View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repositorio Institucional Universidad de Málaga

CERTIFICATE OF PRESENTATION

This is to certify that:

Mar Fernández-Arjona

has participated with the POSTER titled:

Neuraminidase-induced neuroinflammation is largely dependent on microglial TLR4 receptor

Author(s): **M. Fernández-Arjona, J.M. Grondona, P. Fernández-Llebrez, M.D. López-Ávalos**

during the **18th National Meeting of the Spanish Society of Neuroscience**
held in Santiago de Compostela (Spain),
from 4th to 6th of September, 2019.

DIGITALLY SIGNED DOCUMENT

Agnès Gruart

Chair of the Organizing Committee
President of the Spanish Society of Neuroscience

PS2-97

NEURAMINIDASE-INDUCED NEUROINFLAMMATION IS LARGELY DEPENDENT ON MICROGLIAL TLR4 RECEPTOR

M. Fernández-Arjona [1], J.M. Grondona [1], P. Fernández-Llebrez [1], M.D. López-Ávalos [1]
[1] Facultad de Ciencias, Universidad de Málaga, Málaga, Spain
[1] Instituto de Investigación Biomédica (IBIMA), Málaga, Spain

The sialidase neuraminidase (NA) cleaves terminal sialic acid from glycoproteins and glycolipids. Among its various locations, it is present in the envelope/membrane of some bacteria/viruses (e.g. influenza virus), where it is involved in infectiveness and dispersion. The injection of NA within the brain lateral ventricle represents a model of acute sterile inflammation. The relevance of the toll-like receptors TLR2 and TLR4 (particularly those in microglial cells) in such process was investigated using mouse strains deficient in these receptors. In septofimbria and hypothalamus, IBA1-positive and IL-1β-positive cell counts increased after NA injection in wild type (WT) mice. In $TLR4^{-/-}$ mice such increases were largely abolished, while only slightly affected in $TLR2^{-/-}$ mice. Similarly, the NA-induced expression of IL-1β, TNFα and IL-6 (evaluated by qPCR) was completely blocked in $TLR4^{-/-}$ mice, and only partially reduced in $TLR2^{-/-}$ mice. Microglia was isolated from the three mouse strains and exposed to NA or to specific TLR2 and TLR4 agonists (Pam3CSK4 and LPS respectively) *in vitro*. NA induced a cytokine response (IL-1β, TNFα and IL-6) in WT microglia, but was unable to do so in $TLR4^{-/-}$ microglia; TLR2 deficiency partially affected the NA-induced microglia response. To investigate if such response of microglial cells to NA was dependent on the sialidase activity of the enzyme, WT microglia was exposed *in vitro* to NA previously inactivated with heat, or inhibited with two different sialidase inhibitors (oseltamivir phosphate and N-acetyl-2,3-dehydro-2-deoxyneuraminic acid). In all cases, NA-induced microglia activation was dependent on the intact sialidase activity of NA. Therefore, we conclude that NA is able to directly activate microglial cells, mostly through TLR4 receptor and due to its sialidase activity. Accordingly, the inflammatory reaction induced by NA *in vivo* is partially dependent on TLR2, while TLR4 plays a crucial role.

Neuraminidase-induced neuroinflammation is largely dependent on microglial TLR4 receptor

Mar Fernández-Arjona, Jesús M. Grondona, Pedro Fernández-Llebrez, María Dolores López-Ávalos

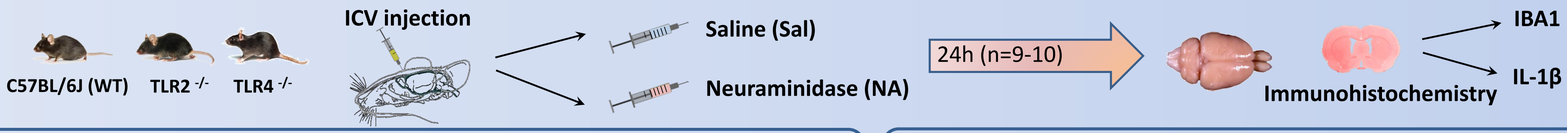
Departamento de Biología celular, Genética y Fisiología, Universidad de Málaga
Instituto de Investigaciones Biomédicas de Málaga-IBIMA

