Experimental Neurology 310 (2018) 14–21

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research paper

# Dephosphorylated rather than hyperphosphorylated Tau triggers a pro-inflammatory profile in microglia through the p38 MAPK pathway

Juan Ramón Perea[a,b], Jesús Ávila[a,b], Marta Bolós[a,b,*]

[a] Department of Molecular Neuropathology, Centro de Biología Molecular "Severo Ochoa", CBMSO, CSIC, Madrid, Spain
[b] Network Center for Biomedical Research on Neurodegenerative Diseases (CIBERNED), Madrid, Spain

ARTICLE INFO

*Keywords:*
Alzheimer's disease
Tau
Microglia
Inflammation
Mitogen-activated protein kinase (MAPK)

ABSTRACT

Tauopathies are a broad set of neurodegenerative dementias characterized by the aggregation of Tau protein. Activated microglia and elevated levels of pro-inflammatory molecules are also pathological hallmarks of tauopathies. In these diseases, intracellular Tau is secreted to the extracellular space, where it interacts with other cells, such as neurons and glia, promoting inflammation. However, the mechanism through which extracellular Tau triggers pro-inflammatory responses in microglia remains unknown. Primary microglia cultures were treated with extracellular Tau in its hyperphosphorylated, dephosphorylated or non-phosphorylated form. Protein cytokine arrays, real-time PCR, inhibition of the p38 MAPK pathway, phosphatase assays, and quantification of proteins through immunoblotting were used to analyze the effect of extracellular Tau on the pro-inflammatory response of microglia. The main finding of this work is that extracellular non-phosphorylated and dephosphorylated forms of Tau, rather than hyperphosphorylated Tau, activate the p38 MAPK pathway in microglia, thus triggering a pro-inflammatory response in these cells.

## 1. Introduction

The abnormal accumulation of intracellular hyperphosphorylated Tau protein is the common pathological hallmark of a set of neurodegenerative diseases collectively referred to as tauopathies (Medina et al., 2016). Alzheimer's disease (AD) is the most common form of tauopathy. AD is a fatal neurodegenerative disorder characterized by progressive cognitive and functional impairment, and memory loss (Whitehouse et al., 1982). In the AD brain, Tau is hyperphosphorylated, meaning that it is phosphorylated to a higher degree at physiological sites, as well as at pathological sites (Bussiere et al., 1999; Hasegawa et al., 1996; Hoffmann et al., 1997; Jicha et al., 1997). Together with amyloid-β (Aβ) plaques, Tau aggregates (neurofibrillary tangles (NFTs)) are the defining histopathological hallmarks of AD (Selkoe et al., 1982; Tomlinson et al., 1970).

Recent research has shown that the most toxic form of Tau is not NFTs themselves, but rather the smaller aggregates, called Tau oligomers, which are likely to initiate neurodegeneration in tauopathies (Andorfer et al., 2005; Cowan and Mudher, 2013; Fox et al., 2011; Ishihara et al., 1999; Ishihara et al., 2001; Le Corre et al., 2006; Spires-Jones et al., 2009; Wittmann et al., 2001). Intracellular Tau can be released into the extracellular space (Perez et al., 2016; Yamada et al., 2014) and can affect neighboring cells, such as neurons (Gomez-Ramos et al., 2006) and glia (Bolos et al., 2015; Leyns and Holtzman, 2017). In this regard, we have recently shown that extracellular soluble Tau, mainly composed of monomers and dimers, has devastating effects on the structural plasticity of hippocampal granule neurons (Bolos et al., 2017b). This finding thus supports the notion that impaired clearance of extracellular Tau contributes to the progression of AD (Alonso et al., 2016; Hyman, 2014; Medina and Avila, 2014a, 2014b; Mirbaha et al., 2015).

Microglia are the resident immune cells in the brain, and they participate in neuroprotection and the maintenance of homeostasis (Bolos et al., 2018; Ransohoff and El Khoury, 2016). Under pathological conditions, microglia are activated and become ramified. These cells have the capacity to proliferate, migrate, and efficiently phagocytose pathogens and cellular debris (Aloisi, 2001; Diaz-Aparicio et al., 2016; Kreutzberg, 1996; Sierra et al., 2014). Furthermore, activated microglia can release a host of pro-inflammatory cytokines, including interleukin (IL) 1-beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-6, IL-18, and interferon gamma (IFN-γ), among others (Gonzalez-Scarano and Baltuch, 1999; Hanisch, 2002; Ransohoff, 2016).

