VIII Reunión de la Red Glial Española

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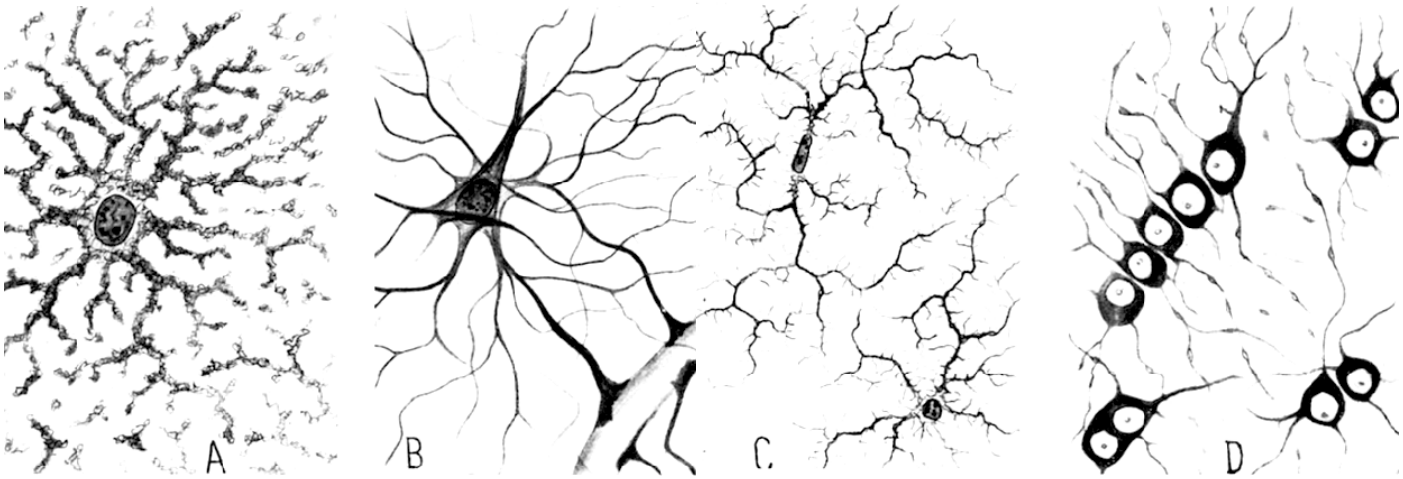

PROGRAMA
Martes 22 de Septiembre (sala 4)

16.00 – 16.20 SESIÓN TÉCNICA:

Método Ubc- StarTrack para la identificación de clones gliales procedentes de un progenitor
María Figueres-Oñate, Instituto Cajal, Madrid

16.20 – 17.00 SESIÓN NEUROPROGENITORES

16.20 *Study of human primary Glioma Stem Cells invasion in the presence of cell-penetrating peptides based on the interaction between connexin43 and c-Src*
Myriam Jaraíz-Rodríguez, Instituto de Neurociencias de Castilla y León, Salamanca

16.30 *Neural stem cell-derived reactive astrocytes induced by seizures in LPAR1-EGFP mice*
Roberto Valcárcel Martín, Achucarro Basque Center for Neuroscience, Bizkaia

16.40 *Aged neural stem cells in the hippocampus*
Soraya Martín Suárez, Achucarro Basque Center for Neuroscience, Bizkaia

16.50 *Aplicación de células madre mesenquimales en un modelo animal de hidrocefalia congénita con reacciones astrocitarias*
María García Bonilla, Universidad de Málaga, Málaga

17.00 – 17.30 SESIÓN ASTROCITOS

17.00 *Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways*

Ricardo Martín, Instituto Cajal, Madrid

17.10 *Optogenetic boosting of astrocyte-neuron signaling*

Sara Mederos, Instituto Cajal, Madrid

17.20 *Understanding ApoD neuroprotective function: ApoD distribution in pH-dependent sub-domains of the astroglial lysosomal compartment upon metabolic and oxidative stress*
Raquel Pascua Maestro, Instituto de Biología y Genética Molecular, Valladolid

17.30 *Alzheimer amyloid-beta internalization by astrocytes requires clathrin-mediated endocytosis*
Marta Domínguez-Prieto, Instituto de Neurociencias de Castilla y León, Salamanca

