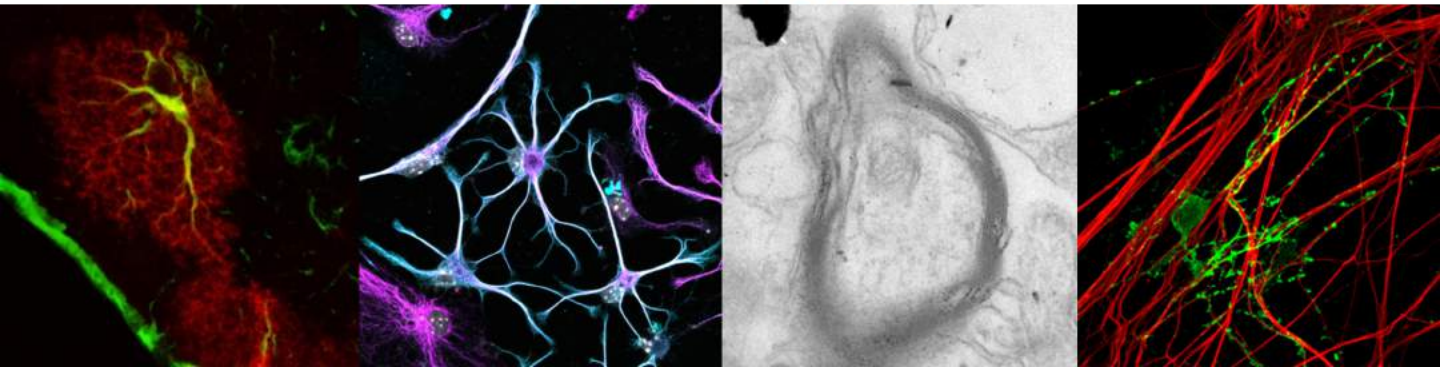

X meeting of the Red Glial Española
Santiago de Compostela 2019

Wednesday 4th of September (room Obradoiro)

8:30-8:45 Welcome and inauguration

8:45-10:30 Oral presentations

SESSION 1: GLIA-NEURON CROSSTALK.

8:45 ***Celia Luchena Moreno*** *(Achucarro B.C.N., Leioa):* B-amyloid peptide causes synaptic loss and a dysregulation of the complement system in neurons and glial cells in vitro.

9:00 ***Jimena Baleriola*** (*Achucarro B.C.N., Leioa*): Extracellular vesicles regulate the local neuronal translatome

9:15 ***Marta Navarrete*** (*Instituto Cajal, CSIC, Madrid*): Astrocytes as key drivers in NMDA receptor-dependent long term depression.

SESSION 2: MICROGLIA

9:30 ***Mar Márquez*** (*Achucarro B.C.N., Leioa*): Mitochondrial functional, metabolic and morphological changes in phagocytic microglia.

9:45 ***Miriam Corraliza Gómez*** (*IBGM (Uva-CSIC*), Valladolid): Role of insulin degrading enzyme (IDE) in microglial cells at the confluence of Alzheimer's disease (AD) and type 2 diabetes (T2D).

SESSION 3: GLIAL CELLS AND PATHOLOGY

10:00 ***Sergio Casas*** *(Instituto Cajal- CSIC, Madrid*): Glioblastoma cells vampirize WNT from neurons and trigger a JNK/MMP signaling loop that enhances glioblastoma progression and neurodegeneration

10:15 ***Jörg Michael Mey*** (*Hospital Nacional de Parapléjicos, Toledo*): Effect of human bone marrow-derived cell implants on glia cells affter spinal cord contusion injury in rats

10:30-10:45 Tribute to Rio-Hortega and Achucarro on the centenary of their discovery of microglia and oligodendrocytes (Fernando de Castro; Instituto Cajal-CSIC, Madrid)

10:45-12:15 Coffe break and poster session *(Hall)*

12:15-12:45 V Laia Acarín Award *(room Obradoiro)*

Sara Mederos *(Instituto Cajal-CSIC, Madrid):* Melanopsin for precise optogenetic activation of astrocyte-neuron networks

12:45-13:30 Assembly

Localization:

Palacio de Congresos e Exposicións de Galicia. Rúa de Miguel Ferro Caaveiro, s/n, 15707 Santiago de Compostela, A Coruña. Tel. 981519988. www.palaciosantiago.com

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ORAL PRESENTATIONS

CO1. B-AMYLOID PEPTIDE CAUSES SYNAPTIC LOSS AND A DYSREGULATION OF THE COMPLEMENT SYSTEM IN NEURONS AND GLIAL CELLS IN VITRO

C. Luchena[1,2], J. Zuazo-Ibarra[1,2], E. Alberdi[1,2,3], C. Matute[1,2,3], E. Capetillo-Zarate[1,2,3,4,5]

[1]Achucarro Basque Center for Neuroscience. Leioa, Spain.
[2]Facultad de Medicina y Enfermería, Universidad del País Vasco. Leioa, Spain.
[3]CIBERNED, Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas. Spain.
[4]IKERBASQUE, Basque Foundation for Science. Bilbao, Spain.
[5]Department of Biochemistry, Weill Cornell Medical College, Cornell University. New York, USA.

Synaptic dysfunction is a key hallmark of Alzheimer's disease (AD), and it is the best pathological correlate with cognitive decline. Yet the cellular mechanism(s) by which β- amyloid (Aβ) affects synapses remains unclear. Additional to the beneficial role of glial cells by eliminating Aβ, recent discoveries suggest that these cells might participate via the complement cascade in early synapse loss in AD models. However, contribution of neurons and glial cells in the complement cascade and its connection with Aβ-induced synaptic loss needs further clarification.

In order to study how microglia and astrocytes influence Aβ-induced synaptic loss and expression of complement proteins in AD, we performed immunofluorescence techniques to measure pre- and post-synaptic markers in neurons with and without glial cells, as well as the expression of complement proteins C1q and C3 in neurons, microglia and astrocytes in presence or absence of Aβ oligomers (Aβo).

We found that Aβo induced a reduction in pre- and post-synaptic markers in primary cultured neurons, while it did not alter its C1q and C3 levels. In contrast, in neuron-microglia co-cultures, C1q intensity was greater in both neurons and microglia and the post-synaptic reduction induced by Aβo was partially ameliorated compared to neurons alone. In addition, in neuron-astrocyte co-cultures, Aβo induced an increase of C3 and pre-synaptic marker levels were restored. Interestingly, in the triple co-culture of neurons, microglia and astrocytes, Aβo decreased C1q intensity in neurons and synaptic damage remained.

Overall, these results indicate that communication between neurons and glial cells plays a key role in synapse pathology. Besides, presence of Aβo produces a dysregulation of complement proteins in neurons and glia. Further study of the link between synaptic loss and the complement cascade is necessary for understanding the role of this pathway in synapse pathology in AD.

Supported by the Basque Government (PIBA PI-2016-1-0009, ELKARTEK KK-2017-00067) and CIBERNED. C. Luchena and J. Zuazo-Ibarra are recipients of fellowships from Tatiana Pérez de Guzmán el Bueno Foundation and the Basque Government respectively.

CO2. EXTRACELLULAR VESICLES REGULATE THE LOCAL NEURONAL TRANSLATOME

Gamarra, M[1], Rodrigues-Batista, A[1,2], González, E[3], Falcón, J.M[3] and Baleriola, J[1,4,5]

[1]Achucarro Basque Center for Neuroscience, Leioa, Spain.
[2]Universidade do Minho, Braga, Portugal.
[3]CIC-Biogune, Derio, Spain.
[4]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.
[5]Facultad de Biología Celular e Histología, Universidad del País Vasco UPV/EHU, Leioa, Spain.

Previous work shows that transcription factor ATF4 translated in axons exposed to Aβ oligomers (oAβ). Pharmacological inhibition of local translation or local application of Atf4-targeting siRNAs is sufficient to reverse Aβ-induced neurodegeneration. Thus, inhibiting ATF4 production in axons could virtually stop the propagation of Aβ-induced pathology from axons to somata. However, somatic ATF4 plays an adaptative role in Aβ-treated neurons. The distinct effects elicited by ATF4 based on its localized synthesis makes this transcription factor (and likely others with similar characteristics) not suitable for therapeutic intervention unless local translation is targeted without affecting somatic ATF4. Based on studies performed on the peripheral nervous system, we hypothesize that astroglia remotely controls the local neuronal translatome by transferring extracellular vesicles (EVs) to neurites. From this perspective, targeting glial EVs rather than directly interfering with local translation might be a novel approach to slow down Aβ pathology.

We used Boyden chambers to separate somata from neurites and we analyzed the effect of co-culturing astrocytes on local protein levels. We additionally treated hippocampal neurons with conditioned media retrieved from neuronal or neuron-astrocyte co-cultures exposed to vehicle or to oAβ. Results from both approaches suggest that the presence of astrocytes enhance local translation in neurites in both physiological and pathological conditions. To determine whether EVs mediate the aforementioned effect on translation, we depleted the conditioned media from EVs by ultracentrifigation. Interestingly, neurons treated with EV-depleted media from neuronal cultures showed lower neuritic protein production than conditioned media containing EVs regardless of the environment where the EVs were produced (healthy vs. inhealthy). However, EV-depleted media released by astrocytes had a differential effect on neurons depending on the context. These results suggest that glial EVs play distinct roles on local translation in neurites depending on whether they are released in a physiological or a pathological context.