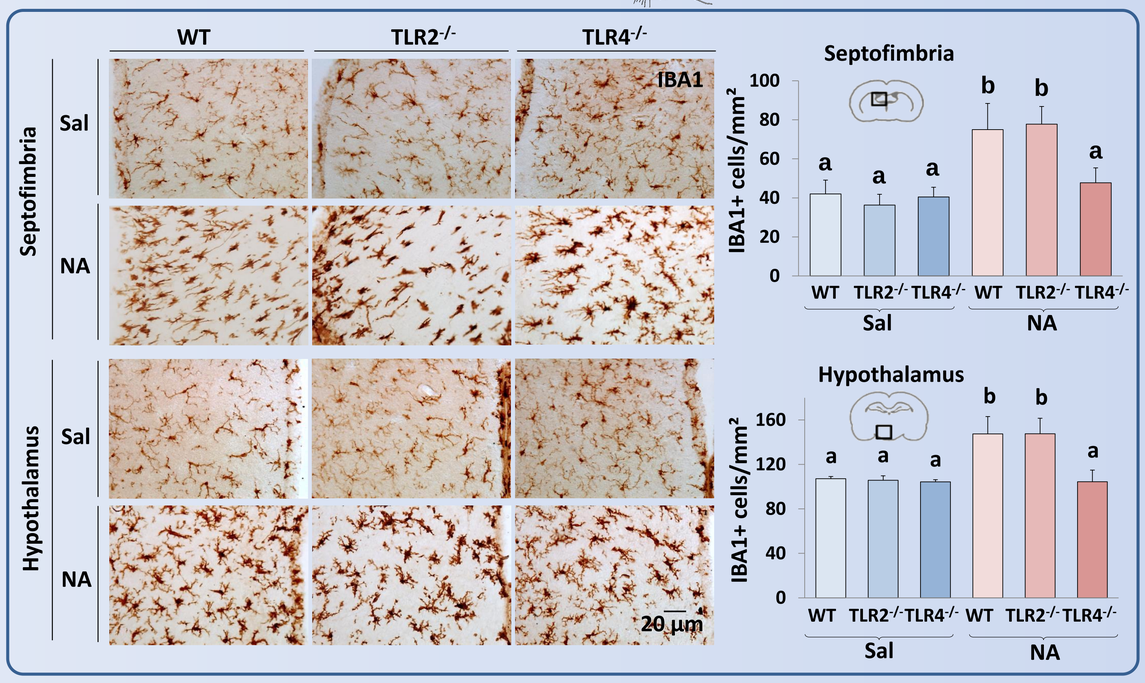
marfernandez@uma.es

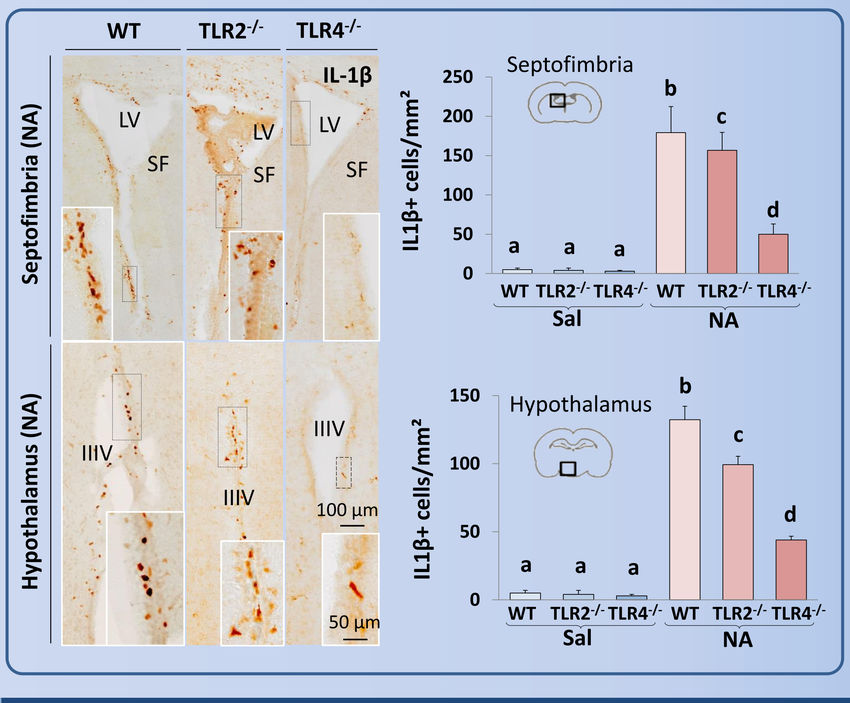
18TH NATIONAL MEETING OF THE SPANISH SOCIETY OF NEUROSCIENCE
SANTIAGO DE COMPOSTELA
4 - 6 SEPTEMBER, 2019

The sialidase neuraminidase (NA) cleaves terminal sialic acid from glycoproteins and glycolipids. Among its various locations, it is present in the envelope/membrane of some bacteria/viruses (e.g. influenza virus), where it is involved in infectiveness and dispersion. The injection of NA within the brain lateral ventricle represents a model of acute