INSTITUTO DE BIOLOGIA CELULAR Y NEUROCIENCIA
Laboratorio de Neuropatología Molecular
Facultad de Medicina - Universidad de Buenos Aires

Buenos Aires, July 13th of 2015

International Society for Neurochemistry
Committee for Aid and Education in Neurochemistry (CAEN)

Dear Sirs,

I want to acknowledge for this great opportunity that allows me to visit Dr Felipe Court laboratory at the Pontificia Universidad Católica de Chile in Santiago, during May and June of this year, to develop the project "*Characterization of exosomes released by reactive astrocytes and their impact in neuronal survival and glial activation*".

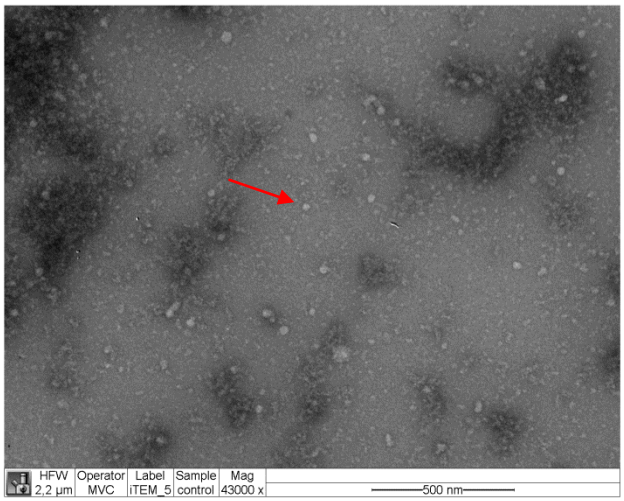
I started in the laboratory on May 18th. During mi first week, I made primary culture of cortical astrocytes and microglia from rat prenatal pups of 5 days, for perform my experiments.

Once astrocytes reached confluency in T75 flasks, they were subjected to different conditions, which consisted of exposing astrocytes to bacterial LPS to induce reactive gliosis converting them in a proinflammatory phenotype; or a control condition without treatment. After 16 hours, the medium was changed to DMEM complete EXO FREE, and astrocytes were incubated at 37°C for 48 hours. At this point the extraction and purification of exosomes was performed.

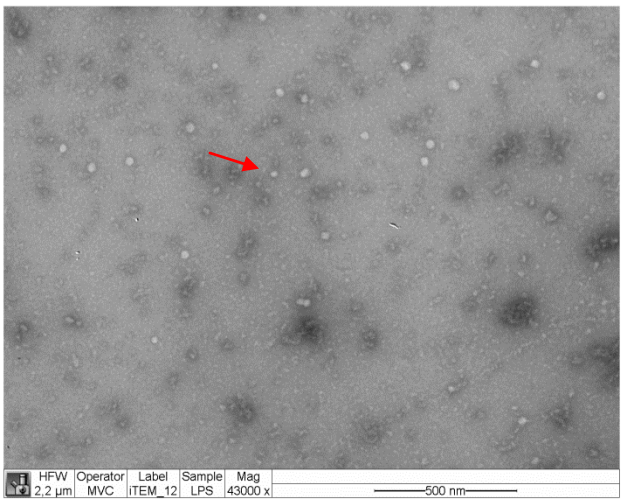
The results showed that a higher concentration of exosomes was obtained from those astrocytes previously treated with LPS, compared to controls that did not receive any stimulus.

Then, exosomes were characterized by negative staining Electron Microscopy to confirm that the morphology and size of those corresponded to the previously observed in the literature. The term exosome was adopted in the late 1980s for small (30-100nm) vesicles of endosomal origin (Johnstone et al. 1987). Exosomes observed by cryo-EM have round shape (Conde-Vancells et al. 2008, Raposo & Stoorvogel 2013).

Control exosomes



LPS exosomes



In the next experiment, the astroglial exosomes obtained from control or LPS-exposed astrocytes, were used to treat naïve primary cultures enriched in astrocytes. After 48 hours, astrocytes were characterized under two main criteria: morphology and astroglial activation by inmunofluorescence (GFAP overexpression, NFkB activation).