CO3. ASTROCYTES AS KEY DRIVERS IN NMDA RECEPTOR-DEPEDENT LONG TERM DEPRESSION

M. Navarrete[1,2], M.I. Cuartero[1], R. Palenzuela[1,3], J.E. Draffin[1], A. Konomi[1], I. Serra[2], S. Colié[4], S. Castaño-Castaño[5], M.T. Hasan[5,6], Á.R. Nebreda[4,7] and J.A. Esteban[1]

[1]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid 28049, Spain.
[2] Instituto Cajal (CSIC), Madrid 28002, Spain
[3]School of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain.
[4]Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, 08028 Barcelona, Spain.
[5]Achucarro Basque Center for Neuroscience, 48940 Leioa, Spain.
[6]Ikerbasque – Basque Foundation for Science, 48013 Bilbao, Spain.
[7]ICREA, Pg. Lluís Companys 23, 08010 Barcelona, Spain.

NMDA receptor-dependent long-term depression (LTD) in the hippocampus is a well-known form of synaptic plasticity that has been linked to different cognitive functions. The core mechanism for this form of plasticity is thought to be entirely neuronal. However, we now demonstrate that astrocytic activity drives LTD at CA3-CA1 synapses. We have found that LTD induction enhances astrocyte-to-neuron communication mediated by glutamate, and that Ca^{2+} signaling and SNARE-dependent vesicular release from the astrocyte are required for LTD expression. In addition, using optogenetic techniques, we show that low-frequency astrocytic activation, in the absence of presynaptic activity, is sufficient to induce postsynaptic AMPA receptor removal and LTD expression. Using cell-type-specific gene deletion, we show that astrocytic p38α MAPK is required for the increased astrocytic glutamate release and astrocyte-to-neuron communication during low-frequency stimulation. Accordingly, removal of astrocytic (but not neuronal) p38□ abolishes LTD expression. Finally, this mechanism modulates long-term memory *in vivo*. These results present a fundamental change of paradigm, in which the axis composed of presynaptic neuron-astrocyte-postsynaptic neuron defines an obligatory relay for information processing leading to synaptic plasticity.

CO4. MITOCHONDRIAL FUNCTIONAL, METABOLIC AND MORPHOLOGICAL CHANGES IN PHAGOCYTIC MICROGLIA

Mar Márquez-Ropero[1,2], Víctor Sánchez-Zafra[1,2], Irune Díaz-Aparicio[1,2], Jorge Valero[1,3], Fernando García-Moreno [1,3] , Amanda Sierra[1,2,3]

[1] Achucarro Basque Center for Neuroscience, Leioa, Spain

[2] University of the Basque Country EHU/UPV, Leioa, Spain

[3] Ikerbasque Foundation, Bilbao, Spain

Microglial cells are the brain professional phagocytes, in charge of removing apoptotic cell debris during development, in adult neurogenic niches and during neurodegenerative conditions. Phagocytosis is essential for the maintenance of tissue homeostasis because it prevents spillover of toxic intracellular contents and has immunomodulatory effects. However, the impact of engulfing and degrading apoptotic cargo in microglial physiology and function has not been described yet. We first addressed global transcription changes induced by phagocytosis of apoptotic cells in cultured microglia using gene arrays and found a significant modulation of metabolism, which suggested an upregulation of glycolytic genes and a downregulation of oxidative phosphorylation genes. We further confirmed the reduction of mitochondrial oxidative phosphorylation after phagocytosis of apoptotic cells using a Seahorse extracellular flux analyzer. To determine if these metabolic changes were related to mitochondrial alterations, we analyzed the number, morphology, and dynamics of the mitochondrial network using ultrastructural analysis coupled to live imaging. We found that phagocytosis induced a strong remodeling of the mitochondrial network, with fewer and less complex mitochondria. As mitochondria are involved in many key cell functions, this multifunctional analysis will help us to understand how phagocytosis-induced metabolic and mitochondrial remodeling affects microglial function both in healthy conditions and in neurodegenerative diseases.

CO5. ROLE OF INSULIN DEGRADING ENZYME (IDE) IN MICROGLIAL CELLS AT THE CONFLUENCE OF ALZHEIMER'S DISEASE (AD) AND TYPE 2 DIABETES (T2D)

M. Corraliza-Gómez, D. Sánchez, E. Arranz, I. Cozar-Castellano and M.D. Ganfornina

Instituto de Biología y Genética Molecular, Universidad de Valladolid-CSIC, Valladolid, Spain.

AD and T2D are two chronic progressive pathologies with an alarming increase in their prevalence worldwide; however, the common mechanisms underlying these pathologies are poorly understood.

Our research focuses on understanding the role of IDE in microglial cells, vigilant monitors of the brain, when challenged with pathological stimuli that occur in T2D and AD such as hyperglycemia, amyloid oligomers, oxidative stress and inflammatory events. We are studying the ability of the murine microglial cell line BV2 to survive to these insults, the inflammatory response that such conditions trigger and how this affects the expression and subcellular localization of IDE, a metalloprotease able to degrade both insulin and amyloid beta *in vitro*. We also compare microglial behavior with macrophages (murine cell line Raw264.7) to better understand the specific functional responses of glial cells.

We found that microglial metabolic activity increases upon chronic high D-glucose treatment. Our data show that chronic hyperglycemia switches microglia, but not macrophages, towards a mild pro-inflammatory phenotype. This condition does not modify IDE protein levels. Surprisingly, LPS treatment significantly reduces the amount of IDE protein specifically in microglial cells, while oxidative stress induced by paraquat has a biphasic dose-dependent effect on IDE.

IDE subcellular localization and secretion mechanisms are of particular relevance and need to be solved to proper interpret IDE function in microglial cells. We have found that IDE partitions in both the membrane and soluble fractions of microglial cells, and we are currently assessing IDE secretion upon glucose and amyloid beta stimulation. Since the activation state of microglial cells can have an impact on IDE expression, and consequently, on its traffic and function, it becomes a relevant player in the progression of neurodegeneration when AD and T2D are combined.

Support: BFU2015-68149-R, VA086G18, SAF2016-77871-C2-1-R

CO6. GLIOBLASTOMA CELLS VAMPIRIZE WNT FROM NEURONS AND TRIGGER A JNK/MMP SIGNALING LOOP THAT ENHANCES GLIOBLASTOMA PROGRESSION AND NEURODEGENERATION

Marta Portela[1*], Varun Venkataramani[2,3,4], Natasha Fahey-Lozano[1], Esther Seco[1], Maria Losada-Perez[1], Frank Winkler[2,3] and Sergio Casas-Tintó[1*]

[1]Instituto Cajal-CSIC. Av. del Doctor Arce, 37. 28002. Madrid. Spain
[2]Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, INF 400, 69120 Heidelberg, Germany
[3]Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
[4]Institute for Anatomy and Cell Biology, Heidelberg University, 69120 Heidelberg, Germany.

Glioblastoma (GB) is the most lethal brain tumor and Wingless-related-integration-site (WNT) pathway activation in these tumors is associated with a poor prognosis. Clinically the disease is characterized by progressive neurological deficits. However, whether these symptoms result from direct or indirect damage to neurons is still unresolved. Using *Drosophila* and primary xenografts as models of human GB, we describe a mechanism that leads to activation of WNT signaling [Wingless (Wg) in *Drosophila*] in tumor cells. GB cells display a network of tumor microtubes (TMs) that enwrap neurons, accumulate Wg receptor Frizzled1 (Fz1), and, thereby, deplete Wg from neurons, causing neurodegeneration. We have defined this process as "vampirization". Furthermore, GB cells establish a positive feedback loop to promote their expansion, where the Wg pathway activates cJun N-terminal kinase (JNK) in GB cells, and in turn JNK signaling leads to the post-transcriptional upregulation and accumulation of matrix metalloproteinases (MMPs), which facilitate TMs infiltration throughout the brain, TMs network expansion and further Wg depletion from neurons. Consequently, GB cells proliferate due to the activation of the Wg-signaling target, β-catenin, and neurons degenerate due to Wg signaling extinction. Our findings reveal a molecular mechanism for TMs production, infiltration and maintenance that can explain both neuron-dependent tumor progression and also the neural decay associated with GB.

CO7. EFFECT OF HUMAN BONE MARROW-DERIVED CELL IMPLANTS ON GLIA CELLS AFTER SPINAL CORD CONTUSION INJURY IN RATS

J. Mey[1,2], S. Wu[1,2], J.P. de Munter[3], E.Ch. Wolters[3], B.W. Kramer[2], L. Romero-Ramírez[1]

[1]Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain
[2]School of Mental Health and Neuroscience, Maastricht University, Netherlands
[3]Neuroplast BV, Maastricht, Netherlands

The objective of this study was to test whether the acute administration of a standardized human bone marrow-derived stem cell preparation (NeuroCells, NC) enhances functional motor recovery after a spinal cord injury (SCI) in rats.