17.40 – 18.20 SESIÓN MICROGLÍA

17.40 F*unción de las células microgliales en cultivos organotípicos de retina de ratón postnatal*
Rosa M. Ferrer Martín, Universidad de Granada, Granada

17.50 *Neuronal hyperactivity uncouples microglial phagocytosis and leads to delayed cell-clearance*
Irune Diaz Aparicio, Achucarro Basque Center for Neuroscience, Bizkaia

18.00 *Microglial phagocytosis is impaired in chronic mouse and human MTLE and correlates with inflammation*
Soledad Beccari Galeano, Achucarro Basque Center for Neuroscience, Bizkaia

18.10 *Deterioro de la respuesta inflamatoria glial en el ratón modelo de senescencia SAMP8*
Rubén Corpas, Institut d´Investigacions Biomèdiques de Barcelona, Barcelona

18.20 – 19.00 ASAMBLEA

RESÚMENES

1. Método Ubc- StarTrack para el la identificación de clones gliales procedentes de un progenitor

María Figueres Oñate y Laura López Mascaraque
Instituto Cajal-CSIC, Madrid

El método se basa en la incorporación genómica de distintos genes codificando distintas proteínas fluorescentes (EGFP, mCherry, mCerulean, mTSapphire, mKO, YFP) tanto en el núcleo como en el citoplasma, ya diseñados para el StarTrack (García-Marques and Lopez-Mascaraque, 2013) en el que estaban reg ulados por el promotor hGFAP. En este caso la expresión esta regulada por un promotor ubicuo (Ubiquitina C, UbC), con el que se obtiene un marcaje de todos los distintos linajes que provengan de una misma célula (astrocitos, oligodendrocitos, celulas Ng2 y neuronas). A fin de conseguir una marca estable, los vectores se han desarrollado con el sistema PiggyBac y el Cre-LoxP, permitiendo que el marcaje sea integrado y transmitido a cada célula hija. Los doce plasmidos recombinantes, junto con el vector que contiene la transposasa y el que contiene la Cre inducible, se insertan en el ventriculo lateral de embriones de ratón y se procede a la electroporacion. Los ratones serán sacrificados a distintos estadios embrionarios, postnatales o adulto dependiendo del objetivo. En conjunto, con el diseño de estos plásmidos regulados bajo la expresión de un promotor ubicuo, pretendemos trazar todas las células que provienen de un mismo progenitor, ya que estas compartirán el mismo marcaje. Los clones se definirán como grupos de células con la misma combinación de proteínas fluorescentes. En función del número de copias que se integren de cada proteína fluorescente, se generará una tonalidad específica compartida por cada clon.

2. Study of human primary Glioma Stem Cells invasion in the presence of cell-penetrating peptides based on the interaction between connexin43 and c-Src

M. Jaraíz-Rodríguez[1], M.D. Tabernero[2], M. González-Tablas[3], A. Otero[4], A. Orfao[3], J.M. Medina[1] and A. Tabernero[1]

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Connexin43 (Cx43) is the main gap junction channel-forming protein in astrocytes. This protein is downregulated in brain tumours called gliomas. Tumour initiation, relapse, and therapeutic resistance in gliomas is attributed to Glioma Stem Cells (GSCs). Interestingly, several cell-penetrating peptides (CPPs) containing different regions of Cx43 involved in c-Src interaction reverse Glioma Stem Cells (GSCs) phenotype and reduce the rate of cell growth. Considering the controversial Cx43 migration properties and the infiltrative nature of these tumours, we have investigated the role of these CPPs in human primary GSC migration and invasion.
Human primary GSCs were obtained from fresh tumour biopsies and were treated with CPPs. Human GSCs G166 were treated with CPPs. Migration was studied using tiny-tumour cultures, Time-Lapse live-cell Imaging and Immunocytochemistry. Invasion was studied using 8.0 µM pore transwell inserts with or without Matrigel. The mechanism involved in migration was studied by Western blot, evaluating the activity of Focal Adhesion Kinase (FAK).
Our findings indicate that our CPPs reduced the rate of human primary GSCs and G166 GSCs migration and invasion. In addition, CPPs inhibited c-Src activity in these cells and consequently decreased FAK phosphorylation necessary to establish adequate focal adhesions in order to migrate. It should be mentioned that FAK is activated by Src-mediated phosphorylation.
In conclusion, our results show that c-Src plays an essential role in the effects of Cx43 on migration and suggest these CPPs by inhibiting migration and invasion could be the basis for promising therapies.