Mitogen-activated protein kinases (MAPKs) are members of specific signaling cascades that serve as convergent points for numerous and diverse extracellular signals and are thus critical integrators of signaling events (Arthur and Ley, 2013; Bradham and McClay, 2006). Recent

* Corresponding author at: Department of Molecular Neuropathology, Centro de Biología Molecular "Severo Ochoa", CBMSO, CSIC, Madrid, Spain.
*E-mail address:* mbolos@cbm.csic.es (M. Bolós).

https://doi.org/10.1016/j.expneurol.2018.08.007
Received 27 March 2018; Received in revised form 4 July 2018; Accepted 15 August 2018
Available online 20 August 2018
0014-4886/ 

years have witnessed increasing interest in p38 MAPKs (p38) in the AD research field. Given their involvement in inflammation and cell death pathways, these kinases may contribute to the pathogenic events that occur in the AD brain (Correa and Eales, 2012; Sun et al., 2003; Wang et al., 2014; Zhu et al., 2002). Several studies report the relationship between Aβ 1–42, mainly oligomers, and p38 activation in microglia (Bachstetter et al., 2011; Lee and Kim, 2017). The increased levels of oligomeric Aβ in the AD brain may activate signaling cascades that increase p38 activity (Adolfsson et al., 2012). However, the effect of Tau on p38 activation and its contribution to microglia-mediated inflammation remain unknown.

Here we show that extracellular Tau modulates the pro-inflammatory profile of microglia by regulating p38 activity in these cells. Furthermore, this effect was produced only when Tau was dephosphorylated, while it was absent when this protein was hyperphosphorylated.

## 2. Results

### 2.1. p38 MAPK is a target of Tau in microglia

We previously reported that extracellular Tau is internalized by microglia (Bolos et al., 2015; Bolos et al., 2017a). However, the effect of Tau on these cells remains unclear. In AD, which is also considered as an inflammatory disease (Bolos et al., 2017c; McGeer et al., 1996), Aβ activates p38 (Culbert et al., 2006; McDonald et al., 1998). Therefore, we studied whether Tau modulates p38 expression in these cells. To this end, primary microglia cultures were treated with Tau derived from *E. coli* (non-phosphorylated (npTau)) at 1 μM or vehicle (phosphate saline buffer (PBS)) for 0, 30, 120, or 240 min. The phosphorylation of p38 in Thr180/Tyr182 residues, which implies kinase activation, was analyzed as (p-p38/GAPDH)/ (p38/GAPDH). An increase in phosphorylated p38 (p-p38) was observed after 30 min of treatment with non-phosphorylated Tau (npTau) (Fig. 1A–B). In contrast, this effect was not replicated when cells were treated with PBS under the same conditions (Fig. 1A–B). Although there was an increase in p-p38 after treatment with npTau at the other times tested, the differences, as shown by 2-way ANOVA, were not statistically significant when compared with the control cells treated with PBS. Therefore, 30 min of treatment was used in the rest of the experiments.

To study whether this effect was replicated *in vivo*, 2-month-old C57Bl/6 mice were stereotaxically injected with npTau or PBS (Fig. 1C). After 2 weeks, the amount of p-p38, analyzed following the same protocol as for *in vitro* experiments, was increased in the brain of these mice compared to control ones injected with PBS (Fig. 1D–E). However, to confirm that the activation of p38 was an effect mediated by Tau specifically in microglial cells, which was the main objective of this work, we performed the rest of the experiments using an *in vitro* model of microglial primary cultures because the tissue derived from brain contains other types of cells.