Otherwise healthy male Wistar rats were treated with sub-occipital intrathecal NC, i.p. injections of methyl prednisolone or vehicle (NaCl) within one hour after a contusion injury (Infinite Horizon impactor, 2N) at the spinal thoracic level T9. One dose of NC consisted, on average, of 1.8 million non-manipulated cells in 100 µL suspension, which was processed out of fresh human bone marrow from the iliac crest of healthy volunteers. General health, weight and urodynamic functions were monitored daily. The recovery of motor function was assessed using the BBB and the Rotarod locomotor function tests during a 9-week surveillance period. Neuropathic pain (von Frey) was assessed at 9 weeks. After this time, rats were perfused, and the spinal cord tissue was studied histologically.

The NC-treated rats did not reject the human implants and showed no sign of sickness behavior. They displayed better recovery of SCI-induced motor deficits as compared to MP- treated animals, reaching significance at 4 dpo and weeks 1-5. None of the NC treated rats suffered from allodynia or hyperalgesia after 9 weeks, whereas this occurred in 1/5 MP- treated and 3/10 vehicle–treated animals. The recovery of bladder control was similar in all groups. Histological evaluation of treatment effects on tissue loss, scar formation and the distribution of Iba1-positive microglia/macrophages indicate that stem cell implants reduced the inflammatory response but did not affect astrogliosis and tissue degeneration.

Intrathecal NC treatment proved to be a safe intervention of SCI in wt rats. NC-treated rats recovered their motor functions significantly earlier and better than MP-treated rats. There were no significant differences in general health status and recovery of bladder control between groups.

POSTERS

P01. ROLE OF IRF5 TRANSCRIPTION FACTOR IN MULTIPLE SCLEROSIS MODELS OF DEMYELINATION

A. Zabala, A. Montilla, V. Tepavcevic, A. Sierra, F. Soria, C. Matute and M. Domercq

Achucarro Basque Center for Neurosciences, Ciberned and Departamento de Neurociencias, Universidad del País Vasco, Leioa, Spain

Understanding the mechanism of microglia-specific responses during pathology is vital to promote regenerative responses. We have previously observed that P2X4 receptor modulates microglia response and that the potentiation of P2X4R signaling in microglia ameliorated experimental autoimmune encephalomyelitis (EAE) symptoms, an animal model of multiple sclerosis (Zabala et al., 2018). Microglia conversion into the $P2X4R^{+}$ reactive state is driven by interferon regulatory factor 5 (IRF5), a transcription factor involved in innate immune activation. Interestingly, IRF5 has been associated to multiple sclerosis pathology. Compared to wild type mice, $IRF5^{-/-}$ mice showed an initial delay in the appearance of neurological symptoms after EAE induction but, on the contrary, an absence of improvement in the recovery phase. $IRF5^{-/-}$ mice had an increase in myelin damage, in axonal damage and in the number of $Iba1^{+}$ positive cells in the lesions. Confocal imaging analysis revealed that $IRF5^{-/-}$ mice had a higher accumulation of myelin debris in microglia/macrophages. In parallel, chemical-induced demyelination by lysolecithin, trigger a secondary pathological inflammation and immune activation in $Irf5^{-/-}$ mice that results in a deficient recruitment of oligodendrocyte progenitors to the lesion. These findings reconciles with the inside-out hypothesis of MS pathogenesis and point to a complex role of IRF5 that evolves from detrimental to beneficial, a finding in correlation with the complex and evolving role of microglia/macrophages along the pathology.

Acknowledgements: This work was supported by Merck-Serono; the Spanish Ministry of Education and Science; the Basque Government; the University of the Basque Country (UPV/EHU). A. Zabala and A. Montilla hold a fellowship from the University of the Basque Country (UPV/EHU) and the Spanish Ministry of Education and Science, respectively.

P02. EFFECTS OF CONNEXIN43 REGION 266-283 IN NEURAL STEM CELLS FROM THE SUBVENTRICULAR ZONE IN AN IN VIVO GLIOMA MODEL

A. Álvarez-Vázquez, R. Talaverón, J. M. Medina, A. Tabernero

Instituto de Neurociencias de Castilla y León (INCYL), Universidad de Salamanca, Salamanca, Spain

Glioblastoma multiforme is one of the most aggressive brain tumours worldwide. Part of its malignancy lies in a subpopulation of tumour cells known as Glioma Stem Cells (GSCs). Aiming to eliminate these cells, the group designed the cell-penetrating peptide TAT-Cx43$_{266-283}$, based on the interaction between connexin43 and the oncogenic protein c-Src, observing significant effects of this peptide on the reduction of tumour cell proliferation and migration.

Several studies have shown a relationship between GSCs and neural stem cells (NSCs) from the subventricular zone (SVZ), one of the remaining neurogenic niches in adulthood. Therefore, in this work we aimed to investigate whether TAT-Cx43$_{266-283}$ affects SVZ NSCs in a glioma model in vivo. In order to do that we studied the number of SVZ NSCs by immunohistochemistry, using the marker nestin, as well as their differentiation to glial (astrocytes) and neural lineage using GFAP and doublecortin, respectively. Our preliminary results suggest that the implantation of tumour cells slightly increased nestin and GFAP levels within the SVZ, and that the treatment with TAT-Cx43$_{266-283}$ reverted this effect. These results suggest a relationship between the tumour and the number of astrocytes generated in the SVZ. In absence of tumour cells, TAT-Cx43$_{266-283}$ did not modify the levels of GFAP and doublecortin in the SVZ, suggesting that TAT-Cx43$_{266-283}$ did not affect differentiation of SVZ NSCs under resting conditions.

P03. RECONSIDERING THE EPENDYMAL REGION IN HUMAN SPINAL CORD AS A SOURCE OF NEURAL REPAIR

D. Garcia-Ovejero[1], A. Arevalo-Martin[1], B. Paniagua-Torija[1], A. Torrillas[1], C. G. Faulkes[2], A.J. Jimenez-Lara[3], E. Molina-Holgado[1]

[1]Hospital Nacional de Parapléjicos, SESCAM, Toledo

[2]. School of Biological & Chemical Sciences, Queen Mary University of London, London

[3]. Facultad de Ciencias, Universidad de Málaga, Málaga

In species that regenerate the injured spinal cord (salamander, zebra fish…), the ependymal region is a source of new cells and a prominent coordinator of regeneration. In mammals, cells at the ependymal region proliferate in normal conditions and react to spinal cord injury, but their actual contribution to repair is controversial.

In the last years we have studied this region in adult humans using a variety of techniques and found that it represents a unique trait of our species. Here we summarize our findings obtained from control and spinal cord injured individuals, together with new data on the process of human central canal closure and a comparative view with other mammal species. Our data challenge the view of the spinal cord ependymal region as a neurogenic niche but may be useful to better understand the biology and cell organization of this structure in humans, and may offer new perspectives especially in the field of ependymal.

P04. MYELOID-DERIVED SUPPRESSOR CELL PERIPHERAL LOAD CAN PREDICT A GREATER ENDOGENOUS REMYELINATION CAPACITY IN THE MURINE MODEL OF MULTIPLE SCLEROSIS

I. Sánchez-de Lara, R. Lebrón-Galán, I. Machín, C. Camacho-Toledano, MC. Ortega*, D. Clemente*

Neuroinmmuno-Repair Group. Hospital Nacional de Parapléjicos-SESCAM. Toledo, Spain.

Multiple sclerosis (MS) is a chronic, autoimmune and demyelinating disease of the central nervous system (CNS). MS is characterized by the presence of demyelinated lesions in the brain parenchyma, being some of them spontaneously repaired by oligodendrocyte precursor cells (OPCs). It has been described that successful remyelination needs from both adaptive and innate immune regulatory cell activity. Myeloid-derived suppressor cells (MDSCs) have emerged as an immune cell population with a crucial role in immune-related disorders, including MS. Our group has data indicating that MDSC abundance at the onset of the clinical course of the MS animal model, experimental autoimmune encephalomyelitis (EAE), is indicative of a later disease severity together with a lesser myelin destruction and axonal damage extent at the peak of clinical symptoms. In the present work, we study the relationship between disease aggressiveness, MDSCs density or activity and the OPC distribution associated to the demyelinated area. In this sense, we have performed a prospective study to analyze if the level of MDSCs in the peripheral blood at the onset of the disease is indicative of a higher density or proliferative rate of OPCs closely associated to the demyelinated area. In the current work, we observe a direct correlation between MDSCs in the blood the first day of symptoms and the density of Arg-I^+ (MDSCs) and $NG2^+$ cells (OPCS) in the spinal cord at the peak of the disease. Our data point to MDSCs as promoting factors for OPC mobilization to the demyelinated area, which is directly related to the severity of the clinical course. In sum, our data indicate the role of MDSCs peripheral load at the onset of the symptoms as a putative bioindicator not only of a milder severity of the future clinical course but also of a less damaged CNS prone to spontaneous remyelination.