3. Neural stem cell-derived reactive astrocytes induced by seizures in LPAR1-EGFP mice

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Radial neural stem cells (rNSCs) persist in the hippocampus of most mammals and are able to generate neurons through adulthood, a process known as adult neurogenesis. We have recently discovered that seizures originated in the hippocampus massively activate rNSCs inducing them to switch to symmetric cell division in order to generate reactive astrocytes (RAs), right as they also differentiate into RAs. In addition, the neurogenic program of rNSCs becomes abolished.

Because RAs are crucial in the brain's response to injury as they are proinflammatory and disrupt synaptic transmission, we aim to characterize rNSC-derived RAs and compare them with those RAs differentiated from parenchymal astrocytes in the hippocampus after seizures. For this purpose we resorted to a transgenic line of mice in which the transgene LPAR1-EGFP labels specifically the rNSCs of the adult hippocampus and the rNSC-derived RAs generated after seizures. Interestingly, RAs derived form parenchymal astrocytes do not express LPAR1-EGFP. Thus, this transgenic line is a valuable tool to study the changes in the neurogenic niche after epileptic seizures.

The differential expression of LPAR1-EGFP suggests that indeed the rNSC-derived RAs are different from parenchymal astrocytes-derived RAs, and their contribution to tissue damage/repair might be different. This holds true at least for several weeks after the initial period of seizures, but afterwards LPAR1-EGFP expression is lost in rNSC-derived RAs. Our observations point out to LPAR1 playing a role in the regulation of the hippocampal neurogenic niche in basal and physiopathological conditions and to the existence of a new type of RA in the damaged hippocampus.

4. Aged neural stem cells in the hippocampus

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Adult hippocampal neurogenesis declines with age, mainly due to a progressive and activation-coupled loss of the radial neural stem cells (rNSCs) that give rise to neurons. We herein compare in the detail the dentate gyrus of young (3 month) and older (12 month) mice, with a special focus on NSCs.

We describe how not only the population of NSC declines dramatically, but also there is an accumulation of NSC with a reactive-like multi-ramified phenotype (mNSCs) that get activated to enter the cell cycle with much lower frequency. Whether this phenotype denotes senescence of an intermediate state in astrocytic differentiation remains to be determined. Interestingly, the relative proportion of activated NSC, and the level of re-entry in cell cycle, in the aged mice remains similar, suggesting the existence of intrinsic mechanisms controlling the activation of the NSC population.

In addition, by measuring the distribution rNSCs in the young and aged mice we determined that their loss is not homogeneous, as the remaining NSCs are present in clusters with an internal distance between them similar to those of younger mice, again pointing out to intrinsic mechanisms governing NSC activation/depletion.

Finally, we observed in the aged mice changes in the number of connexin 43-positive gap junctions between NSCs and granule cells; loss of mitotic potential of neuroblasts; and an accumulation of astrocytes(with a reactive-like phenotype).

Together, our results unveil new properties of adult hippocampal NSCs and provide new insight into the mechanisms of aging of the hippocampus and its adult neurogenic niche.

5. Aplicación de células madre mesenquimales en un modelo animal de hidrocefalia congénita con reacciones astrocitarias

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En la hidrocefalia congénita aparecen reacciones astrocitarias periventriculares asociadas con fenómenos neurodegenerativos. Las células madre mesenquimales de la médula ósea (BM-MSC) tienen capacidad de migrar hacia regiones degeneradas, con condiciones isquémicas y neuroinflamatorias, donde pueden producir factores neuroprotectores. En el presente trabajo, esta propiedad ha sido estudiada en relación con las reacciones astrocitarias en un modelo animal de hidrocefalia congénita (ratón hyh).

Se aislaron BM-MSC de la médula ósea de ratones transgénicos que expresan la proteína monomérica fluorescente roja. Previamente a su aplicación, se analizaron las BM-MSC mediante citometría de flujo e inmunofluorescencia con microscopia confocal. Después se inyectaron en el ventrículo lateral de ratones hyh de 20 días de edad, para ser detectadas tras 24/96 horas mediante microscopia confocal y electrónica.