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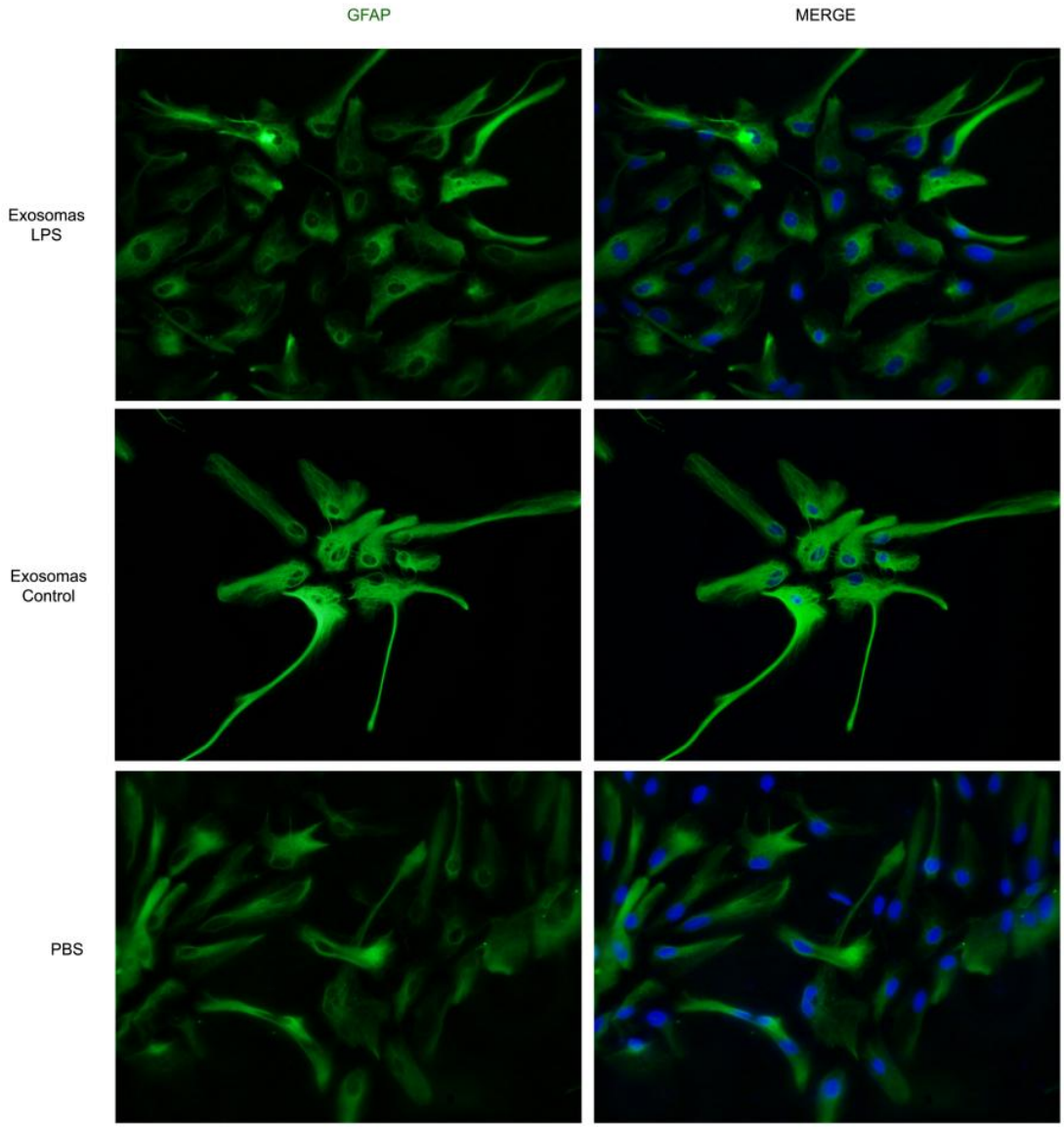

We also analyzed the effects of astroglial exosomes on microglia, specifically looking for changes to the M1 (proinflammatory) or M2 (anti-inflammatory) phenotype of primary cultures enriched in microglia.

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After the visit I brought the tissue culture slides back to my home lab to analyze them by immunocytochemistry and image analysis. I am presently working on the quantification of the morphological alterations observed in microglia and astrocytes treated with the exosomes.

During this short visit I could learn different techniques including my main objective that was to get training in the purification of exosomes from primary cultures of reactive or naïve astrocytes. These preliminary results are important for my thesis project but also will generate a long lasting collaboration between the Court lab and my home lab. The results obtained from this short visit are the initial steps to enrich the prior knowledge about the astroglial cell biology and physiology under phatological conditions in the CNS, where the glial cells conversion to a proinflammatory phenotype is detrimental for neuronal survival, like cerebral ischemia and others neurophatologies.

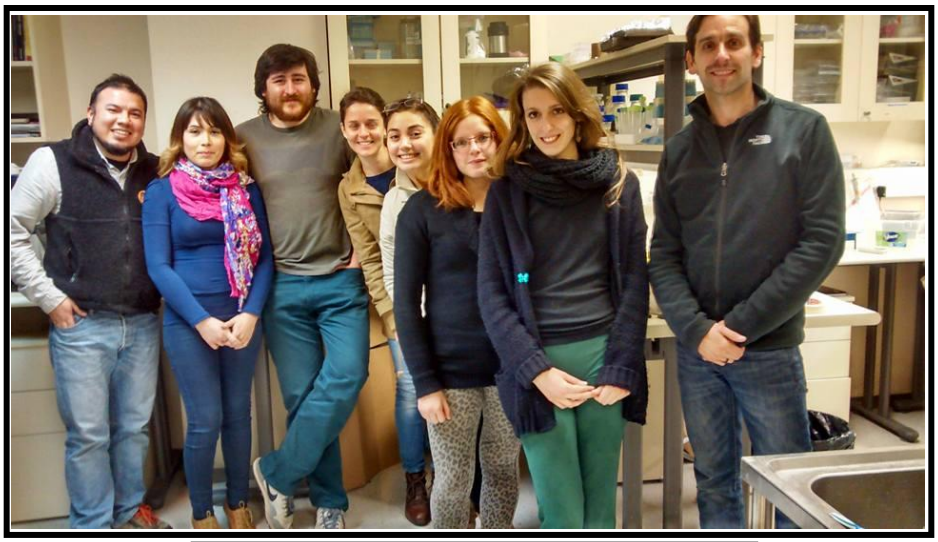
We hypothesize that reactive astrocytes secrete exosomes that can activate both microglia and astroglial population, and this may be related to changes in the content and/or the number of astroglial exosomes released. This would support the idea that exosomes could be important mediators of intercellular communication (Raposo et al. 1996, Zitvogel et al. 1998).

Once again I would like to acknowledge the ISN for this scholarship and this big opportunity, the good predisposition of the host laboratory for welcoming me and my home laboratory for the support.

Please do not hesitate to contact me if you need any additional information.

Best wishes,

Lic. M. Vanesa Cadena



From right to left, I am the second person.