This work was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (PI15/00963; PI18/00357; RD16-0015/0019, partially co-financed by F.E.D.E.R., European Union, “Una manera de hacer Europa”), ARSEP Foundation, Esclerosis Múltiple España (REEM-EME-S5), ADEM-TO and ATORDEM. DC's lab research activity is sponsored by Aciturri Aeronáutica S.L., Vesuvius Ibérica, Fundación Galletas Coral and Embutidos y Jamones España e Hijos. DC, RL-G, and IM are hired by SESCAM. MCO holds a postdoctoral fellowship from the Consejería de Sanidad de Castilla-La Mancha (II-2018_07). CC holds a predoctoral contract funded by ADEM-TO, ATORDEM and the industrial sponsors.

PO5. MYELOID-DERIVED SUPPRESSOR CELLS IN MULTIPLE SCLEROSIS PATIENTS: PUTATIVE BIOINDICATORS FOR THE SEVERITY OF THE CLINICAL COURSE AND NEURO-REPAIR ABILITY OF DEMYELINATING LESIONS

MC. Ortega[1], R. Lebrón-Galán[1], I. Pérez-Molina[2], MR. García-Montero[2], I. Machín[1], D. Clemente[1]

[1] Hospital Nacional de Parapléjicos-SESCAM. Toledo, Spain.
[2] Hospital Virgen de la Salud-SESCAM. Toledo, Spain.

Myeloid-derived suppressor cells (MDSCs) have emerged as a new immune regulatory cell population, comprising a heterogeneous group of immature myeloid cells. MDSCs include two major subsets based on their phenotypic and morphological features: polymorphonuclear and monocytic (M)-MDSC, playing both a crucial role in immune- related disorders, including multiple sclerosis (MS). Data from our group obtained from the EAE model, pointed to M-MDSC abundance in the blood as a putative bioindicator for the severity of the clinical course as well as the degree of demyelination and axonal damage. However, the relationship of M-MDSCs and these two particular aspects in the human pathology needs to be addressed. In this work, we hypothesize that the presence of M-MDSCs in MS patients might also help us to predict the MS progression. The level of M-MDSCs (labeled as $CD11b^{+}CD33^{+}CD14^{+}CD15^{-}HLA\text{-}DR^{-/low}$) in the peripheral blood of MS patients around the time of their first referred relapse (MS-R) was higher than in both controls and MS patients in remission. Interestingly, the abundance of M-MDSCs in MS-R patients inversely correlated with their EDSS at the moment of the relapse. Strikingly, for the very first time we have identified putative M- MDSCs in the CNS of MS patients. M-MDSCs ($CD14^{+}CD15^{-}HLA\text{-}DR^{-/low}$) were associated to those demyelinated plaques with a spontaneous capacity of remyelination, i.e. within the plaque of active lesions and the periplaque of chronic-active lesions.

Moreover, the abundance of MDSCs in active lesions from MS patients with short disease duration showed a direct correlation with the disease length. In parallel, the density of MDSCs was higher in active plaques from non-acute MS patients (disease length < 6 years). In sum, our data point to M-MDSC level as a putative biomarker of the clinical course severity as well as the degree of histopathological damage in the human CNS in the context of MS.

This work was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (PI15/00963; PI18/00357; RD16-0015/0019, partially co-financed by F.E.D.E.R., European Union, “Una manera de hacer Europa”), ARSEP Foundation, Esclerosis Múltiple España (REEM-EME-S5), ADEM-TO and ATORDEM. DC's lab research activity is sponsored by Aciturri Aeronáutica S.L., Vesuvius Ibérica, Fundación Galletas Coral and Embutidos y Jamones España e Hijos. DC, RL-G, IP-M, MRG-M and IM are hired by SESCAM. MCO holds a postdoctoral fellowship from the Consejería de Sanidad de Castilla-La Mancha (II-2018_07).

PO6. GENERATION AND CHARACTERIZATION OF HUMAN IPSC DERIVED ASTROCYTES FROM HEALTHY AND LRRK2 G2019S PARKINSON´S DISEASE DONORS: IMPLICATIONS IN NEURONAL DEGENERATION.

P. Ramos-González [1], F. Cavaliere [1], R. Bugallo[2], J.C. Chara[1], M. Arrasate[2], C. Matute [1]

[1]Facultad de Medicina y Enfermería, Universidad del País Vasco, Achucarro Basque Center for Neuroscience, Bilbao, Vizcaya, Spain.
[2]Centro de Investigación Médica Aplicada (CIMA), Universidad de Navarra, Pamplona, Navarra, Spain.

Parkinson´s Disease is the second most common neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the substantia nigra *pars compacta* and the accumulation of α-synuclein in Lewy Body called cytoplasmic inclusions. Mostly idiopathic, between the 5-10% of the cases appear due to specific mutations in different genes, such as the leucine-rich repeat kinase LRRK2. $LRRK2^{G2019S}$ mutation has been linked to alterations in autophagy and accumulation of α-synuclein during the progression of the disease. It is known that astrocytes participate to the transmission of alpha synuclein and the degeneration of neurons during the disease.

Thus, here, we have generated astrocytes from iPSC lines derived from patients' fibroblasts with the $LRRK2^{G2019S}$ mutation and from healthy age-matched donors. We have developed a modified iPSC differentiation protocol, obtaining approximately a 95% of astrocytes in our cultures. To asses possible implications of astrocytes to the pathogenesis of the disease we have studied metabolic and functional parameters including mitochondrial respiration and glycolitic activity. Morphological and cellular alterations have been studied with CX7 High Content Screening Platform or by electron microscopy.

More importantly, we have performed cocultures between healthy or LRRK2 ^{G2019S} astrocytes with healthy neurons and we have assesed the survival and the risk of neuronal death when they coexist with our astrocytes.

The results of these studies provide new evidences of the implications of astrocytes in the propagation of the disease and in the neuronal degeneration that occurs in Parkinson´s Disease.

P07. PV^{+}-ASTROCYTE SIGNALING MODULATES CORTICAL INHIBITORY SYNAPTIC ACTIVITY

Sara Mederos[1], Julio Esparza[1], Cristina Sánchez-Puelles[1], Alexey Ponomarenko[2], Gertrudis Perea[1]

1. Instituto Cajal (CSIC). Madrid, Spain
2. Institute of Clinical Neuroscience and Medical Psychology. Düsseldorf, Germany.

Astrocytes play critical roles in homeostatic functions. To the moment, a large number of studies have focused on astrocyte communication with principal neurons (PCs) and excitatory synaptic transmission, but the relationship between astrocytes and the aminobutyric acid (GABA) interneurons is poorly known (Perea et al., 2016). We aim to determine how astrocytes respond to a particular subpopulation of interneurons, the parvoalbumin positive cells (PV+), which are known to have crucial implication in the control of network activity, and the consequences of this signaling for the cortical networks (Tremblay et al., 2016). Using electrophysiology and optogenetic techniques in layer 2/3 of prefrontal cortical slices, we have found that:

1- Selective stimulation of PV+ cells triggers transient potentiation of inhibitory synaptic transmission recorded at PCs.

2- Astrocytes respond with calcium elevations mediated by $GABA_B$ activation to the selective stimulation of PV+ cells.

3- By releasing glutamate, astrocytes contribute to the enhancement of inhibitory tone through activation of mGluR1 receptors at presynaptic GABAergic terminals.

4- In vivo data showed that astrocytes are involved in modulation of cognitive processes related with behavioral responses.

Further characterization of these outcomes will open insights into the interneuron-astrocyte signaling and its consequences for the excitatory-inhibitory balance in cortical circuits.

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P08. ENERGETIC FAILURE DRIVES MICROGLIAL PHAGOCYTOSIS DYSFUNCTION IN STROKE

Ainhoa Plaza-Zabala[1], Sol Beccari[1,2], Virginia Sierra-Torre[1,2], Paloma R. Huguet-Rodriguez[2] Carretero-Guillén A[1], Castaño-Castaño S[1], Mazahir T. Hasan[1], Takashi Umekawa[3,4], Ahmed Osman[3], Wei Han[3], Cecilia Dominguez[3], Klas Blomgren[3], Jorge Valero[1,5] Amanda Sierra[1,2,5z]

[1] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Leioa, Spain
[2] University of the Basque Country, Leioa, Spain
[3] Karolinska Institutet, Department of Women's and Children's Health, Stockholm, Sweden
[4] Mie University Graduate School of Medicine, Tsu, Japan
[5] Ikerbasque Foundation, Bilbao, Spain

Microglial phagocytosis is an essential mechanism to maintain tissue homeostasis. In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. In response to a phagocytic challenge induced by excitotoxicity or inflammation, microglia proportionally increase their phagocytic output to counteract the rise in apoptotic cells, thus maintaining apoptosis and phagocytosis tightly coupled to each other. However, this phagocytic potential was blocked in a mouse model of cerebral hypoxia-ischemia. Using CX3CR1-GFP and CCR2-RFP mice, where resident microglia can be discriminated from blood-derived monocytes, we have discovered that microglial phagocytosis is strongly uncoupled from apoptosis as early as 1d after ischemia in both postnatal day 9 (P9) and 3 month old (3m) mice brains. Importantly, we have observed that this blockage occurred before the blood-derived monocyte infiltration which took place 3 days after ischemia. In addition, the mechanisms underlying the microglial phagocytosis impairment were assessed using primary microglial cultures and organotypic hippocampal slices under oxygen nutrient deprivation/reperfusion (OND). We found that OND conditions in primary microglial cultures led to a reduction in the degradation stage, possibly due to a lysosomal dysfunction. Interestingly, in organotypic hippocampal slices, microglial phagocyctosis was impaired under OND conditions for 3 and 6 hours, and rapidly recovered after 1 hour reperfusion. Our hypothesis is that the phagocytic blockage was the result of a reduced microglial surveillance and motility of the processes, which was assessed by 2-photon microscopy. Hence, microglial phagocytic potential is a novel and yet unexplored therapy to promote clearance of apoptotic cells and immunomodulation, in order to accelerate the recovery of the ischemic brain.