Antes de su aplicación, la mayoría de las BM-MSC expresaban nestina, GFAP, NG2 y el factor de factor neurotrófico derivado de la glía (GDNF). Tras la inyección, las BM-MSC se detectaron recubriendo las superficies ventriculares donde existe una reacción astrocitaria y alrededor de los vasos sanguíneos periventriculares. Las BM-MSC fueron encontradas expresando GDNF, al igual que los astrocitos reactivos, lo cual sugiere una posible capacidad neuroprotectora y tal vez una influencia sobre la propia reacción astrocitaria.

Financiado por FIS PI12/0631-FEDER a AJJ.

6. Circuit-specific signaling in astrocyte-neuron netwroks in basal ganglia pathways

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Astrocytes are non-neuronal cells that are emerging as important regulatory elements in brain function by actively exchanging signals with neurons. They respond to neurotransmitters and release gliotransmitters that modulate synaptic transmission. However, the cell- and synapsespecificity of the functional relationship between astrocytes and neurons in particular brain circuits remains unknown. Here we show that in the dorsal striatum, which mainly comprises two subtypes of intermingled neurons (striatonigral and striatopallidal medium spiny neurons, MSNs) and synapses belonging two distinct neural circuits (the basal ganglia direct and indirect pathways), subpopulations of striatal astrocytes selectively respond to the activity of specific MSN subtypes. In turn, these subpopulations of astrocytes release glutamate that selectively activates NMDA receptors in homotypic, but not heterotypic MSNs. Likewise, subpopulations of astrocytes lead to the selective regulation of homotypic synapses through activation of group I metabotropic glutamate receptors. Therefore, bidirectional astrocyteneuron signaling selectively occurs between specific subpopulations of astrocytes, neurons and synapses, which establish circuit-specific functional astrocyte-neuronal networks.

7. Optogenetic boosting of astrocyte-neuron signaling

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Astrocyte signaling has critical impact on brain physiology by releasing neuroactive substances, so-called gliotransmitters (Araque et al., 2014). However, its study is limited by standard experimental approaches to manipulate astrocyte activity. Recently, optogenetic has provided powerful method to non-invasively activate and control neuronal circuits. Here, we manipulate astrocytes with optogenetic tools to control their activity and evaluate their consequences on neuronal physiology. The ectopic expression of channelrhodopsin-2 (ChR2, a light-activated ion channel protein) was targeted specifically to astrocytes by viral transfection (Perea et al., 2014). Using electrophysiological techniques in brain slices, we found that optical activation of astrocytes enhanced local excitatory synaptic transmission in CA1 hippocampal pyramidal neurons. ChR2-stimulated astrocytes induced sustained potentiation of evoked synaptic responses according with the duration of light stimulation. Neuronal activity to light stimulation was recorded in control slices and no significant changes were observed. The pharmacological analysis indicated that astrocyte-induced modulation of synaptic transmission was mediated by activation of glutamatergic receptors at neuronal membranes. Then, optical activation of astrocytes stimulates glutamate release that controls the synaptic strength of pyramidal neurons influencing the operation of particular circuits.

Supported by MINECO: RYC-2012-12014; CSD2010-00045; BFU2013-47265R.

References:

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Perea G, Yang A, Boyden ES, Sur M. Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. Nat Commun. 2014;5:3262. doi: 10.1038/ncomms4262.

8. Understanding ApoD neuroprotective function: ApoD distribution in pH-dependent sub-domains of the astroglial lysosomal compartment upon metabolic and oxidative stress.

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Apolipoprotein D (ApoD) is expressed in the nervous system, increases with aging and neurodegeneration, and is induced in response to serum deprivation or oxidative stress. To understand how ApoD traffic through different subcellular compartments affects glial cells protecting mechanisms, we analyze the time course of ApoD subcellular traffic upon exposure to low-serum and paraquat stimuli in a human astroglioma cell line.

Confocal microscopy analysis of ApoD localization indicates that secretion is followed by interaction with the plasma membrane, endocytosis via both the caveolin and clathrin-mediated pathways, and location in endosomes. No ApoD is immunodetected in mitochondria or nuclei.

Interestingly, an important fraction of ApoD is targeted to lysosomes. Large LAMP2-ApoD-positive organelles indicate ApoD presence in autophagolysosomes as well, which is confirmed by co-localization with LC-3. ApoD presence inside lysosomes and autophagolysosomes is stable over time, suggesting an active role in lysosomal-autophagy function.