sterile inflammation. The relevance of the toll-like receptors TLR2 and TLR4 (particularly those in microglial cells) in such process was investigated using mouse strains deficient in these receptors.

1. IBA1 (+) and IL-1β (+) cells counts after NA injection, in septofimbria and hypothalamus





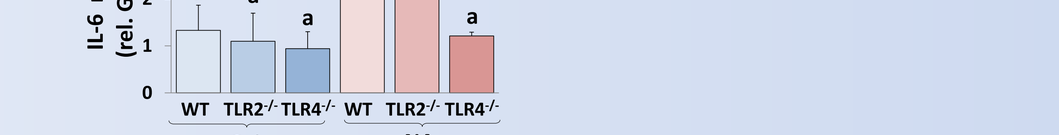


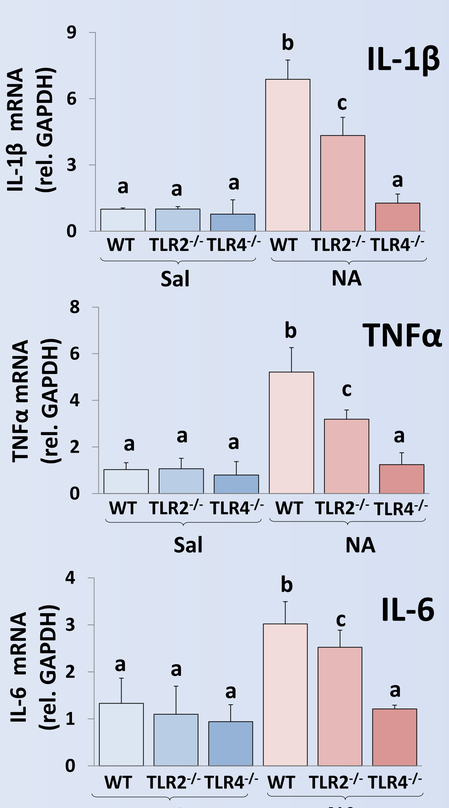
IBA1 (+) and IL-1β (+) cells increase in wild type mice…

…while in TLR4$^{-/-}$ mice such increases are largely abolished,…

… and only slightly affected in TLR2$^{-/-}$ mice.