P09. ROLE OF APOLIPOPROTEIN D IN MICROGLIAL RESPONSE TO OXIDATIVE STRESS AND AMYLOID BETA TRIGGERED DAMAGE.

B. Bendito-Guilarte, M. Corraliza-Gomez, M. Poncela, M, Villa-Valdes, E. Arribas-Rodriguez, M.D. Ganfornina, D. Sánchez

Instituto de Biología y Genética Molecular, Universidad de Valladolid-CSIC, Valladolid, Spain.

The nervous system environment is closely surveyed by microglia, resident phagocytes that show complex phenotypes in brain function. Upon injury, microglia phagocytose cell debris, and their secreted factors modulate the immune response and tissue repair. Apolipoprotein D (ApoD) is secreted by astrocytes and myelinating glia in response to injury, to altered proteostasis or to oxidative stress (OS), factors that are commonly associated with aging and neurodegenerative conditions. ApoD helps to maintain lysosomal functional integrity, which contributes to cell survival and optimizes phagocytic activity after nervous system damage. Because of these intersecting functions, we decided to study the role of ApoD in microglia.

We found no expression of ApoD in microglia, neither under homeostatic conditions nor upon Paraquat (PQ)-triggered OS. We tested the effect of exogenously added ApoD protein, mimicking the natural astrocyte-derived source. ApoD is internalized by BV2 microglial cells, and exerts a pro-survival effect when cells are acutely challenged by PQ-induced OS or ² -amyloid oligomers. However, no protection appears upon chronic exposure to these stimuli. Following internalization, ApoD appears in BV2 cell vesicular compartments. We found a partial colocalization of endocytosed ApoD with the lysosomal/endosomal marker Lamp-2, prompting further investigations on the protein traffic within microglial cells and its eventual degradation.

Since ApoD could alter the tissue repair capacities of microglia, we are currently studying the inflammatory profile of microglia secretome, evaluating cytokines implicated in their neuroprotective role. Finally, we are testing microglial response in animals constitutively deprived of ApoD. We are comparing the cytokine response and phagocytic ability of WT and ApoD-KO microglial primary cultures. Also, using molecular markers, we are assessing microglia functional states in the brain of WT and ApoD-KO mice exposed to PQ or upon physiological aging. Understanding how ApoD acts on microglia is key to properly assess its neuroprotective potential.

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P10. MICROGLIAL ACTIVATION PREVENTS Aβ-INDUCED SYNAPTIC DYSFUNCTION AND REDUCES EXTRACELLULAR Aβ IN ALZHEIMER'S DISEASE

J. Zuazo-Ibarra [1,2], C. Luchena [1,2], E. Alberdi [1,2,3], C. Matute [1,2,3] and E. Capetillo-Zarate [1,2,3,4,5]

[1]. Achucarro Basque Center for Neuroscience, Leioa, Spain
[2]. Facultad de Medicina y Enfermería, Universidad del País Vasco, Leioa, Spain
[3]. CIBERNED, Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas, Spain
[4]. IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[5]. Department of Biochemistry, Well Cornell Medical College, New York, USA

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of progressive cognitive decline in the aged population. Accumulation of β-amyloid (Aβ) peptide and the synaptic dysfunction are the main hallmarks of AD neuropathology. Loss of synapses occurs early in AD and is considered the best pathological correlate of cognitive decline.

The role of microglia, innate immune cells of the brain, in AD remains controversial. In one hand, microglia mediates early synapse loss in AD models. In contrast, activation of microglia by immunotherapy or the cytokine *macrophage colony stimulating factor* (MCSF) results in a more efficient Aβ degradation

To study the role of microglia in Aβ-related synapse pathology we performed immunofluorescence and western blot techniques to measure the levels of pre- and post- synaptic markers in neurons cultured alone or together with microglia in the presence or absence of Aβ oligomers. We also carried out immunoprecipitation for Aβ detection in the culture media.

We observed a significant reduction of both pre-(synaptophysin) and postsynaptic (homer) markers labelling in primary neuron cultures in presence of Aβ compared to controls. However, we did not observe synapse pruning in our model. In the contrary, we found that microglia decreased extracellular amyloid in microglia-neuron co-cultures in presence of extracellular Aβ oligomers although this was not enough to restore synapses in control conditions. Notably, microglial activation by MCSF was not only able to reduce extracellular amyloid load but also to prevent synapse damage.

Overall, these results show the Aβ impact on synaptic loss in neuronal primary culture and that microglia activation is able to reduce extracellular Aβ and to avoid synaptic damage *in vitro*. These results strongly suggest that Aβ oligomers are deleterious to synaptic function by interfering with neurons, and that microglial modulation constitutes an important therapeutic target for the prevention of synapse toxicity.

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P11. PHARMACOLOGICAL BLOCKADE OF IL-34 TO MODULATE MICROGLIAL PROLIFERATION IN NEURODEGENERATIVE DISEASE

M. Martín Estebané [1], J. Obst [1], E. Simon [1], A.R. Prescott [2], V.H. Perry [1], Dementia Consortium Project Partners, D. Gómez Nicola[1],

[1] University of Southampton, Southampton, United Kingdom.
[2] University of Dundee, Dundee, United Kingdom.

The proliferation and activation of microglia, the brain´s main resident macrophages, is a hallmark of many neurodegenerative diseases including Alzheimer's disease and prion disease. Colony stimulating factor 1 receptor (CSF1R) is critically involved in regulating proliferation of microglia, and CSF1R blocking strategies have been recently used to modify microglia in neurodegenerative diseases. CSF1R can be activated by two independent ligands, CSF1 and interleukin-34 (IL-34), and it has been reported that microglia development and maintenance depends on IL-34 signalling.

Our aim is to evaluate novel IL-34 blocking strategies to modulate microglia proliferation in neurodegenerative diseases, using the ME7 model of prion disease. We will determine the effects of different IL-34 blocking strategies (acute vs. chronic and systemic vs. CNS-specific treatment) using anti-IL34 blocking antibodies in health and prion disease and how this compares to anti-CSF1R blockade. For this purpose, we will study peripheral macrophages/monocytes populations and microglial proliferation, activation and gene expression profile by flow cytometry, immunohistochemistry analysis and RNA sequencing.

Our preliminary results show that IL-34 blocking treatment did not alter peripheral macrophages and monocytes populations in healthy mice, avoiding the side effects observed after CSF1R blockade on the systemic compartment. However, we observed changes in microglial proliferation and gene expression after IL-34 blockade in prion-diseased mice, indicating that microglia could be more specifically targeted by reducing IL-34 and that this ligand plays an important role in the modulation of microglia population during neurodegeneration. Our results suggest that control of the microglial response through IL-34 blockade could be a potential therapeutic approach in neurodegenerative diseases.

P12. ADDRESSING THE ROLE OF ASTROGLIAL CB_1 IN MULTIPLE SCLEROSIS

A. Moreno García[1,2,3,5], A Lamela[1,2], A Manterola[1,2,3], A Bernal-Chico[1,2,3], L Robin[5], JC Chara[1,2,3], C Matute[1,2,3], A. Rodríguez Antigüedad[1,3,4], G Marsicano[5], S Mato[1,2,3,4]

[1]Facultad de Medicina y Enfermería, Universidad del País Vasco UPV/EHU, Leioa, Spain
[2]Centro Achucarro de Neurociencias, Leioa, Spain
[3]Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain
[4]Biocruces Bizkaia, Barakaldo, Spain
[5]NeuroCentre Magendie, Bordeaux, France

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) characterized by oligodendrocyte loss, inflammation, gliosis and axonal degeneration that affects both grey and white matter. Astroglial cells regulate inflammation and neurodegeneration in MS through a variety of mechanisms that include the recruitment of peripheral immune cells, the activation of pro-inflammatory pathways in microglia and the regulation of myelin repair processes.

Cannabinoid CB_1 receptors exhibit a wide and heterogeneous pattern of expression in the CNS with high expression levels in neurons and low levels in neuroglial cells, such as astrocytes. Cannabinoids and endocannabinoids acting through neuronal CB_1 receptors engage protection from glutamate excitation and exert symptom control in MS. Conversely, CB_1 receptors expressed in astrocytes promote glutamatergic synaptic signaling with potential negative consequences in a neuroinflammatory context. However, the relevance of CB_1 receptors as regulators of astrocyte function in MS remains largely unexplored.