We have developed a method to analyze ApoD distribution differences in lysosomes after *in vivo* measurement of individual lysosomes pH, and find that metabolic or oxidative stress treatments, not only change lysosomal pH, but also the pattern of ApoD distribution within lysosomal populations. This close relationship between lysosomal pH and distribution of ApoD is currently being analyzed under conditions of pharmacological induction or inhibition of autophagy, bringing light on the role of this classically extracellular apolipoprotein in lysosomal performance.

In the light of these new findings, previous hypotheses on ApoD neuroprotective roles in glial cells must be refined: control of plasma membrane and of the lysosome/autophagosome function must be key elements in the function of this lipid-binding protein.

Support:MICINN(BFU2011-23978),JCyL(VA180A11-2).

9. Alzheimer amyloid beta internalization by astrocytes requires clathrin-mediated endocytosis

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Alzheimer's Disease is characterized by the accumulation and deposition of amyloid-beta (Aβ) within the brain. Aβ accumulation depends not only on the rate of its synthesis but also on the rate of clearance. Since astrocytes have being proposed to participate in Aβ clearance from the brain, we decided to study the effects of different Aβ peptides (Aβ 25-35, Aβ 40, Aβ 42) on rat astrocytes in primary culture. Our results showed that all the peptides assayed significantly decreased astrocyte viability while increasing the production of reactive oxygen species (ROS). In order to examine the localization of Aβ within the cells we carried out immunocitochemistry against Aβ. Unexpectedly, we observed that all the Aβ assayed were avidly internalized by astrocytes. To get inside the molecular mechanisms involved in Aβ internalization, we have challenged the astrocytes with inhibitors of endocytosis showing that both phenylarsine oxide (PAO) and chlorpromazine prevented Aβ internalization, a fact consistent with the idea that Aβ is internalized by clathrin-mediated endocytosis.

10. Función de las células microgliales en cultivos organotípicos de retina de ratón postnatal

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La microglía ejerce un papel neurotóxico en algunos procesos neurodegenerativos, pero su efecto también puede ser neurotrófico. En este estudio se ha abordado el análisis del papel de la microglía en cultivos organotípicos de explantes retinianos. Explantes de retina de ratones de 10 días de edad recibieron uno de los siguientes tratamientos: (1) minociclina para bloquear la activación microglial; (2) LPS para incrementar la activación microglial; (3) liposomas cargados con clodronato (Lip-Clo) para reducir el número de células microgliales. Se analizó la viabilidad de las células del explante y la muerte celular mediante citometría de flujo y TUNEL. Las células microgliales se marcaron con técnicas inmunocitoquímicas y de citometría de flujo con diversos anticuerpos, tales como anti-CD11b y antiCD45. La minociclina reducía la activación de la microglía, bloqueaba su migración hacia la capa nuclear interna y provocaba un descenso en la viabilidad de las células del explante, así como un aumento del marcaje con TUNEL. La eliminación de las células microgliales con Lip-Clo también producía una disminución de la viabilidad celular. Por el contrario, el incremento de la activación microglial con LPS no tenía ningún efecto sobre la viabilidad celular. Estos resultados apoyan que la microglía tiene un efecto predominantemente neurotrófico en los explantes de retina cultivados in vitro.

Financiado por: Proyecto de Excelencia P07-CVI-03008 (Junta de Andalucía) y Proyecto BFU2010-19981 (Ministerio de Economía y Competitividad).

11. Neuronal hyperactivity uncouples microglial phagocytosis and leads to delayed cell-clearance

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In physiological conditions, aging, inflammation and excitotoxicity microglial phagocytosis is fast and efficiently coupled to apoptosis in the adult hippocampus, but becomes impaired in a mouse model of epilepsy by intrahippocampal injection of kainic acid (KA). Here we studied the possible mechanisms underlying this uncoupling. To test whether the phagocytic blockade induced by seizures was mediated by the direct effect of KA, we first analyzed the expression of glutamate ionotropic and metabotropic receptor subunits in FACS-sorted microglia. Hippocampal microglia expressed a residual mRNA amount of most subunits, which is unlikely to lead to the formation of functional receptors. In addition, KA had a small effect on microglial phagocytosis in primary cultures and no effect in organotypic cultures, suggesting that the effects of seizures in phagocytosis in vivo are not directly mediated by KA on microglia. Next, we studied the extracellular nucleotide ATP, a well-known "find-me" signal released by apoptotic cells as well as during seizures. We were able to mimic the uncoupling observed in vivo by disrupting ATP gradients in organotypic slices, suggesting that neuronal hyperactivity interferes with "find-me" signals via ATP. Ultimately, microglial phagocytosis impairment leads to the accumulation of non-phagocytosed apoptotic cells and correlates with the development of an inflammatory response in vivo. These results suggest that the impairment of microglial phagocytosis contributes to the early pathophysiology of epilepsy and possibly other neurodegenerative and neurological disorders characterized by neuronal death and inflammation.