### 2.2. Hyperphosphorylated Tau has no effect on p38 in microglia

In tauopathies such as AD, Tau is hyperphosphorylated (Grundke-Iqbal et al., 1986; Medina and Avila, 2014a). Due to the importance of this posttranslational modification of this protein in these diseases, the activation of p38 (p-p38) in microglia was tested in the presence of hyperphosphorylated Tau (hypTau), which was isolated from sf9 cells (Gomez-Ramos et al., 2004). We observed that npTau activated p38 in microglia, as described above (Fig. 2A–B). However, p38 was not activated in the presence of hypTau. Therefore, we next examined the difference, in terms of phosphorylation, between the two forms of Tau using the PHF-1 antibody (which recognizes S396/S404 phosporylated residues of Tau) (Fig. 2C). The western blot showed that there was no

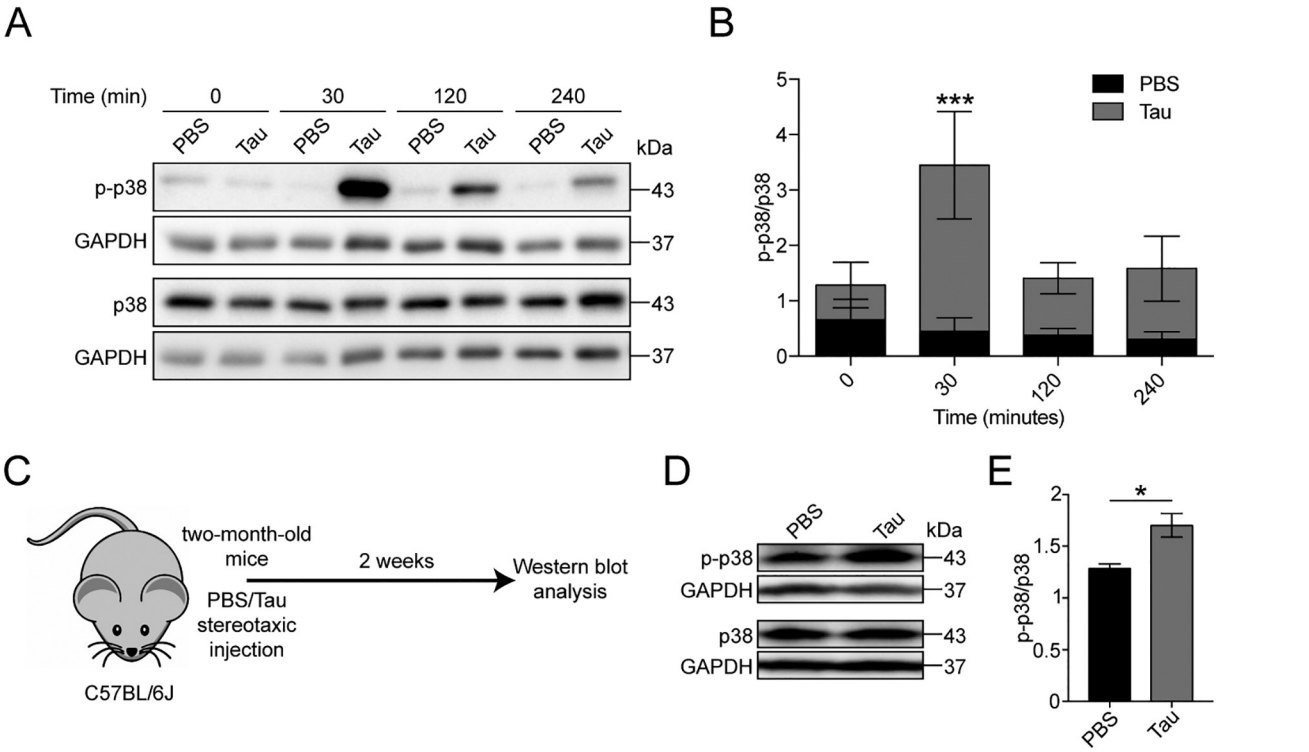


**Fig. 1.** Treatment with Tau activates p38 *in vitro* and *in vivo*. Representative western blot (A) and quantification (B) of the time course of p38 activation from 0 to 240 min. Microglia were treated with PBS (control) or non-phosphorylated Tau (npTau) for different times, and phosphorylated p38 (p-p38) was analyzed as a measure of activation of the kinase. Note that after 30 min of treatment with Tau, microglia showed an increase in p-p38 compared with those treated with PBS. An $N = 4$ independent experiments were performed. (C) Experimental design of *in vivo* experiments. Representative western blot (D) and quantification (E) of p38 activation in the tissue of injected mice. Bars show means ± S.E. $*p \leq 0.05$, $***p \leq 0.001$.