Here we addressed the role of astroglial CB_1 receptors during demyelination *in vivo* using conditional mutant mice lacking CB_1 receptors in cells expressing the astrocyte protein GFAP (GFAP-CB_1R-KO). Chronic experimental autoimmune encephalomyelitis (EAE) was induced in GFAP-CB_1R-KO and wild-type littermates (GFAP-CB_1R-WT) by immunization with myelin oligodendrocyte glycoprotein in Freund's adjuvant supplemented with *Mycobacterium tuberculosis*. GFAP-CB_1R-KO mice exhibited attenuated clinical disability during EAE disease progression as compared to GFAP-CB_1R-WT mice. Histological analysis at the experimental endpoint evidenced that astrocytic CB_1 receptor deletion decreased lesion load, myelin damage, T cell infiltration and microglial reactivity in spinal cord white matter, and to a lesser extent in cortical grey matter, of EAE mice. Our results unveil that astroglial CB_1 receptors exacerbate EAE disease pointing to a previously unexpected role of this receptor population in MS.

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P13. SEROTONINERGIC MODULATION OF ASTROCYTE-NEURON SIGNALING

C. González-Arias, C. Sánchez-Puelles, J. Ramírez-Franco, G. Perea

Cajal Institute (CSIC), Madrid, Spain

The serotonergic (5HT) system plays crucial roles in several brain functions. Although recent evidences have shown that astrocytes actively contribute to neuronal function by exchanging substances with the synaptic elements, the contribution and impact of serotonergic system to the neuron-astrocyte signaling is poorly known.
Therefore, we aim to investigate the role of serotonin (5-HT) onto astrocyte-neuron signaling in layer 2/3 of mouse medial prefrontal cortex (mPFC) by using electrophysiology and Ca^{2+} imaging techniques in mPFC slices.

We have found that local application of 5-HT (1 mM; 1 bar, 10 s) reliably stimulate astrocytic Ca^{2+} elevations that were sensitive to the 5-HT2 receptor antagonist cyproheptadine (1µM). These Ca^{2+} elevations were absent in astrocytes from *Ip3r2*$^{-/-}$ mice, which show a downregulated astrocyte Ca^{2+} signaling. Moreover, the 5-HT-induced Ca^{2+}events triggered glutamate release from astrocytes and the generation of slow inward currents (SICs) in nearby principal neurons. Additionally, local application of 5-HT promotes a transient synaptic depression of excitatory postsynaptic currents (EPSCs) via presynaptic 5-HT1B receptors (1, 2). However, the 5-HT2 receptors blockage evoked a significant synaptic depression after 5- HT stimulation, similar to the observed response in *Ip3r2* $^{-/-}$ mice, which might indicate the contribution of astrocytes to the 5-HT-induced EPSC modulation in wildtype mice. Likewise, in the presence of LY367385 (100 µM), a selective blocker of group I metabotropic glutamate receptors, 5-HT induced a prolonged EPSCs depression in wildtype mice similar to those results obtained from *Ip3r2* $^{-/-}$ mice. These data suggest that glutamate released by astrocytes after 5-HT stimulation would activate the neuronal group I mGluRs that would enhance synaptic transmission.

Altogether, these results suggest a prominent role of astrocytes in the modulatory effects of 5- HTergic system. Thus, dysfunctions of astrocyte-neuron signaling triggered by 5-HT might be involved in the pathology related 5-HTergic system.

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P14. IMMORTALIZATION OF A CELL LINE WITH NEURAL STEM CELL (NSC) CHARACTERISTICS DERIVED FROM MOUSE EMBRYO BRAIN

Cristina Almengló, Pilar Caamaño, Tamara González-Mosquera, Marcos Seoane, Máximo Fraga, Jesús Devesa, Víctor M. Arce, José A. Costoya,

Departaments of Fisioloxia and Ciencias Forenses, Anatomía Patolóxica, Xinecoloxía e Obstetricia, e Pediatría. Universidade de Santiago de Compostela.
Fundacion Publica Galega de Medicina Xenomica,
Research and Development, Foltra Medical Center

Neural stem cells (NSC) have been extensively used as a tool to investigate the mechanisms responsible for neural development and repair and proposed as replacement therapies in various neurodegenerative diseases. Nevertheless, because of their anatomical location and relative rarity, methods for isolation and characterization of NSC are usually time consuming and have some technical limitations. To overcome this problem, we have developed several immortalized cell lines from mouse embryo brains, which display NSC characteristics.

Methods

Primary monolayer cultures of were initially established from 13.5 dpc (day post coitum) C57BL/6 mice embryos (approval ID15005AE/07/FUN01/FIS02/JACP1). Cells were routinely cultured in DMEM and immortalized with the 3T3 protocol. Growth rate was determined in primary, immortalized or high passage immortalized cells by measuring the rate of BrdU incorporation. Metaphase spreads were prepared from either primary or high passage (> 30 passages) immortalized cells.

Results and discussion

Immortalized NSC can be routinely cultured for long periods of time, while retaining their ability to form neurospheres. However, and in contrast to other immortalized primary cells such as fibroblasts or Schwaan cells, high passage immortalized NSC show an increase in proliferation rate, together with other phenotypical modifications such as morphological changes or numerical chromosome abnormalities. Altogether, these findings suggest that cellular transformation may be present in long-term immortalized NSC.

P15. IGF1 GENE THERAPY DELAYS REPRODUCTIVE SENESCENCE

FJD Dolcetti[1], E. Falomir-Lockhart[1], M. L. Herrera[2], MA Arévalo[3] and MJ Bellini[1]

[1]INIBIOLP-CONICET, Facultad de Medicina, Universidad Nacional de La Plata, La Plata, BA, Argentina.
[2]IFEC–CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Córdoba, Argentina.
[3] Instituto Cajal, CSIC, Madrid, Spain

Background: The hypothalamus, a region known to regulate many basic functions such as growth, development, reproduction and metabolism, is thought to be a regulatory center of aging. Evidence demonstrates that the inhibition or activation of the transcription factor NF-κB in microglia or in neurons of the basal hypothalamus (HMB) affects life expectancy and the "beginning" of aging, as well as the release of GnRH. There is solid evidence that middle age (MA) rats have reduced activation of $GnRH^+$ neurons, GnRH release, and an abnormal LH surge. These findings provide a link between inflammation, response to stress and systemic and cerebral aging.

Material and methods: We performed intrahypothalamic stereotactic injection to implement long-term anti-inflammatory gene therapy for IGF1 in the HMB of MA female rats (8 months) up to 12 months, in order to modulate the inflammatory response mediated by NF-kB and delay the appearance of reproductive cessation. The cyclicity of the animals was monitored daily by colpocytology. For immunohistochemical analysis, non-contiguous brain slides were selected and processed with anti-GnRH, anti-Iba1 or anti-Kisspeptin antibodies.

Results: Our results show that, at the end of the experiment, rats treated with IGF1 present a higher proportion of cycling rats compared to control group. We also observed that IGF1 group has a higher number of axonal projections of the $GnRH^+$ neurons and a higher number of $Kisppeptin^+$ neurons in the hypothalamic anteroventral periventricular nucleus.

Conclusions: These results suggest that IGF1 prolongs the reproductive life of MA rats, maintaining $GnRH^+$ and $Kisppeptin^+$ neurons functionality.

P16. THE RELATIONSHIP BETWEEN HIF ACTIVATION, LIPID DROPLET FORMATION AND CELL DEATH FOLLOWING THE STIMULATION OF NO-cGMP MEDIATED PATHWAY IN LN18 HUMAN GLIOBASTOMA-DERIVED CELLS

M. Danti[1], L. Martínez-Escardó[1,2], VJ. Yuste[1,2], E. Claro[1,2], MA. Baltrons[1]
[1]. Facultad de Medicina. Universidad Autónoma de Barcelona, Barcelona
[2]. Instituto de Neurociencias, Universidad Autónoma de Barcelona, Barcelona

Glioblastoma multiforme is the most aggressive brain tumor with a poor prognosis. Hypoxia, which activates HIF-1α pathway, is a common feature in malignant gliomas and has been linked with tumor cell survival and therapy resistance. The capacity to generate lipid droplets (LDs) is positively linked to the ability of cells to survive oxidative stress under hypoxia conditions. In accordance with this, we have previously shown that LDs generated during nutrient deprivation-triggered cellular stress have a clear cytoprotective role.

It is well known that NO is a cytotoxic molecule when is produced at high concentrations after the induction of NO-synthase type-2. Its induction has been described in glioma cells exposed to pro-inflammatory cytokines, as well as in patients' post-mortem tissue. However, whether the NO-pathway is beneficial or detrimental in cancer is still an open question since positive, negative or biphasic effects on apoptosis/cell death have been described in different cell types exposed to NO. Considering there are several reports demonstrating that NO modulates HIF-1 activity, we hypothesize that LD generation under hypoxic conditions or/and NO generation could also modulate the NO-effect on cell death.