12. Microglial phagocytosis is impaired in chronic mouse and human MTLE and correlates with inflammation

Sol Beccari[1,2], Oihane Abiega[1,2], Irune Diaz-Aparicio[1,2], Laura Zaldumbide[3], Lara Galbarriatu[3], Ainhoa Marinas[3], Mirjana Maletic-Savatic[4], Carlos Matute[1,2], Juan M. Encinas[1,2,5], Amanda Sierra[1,2,5].

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In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. We have observed that during aging, inflammation, and excitotoxicity, microglia responded to the increase in apoptosis by adjusting proportionally their phagocytosis. Conversely, in a mouse model of mesial temporal lobe epilepsy (MTLE) by intrahippocampal administration of kainic acid (KA), microglial phagocytosis was reduced as early as 6 hours after injury, and continued to be impaired in the long term. Importantly, this phagocytic blockade led to the accumulation of non-phagocytosed apoptotic cells, and contributed to the development of an inflammatory response. Unexpectedly, in the subacute phase (3-7 days) of MTLE, microglia showed a hypertrophic, seemingly amoeboid morphology that was related to the cells becoming multinucleated. Further, we also detected some cases of phagoptosis or engulfment or non-apoptotic cells. In later stages (4 months) of MTLE, microglial phagocytosis remained impaired. Importantly, the microglial phagocytosis impairment was observed in human hippocampal tissue from MTLE patients. In the human tissue, we found the same kind of phagocytosis observed in the mouse brain by terminal or en passant branches of microglia. In addition, we observed a unique type of phagocytosis in which several microglial nuclei formed a surrounding the apoptotic cell, in an aster-like structure. These results demonstrate that the impairment of microglial phagocytosis is a novel mechanism contributing to the pathophysiology of epilepsy.

13. Deterioro de la respuesta inflamatoria glial en el ratón modelo de senescencia SAMP8

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La neuroinflamación crónica asociada al envejecimiento contribuye al desencadenamiento y progresión de enfermedades neurodegenerativas. El ratón de senescencia acelerada SAMP8 muestra neuroinflamación junto con pérdida cognitiva. El objetivo es analizar la respuesta inflamatoria de células microgliales y astrocitos de ratones SAMP8 en comparación con la cepa control SAMR1. *In vitro* se han estudiado cultivos gliales mixtos y de microglía en respuesta a lipopolisacárido/interferón-γ (LPS/IFN). *In vivo* se ha estudiado la respuesta a LPS a 6 y 12 meses de edad. Los niveles de las citoquinas proinflamatorias IL6, IL1β y TNFα se determinaron mediante ELISA, su expresión génica por qRT-PCR y la generación de óxido nítrico (NO) mediante el método de Griess. Los cultivos mixtos y de microglía de SAMP8 mostraron mayor producción de citoquinas y NO en respuesta a LPS/IFN, y un aumento de NO en microglía en condiciones basales, indicando alteraciones de mecanismos inflamatorios gliales. El tejido cerebral de SAMP8 de 6 meses mostró mayores niveles de citoquinas que SAMR1 y el LPS exacerbó la respuesta inflamatoria. En SAMP8 de 12 meses se detectó una disminución de los marcadores inflamatorios y menor activación frente a LPS. Estos resultados sugieren que la neuroinflamación crónica temprana en el cerebro de SAMP8 depende de glía hiperactivada y que la respuesta inflamatoria a estímulos proinflamatorios externos se muestra afectada en edad de avanzada senescencia. Alteraciones en los mecanismos inflamatorios gliales asociados al envejecimiento disminuyen los mecanismos de defensa a lesiones externas contribuyendo a la fragilidad y la neurodegeneración.

Agradecimientos: CSD2010-00045 y SAF2012-39852, MINECO, y ERDF