2. Citokine expression in hypothalamus after NA injection



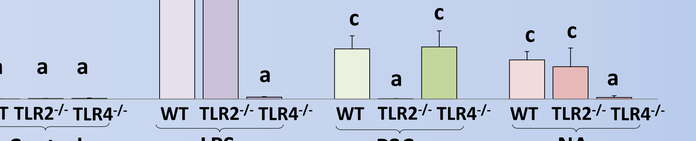


IL-1β, TNFα and IL-6 expression increase in WT mice…

… while in TLR4$^{-/-}$ mice such increases are completely blocked,…

… and only partially reduced in TLR2$^{-/-}$ mice.

3. *In vitro* stimulation of microglia with NA and TLR2/TLR4 agonists

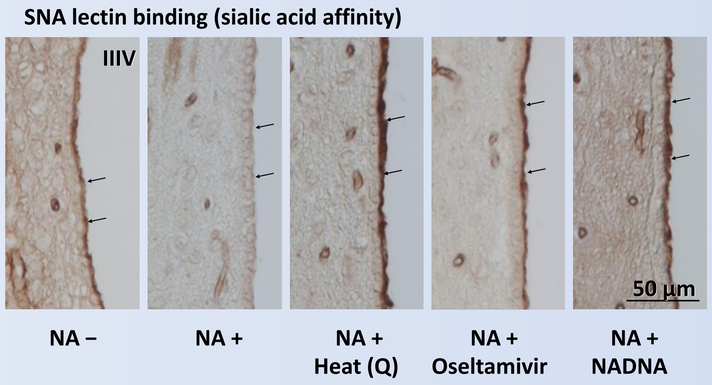


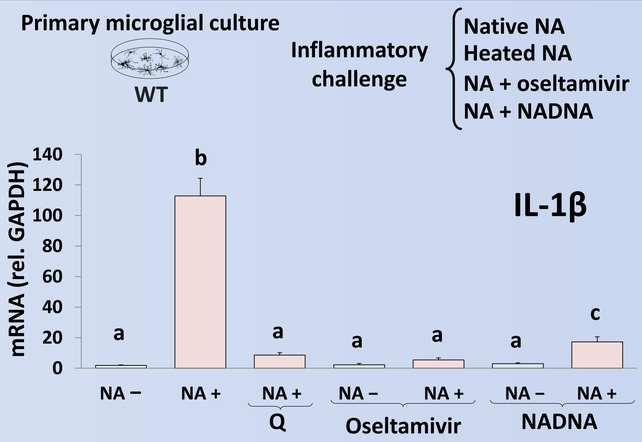
NA induced a cytokine response in WT microglia,…

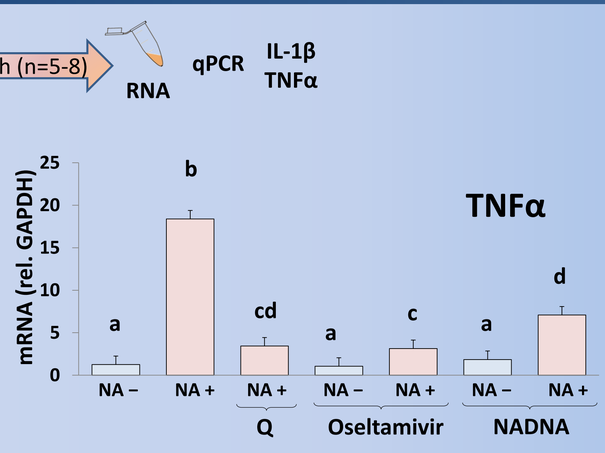
…but was unable to do so in TLR4$^{-/-}$ microglia,…

…while TLR2$^{-/-}$ microglia was partially activated by NA.

4. Microglia response to inactivated / inhibited NA







NA-induced microglia activation is dependent on the sialidase activity of NA

5. Conclusions

1. **The inflammatory reaction induced by NA *in vivo* is partially dependent on TLR2, while TLR4 plays a crucial role.**
2. **Neuraminidase is able to directly activate microglial cells, mostly through TLR4 receptor.**
3. **The sialidase activity of NA is critical for NA-induced microglial activation.**

Grants:
Junta de Andalucía (Spain) P11-CVI-07637
MINECO (Spain) SAF2017-83645