Human glioblastoma LN18 cells were treated with the NO-donor sodium nitroprusside (SNP; 0.025 to 1 mM) for 24 h, stained with PI and cell death was analyzed microscopically. As described, SNP induced dose-dependent cytotoxicity, reaching around 30-40% of cell death at 0.5 mM and 80% at 1 mM. Then, the cells were fixed and stained with Nile-red to analyze the LD content by fluorescence microscopy. Surprisingly, in SNP-treated cells we observed numerous Nile red-stained LDs in the cytoplasm of either living and dead cells, mainly when high concentrations of SNP were used. To study the role of HIF-1α in these SNP-effects, we employed YC-1, a well-known HIF-1α inhibitor. We found that YC-1 pre-treatment (100 μM; 1 h) completely abrogated SNP-mediated LD accumulation. Interestingly, YC-1 increased the SNP- cytotoxic effect by 2-3 folds.

Taken together, these preliminary results suggest that the balance between HIF1-α pathway, LD metabolism, and NO signaling may modulate the NO-effect on cell death and may lead to the possibility of combining HIF/LD generation inhibitors to increase the NO effect at lower concentrations for therapeutic purposes.

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P17. APOLIPOPROTEIN D-MEDIATED PRESERVATION OF LYSOSOMAL FUNCTION PROMOTES CELL SURVIVAL AND DELAYS MOTOR CONTROL IMPAIRMENT IN NIEMANN-PICK TYPE A DISEASE.

R. Pascua-Maestro[1], M. Corraliza-Gomez[1], C. Fadrique-Rojo[1], M.D. Ledesma[2], D. Sanchez[1], M.D. Ganfornina[1]

[1] Instituto de Biología y Genética Molecular, Universidad de Valladolid-CSIC, Valladolid, Spain.
[2] Centro de Biología Molecular Severo Ochoa, CSIC, Madrid, Spain.

Lysosomal Storage Diseases (LSD) are genetic, early onset, neurodegenerative diseases that result in systemic and nervous system dysfunction. We have shown that the Lipocalin Apolipoprotein D (ApoD) is essential for the maintenance of lysosomal functional integrity in glial cells. ApoD ensures cell survival upon oxidative stress (reverting membrane permeabilization and loss of pH gradients), adequate compaction of myelin (controlling glycolipid recycling processes), and proper phagocytic activity after nervous system injury. The crucial role of ApoD within lysosomes led us to study its effects on the Niemann Pick type A disease (NPA), caused by loss of function mutations in the acid sphingomyelinase gene, and resulting in sphingomyelin accumulation in lysosomal membranes. NPA patients rapidly develop progressive neurodegeneration, cerebellar atrophy and myelin deficiencies.

We demonstrate that, as in glial cells and neurons, ApoD is targeted to lysosomes of fibroblasts from NPA patient. While oxidative stress promotes ApoD entry into the lysosomal compartment of healthy cells, such accelerated targeting is lost in diseased cells, contributing to the vulnerability of NPA lysosomes. By measuring cathepsin B activity, galectin-3 subcellular location and lysosomal pH, we demonstrate that exogenously added ApoD is able to significantly reduce lysosomal permeabilization and NPA-promoted lysosomal alkalinization. ApoD addition reverts the accumulation of oxidized products in lysosomes and overall lipid peroxidation levels in NPA cells, resulting in a significant increase in cell survival. As predicted by these protective effects at the cellular level, lack of ApoD in a NPA mouse model accelerates the appearance of behavioral deficits, with enhanced loss of cerebellar-dependent motor control. We are currently analyzing ApoD influence on myelination, gliosis, and Purkinje cell survival. Our results reveal that ApoD protection of lysosomal integrity is able to counteract biological deterioration of NPA disease at the cellular and organismal levels, and open therapeutic opportunities for this devastating disease.

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P18. A CHEMOGENETIC TOOL TO STUDY MYELIN PLASTICITY

Ana Palma Leiva, Carlos Matute, Federico Soria, Alberto Pérez-Samartín and María Domercq

Achucarro Basque Center for Neurosciences, Ciberned and Departamento de Neurociencias, Universidad del País Vasco-UPV/EHU, 48940-Leioa, Spain

Myelin sheaths, specialized segments of oligodendrocyte (OL) plasma membranes in the central nervous system (CNS), facilitate fast, saltatory conduction of action potentials down axons. Classical view supports that oligodendrocytes perform myelination following an innate program and in the absence of exogenous signaling. However, there are new evidences suggesting that changes in myelination during development continue during adult life (Hill et al. 2018). New myelination is thought to depend on oligodendrocyte progenitor cells, giving rise to nascent myelinating oligodendrocytes. Mature oligodendrocytes are largely regarded as being uninvolved. In order to address the role played by mature oligodendrocytes in myelination and remyelination, we have successfully developed transgenic mice, using the CreERT2-lox technology, overexpressing the DREADD receptor M3 mAChR under the promotor of PLP, specific of mature oligodendrocytes. Stimulation of PLP^+ $hM3Dq^+$ oligodendrocytes with the ligand clozapine-N-oxide (CNO) induced a massive increase in cytosolic calcium, branching of oligodendrocytes and higher myelin basic protein synthesis. Promoting remyelination is a major therapeutic goal, both to restore function and to protect axons from degeneration. Using these transgenic mice we plan to analyze the impact of oligodendrocyte stimulation in myelin plasticity and in demyelinating disease models. Indeed, preliminary results showed that chemogenetic oligodendrocyte stimulation ameliorates clinical symptoms after experimental autoimmune encephalomyelitis induction. In conclusion, the chemogenetic tool designed here could help to elucidate the role played by mature oligodendrocytes in myelin plasticity and during remyelination after demyelination.

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P19. NOVEL INSIGHTS INTO THE NEUROLOGICAL PATHOPHYSIOLOGY OF MCT8-DEFICIENCY

M. Guillen-Yunta[1,3], A. García-Aldea[1], V. Valcárcel-Hernández[1], Grijota-Martínez[1,2], A. Guadaño-Ferraz[1,2]

1. Instituto de Investigaciones Biomédicas “Alberto Sols”, Consejo Superior de Investigaciones Científicas
(CSIC)-Universidad Autónoma de Madrid (UAM), Madrid, Spain
2. Center for Biomedical Research on Rare Diseases (Ciberer) Unit 708, Instituto de Salud Carlos III, Madrid, Spain.
3. Universidad Autónoma de Madrid (UAM), Madrid, Spain.

Thyroid hormones (TH) are essential in the development of the brain, regulating processes such as the differentiation of neural cells and myelination. TH are secreted to the blood from the thyroid gland, mainly as T4, which in the brain is converted in the astrocytes into T3, the nuclear active form, by the enzyme deiodinase type 2 (DIO2). The Allan-Herndon-Dudley Syndrome (AHDS or MCT8 deficiency) is an X-linked rare disease caused by mutations in the monocarboxilate transporter 8 (MCT8), a transmembrane transporter specific for TH.

AHDS is characterized by altered serum TH levels and severe neurological damage including profound psychomotor impairment. Evidences strongly suggest that the neurological syndrome in MCT8 deficiency is mainly due to cerebral hypothyroidism, since TH access across brain barriers is impaired. Mct8-deficient mice replicate the alterations in circulating TH levels but not the neurological syndrome observed in AHDS patients, due to a compensatory mechanism involving the Dio2 enzyme. This led us to characterize the neurological phenotype of the double *Mct8/Dio2* knockout mouse (KO) to validate it as a possible model of the disease.

Previous results from brain samples of an 11-year-old MCT8-deficient subject diagnosed with AHDS showed a severely altered expression of neuronal proteins, together with a delay in myelination. The goal of this work was to analyse glial populations and myelination in Mct8 absence in mice. To this aim, we used postnatal day 21 (P21) and P90 *Mct8/Dio2*KO mice as an animal model for Mct8 deficiency.

Our results suggest a neuroinflammatory state in *Mct8/Dio2*KO mice. Evidences suggest that the lack of TH in *Mct8/Dio2*KO mice brain alters the development of neuroglial cells, which could lead to the sustained neuroinflammatory state observed. Myelin studies were also consistent with human data, showing low and aberrant myelination in *Mct8/Dio2*KO mice at P21 that is not fully reversed at P90.

P20. TAT-CX43$_{266-283}$ PEPTIDE IMPAIRS MALIGNANT GROWTH IN MOUSE MODELS OF GLIOMA IN VIVO

R. Talaverón, M. Jaraíz- Rodríguez, L. García –Vicente, S. G. Pelaz , J.M. Medina. A. Tabernero.

Instituto de Neurociencias de Castilla y León (INCYL), Universidad de Salamanca,Salamanca, Spain.

Malignant gliomas are the most frequent primary brain tumors and remain among the most incurable cancers. Connexin43 (Cx43) is an integral membrane protein that forms gap junctions and is widely expressed in astrocytes. This protein is down-regulated in high-grade gliomas and, particularly, in glioma stem cells (GSCs). When Cx43 is restored, the stem cell phenotype of GSCs is reversed and their tumorigenicity is reduced. We previously reported that TAT-Cx43$_{266-283}$, a cell-penetrating peptide based on Cx43, retains the ability to recruit Src together with its endogenous inhibitors PTEN and CSK, causing c-Src inhibition and exerts potent anti-tumor effects in different types of glioma cells in vitro. TAT-Cx43$_{266-283}$, by inhibiting c-Src, also reduces the expression of Sox-2, the formation of neurospheres, the rate of proliferation, migration and invasion in different types of GSCs, including primary GSCs derived from patients. Here, we studied the effect of TAT-Cx43$_{266-283}$ when human glioma stem cells were intracranially injected into NOD/SCID mice. We analyzed human nestin, stem 121 and Sox-2 by immunohistochemistry at different days post-implantation. We observed that TAT-Cx43$_{266-283}$ reduced the expression of the stemness markers nestin and Sox-2 in GSCs at 7 days post-implantation. Consistent with the role of Sox-2 as a transcription factor required for GSC tumorigenicity, TAT-Cx43$_{266-283}$ strongly reduced the number and stemness of human glioma cells at 30 days post-implantation in NOD/SCID mice. Taken together, our results confirm that TAT-Cx43$_{266-283}$ reduces the growth, invasion, and progression of malignant gliomas, which highlights the importance of this compound for the design of new therapies against malignant gliomas.

P21. IMMORTALIZATION OF A CELL LINE WITH NEURAL STEM CELL (NSC) CHARACTERISTICS DERIVED FROM MOUSE EMBRYO BRAIN

Tamara González-Mosquera, Cristina Almengló, Pilar Caamaño, Marcos Seoane, Máximo Fraga, Jesús Devesa, Víctor M. Arce, José A. Costoya,

Departaments of Fisioloxia and Ciencias Forenses, Anatomía Patolóxica, Xinecoloxía e Obstetricia, e Pediatría. Universidade de Santiagode Compostela.
Fundacion Publica Galega de Medicina Xenomica,
Research and Development, Foltra Medical Center

Neural stem cells (NSC) have been extensively used as a tool to investigate the mechanisms responsible for neural repair, and they have been considered as the source for a series of promising replacement therapies in various neurodegenerative diseases. However, their use is limited by their relative rarity and anatomical localization, and also because the methods for isolation and characterization are usually time consuming and have some technical limitations.

Methods

Primary monolayer cultures of either MEA (mouse embryo astrocytes) or MEF were initially established from 13.5 dpc (day post coitum) C57BL/6 mice embryos (approval ID15005AE/07/FUN01/FIS02/JACP1). Cells were routinely cultured in DMEM, and immortalized with the 3T3 protocol. Neurosphere formation in immortalized cells was induced with the neurosphere assay, using semi-synthetic medium supplemented with EGF and bFGF.

Results and discussion

When continuously passed, both MEA and MEF entered in a “crisis” period characterized by the presence of senescent cells and an increase in the expression of p21. Also in both cases, a number of cells were able to overcome this crisis period thus resulting in eventually immortalized cells. However, in contrast with MEF, in which immortal cells emerge at low frequency, explanted MEA give rise to immortal cells with a 100% oddity. Also in contrast with immortalized MEF, which show decreased p21 levels, increased p21 expression is maintained in immortalized MEA. These immortalized MEA express the characteristics markers of all neural populations and, depending on the growth conditions, can be induced to differentiate or to form neurospheres. Therefore, immortalized MEA display *bona fide* NSC-like characteristics and may provide an invaluable tool to supply a consistent and renewable source of undifferentiated CNS precursors.

P22. ACUTE Δ9-TETRAHYDROCANNABINOL ADMINISTRATION ACCELERATES OLIGODENDROCYTE DEVELOPMENT AND REGENERATION

T. Aguado[1,2]*, A. Huerga-Gómez[1,2]*, A. Sánchez-De la Torre[1,2], Ana Bernal-Chico[3], Susana Mato[3], M. Guzman[1,2], I. Galve-Roperh[1,2], J. Palazuelos[1,2]

[1]Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Instituto Universitario de Investigación Neuroquímica (IUIN), Complutense University, 28040, Madrid, Spain; [2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), 28049 Madrid, Spain. [3]Achucarro Basque Center for Neuroscience, Dept. of Neurosciences, University of the Basque Country UPV/EHU, Leioa, Spain.

Research on demyelinating disorders has consistently demonstrated that cannabinoid agonists have the potential to relieve symptomatology and to control inflammatory-mediated neurodegeneration in Multiple sclerosis (MS) animal models and patients. Thus, the contribution of oligodendroglial cannabinoid receptors to the beneficial effects of cannabinoids in MS animal models has mostly focussed on their capacity to prevent cell survival of oligodendrocytes (OLs) and their progenitors (OPs) against excitotoxic injury. However, very few attention has been paid to the potential of targetting OPs by cannabis-based compounds to promote OL regeneration and functional recovery in demyelinating disorders.

Here, we studied the effect of administering Δ^9-Tetrahydrocannabinol (Δ^9-THC), the most abundant cannabinoid present in the plant *Cannabis sativa*, in OPs cell cycle dynamics and OL differentiation, both, during the critical periods of postnatal CNS myelination and under demyelination condition, by using the Cuprizone model.

First, we found that acute Δ^9-THC administration induced OPs cell cycle exit and differentiation, accelerating the process of OL maturation, subcortical white matter (SCWM) myelination and motor function development. Further, we found that acute Δ^9-THC administration following 6 weeks of Cuprizone-induced demyelination promoted OPs cell cycle exit and differentiation, and accelerated SCWM remyelination and motor function and cognitive recovery. CB_1 and CB_2 selective antagonists administration prevented the THC-mediated induction in OL development and regeneration suggesting that CB_1 and CB_2 cannabinoid receptors mediates Δ^9-THC actions in OL lineage cells.

Overall, our results show that acute Δ9-THC administration induces OP cell cycle exit and differentiation, and accelerates OL development during CNS myelination, but also accelerates OL regeneration during CNS remyelination, addressing the therapeutic potential of targetting cannabis-based compounds to promote OL regeneration and functional CNS remyelination in demyelinating disorders.

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LISTADO DE ASISTENTES

Nombre	email
Aguado Sánchez, Tania	taguado@ucm.es
Almengló, Cristina	cristina.almenglo@usc.es
Alvarez Vazquez, Andrea	andrea.av@usal.es
Baleriola, Jimena	jimena.baleriola@achucarro.org
Baltrons, Mª Antonia	mariaantonia.baltrons@uab.cat
Capetillo-Zarate, Estíbaliz	estibaliz.capetillo@ehu.eus
Casas, Sergio	scasas@cajal.csic.es
Castellano López, Bernardo	bernardo.castellano@uab.es
Clemente López, Diego	dclemente@sescam.jccm.es
Corraliza Gómez, Miriam	corraliza@ibgm.uva.es
Costoya, José A.	josea.costoya@usc.es
Cruz Dolcetti, Franco Juan	franco.dolcetti@gmail.com
de Castro, Fernando	Fdecastro@cajal.csic.es
Domercq, Maria	maria.domercq@ehu.eus
Férnandez Gómez, Beatriz	beatriz.fernandez@cajal.csic.es
Ganfornina Álvarez, Mª Dolores	opabinia@ibgm.uva.es
García Aldea, Ángel	agarciaaldea@iib.uam.es
García ovejero, Daniel	dgarciao@sescam.jccm.es
García Segura, Luis Miguel	lmgs@cajal.csic.es
González Arias, Candela	candela.gonzalez@cajal.csic.es
Gonzalez de Mingo, Berta	berta.gonzalez@uab.cat
González-Mosquera, Tamara	t.gonzalez.mosquera@gmail.com
Guadaño, Ana	aguadano@iib.uam.es
Guillén Yunta, Marina	marinagyun96@gmail.com
Luchena Moreno, Celia	celia.luchena@ehu.eus
Márquez, Mar	mar48_nera@hotmail.com
Martín Estebané, Maria	m.martin-estebane@soton.ac.uk
Mato, Susana	susana.mato@ehu.es
Matute, Carlos	carlos.matute@ehu.es
Mederos, Sara	smederos@cajal.csic.es
Mestre, Leyre	leyre@cajal.csic.es
Mey, Jörg Michael	jmey@sescam.jccm.es
Montero Pedrazuela, Ana	amontero@iib.uam.es
Moreno García, Álvaro	alvaro.morenog@ehu.eus
Moura Neto, Vivaldo	
Navarrete Llinás, Marta	mllinas@cajal.csic.es
Nocera, Sonia	Sonia.nocera@cajal.csic.es
Ortega Muñoz, Mª Cristina	mcristinao@sescam.jccm.es
Palazuelos, Javier	j.palazuelos@ucm.es
Perea, Gertrudis	gperea@cajal.csic.es
Plaza-Zabala, Ainhoa	ainhoa.plaza@achucarro.org
Ramos González, Paula	paularamosgon@gmail.com
Sánchez Romero, Diego	lazarill@ibgm.uva.es

Sierra de la Torre, Virginia	virginiasierra1@gmail.com
Sierra, Amanda	amanda951@gmail.com
Talaverón, Rocío	rtalaveron@usal.es
Valcárcel Hernández, Victor	vvalcarcel@iib.uam.es
Wang, Ting	brenda504950535@gmail.com
Wang, Yu	wangyu_493889962@yahoo.com
Zabala, Alazne	Alazne.zabala@ehu.es
Zuazo Ibarra, Jone	jone.zuazo@ehu.eus