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Modulation of LPS-induced memory insult, γ -secretase and neuroinflammation in 3xTg mice by 5-Lipoxygenase

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Abstract

Besides amyloid and tau pathology, a constant feature of Alzheimer's disease (AD) is an intense inflammatory response, which is considered an active player in its pathogenesis. The 5-Lipoxygenase (5LO) is a proinflammatory enzyme and an endogenous modulator of AD-like phenotype in mouse models of the disease. To further understand the role of 5LO in AD pathogenesis, we exposed the 3xTg and 3xTg/5LO knockout mice to lipopolysaccharide (LPS), a known inducer of neuroinflammation, and evaluated its effect on their AD-like phenotype.

3xTg mice treated with LPS manifested a worsening of behavior, γ -secretase up-regulation, and increased neuroinflammatory responses. These effects were completely prevented in 3xTg mice genetically deficient for 5LO. By contrast, the absence of 5LO did not protect against increase in tau phosphorylation at specific epitopes that were mediated by the activation of the cyclin-dependent kinase 5.

Our data demonstrate that the 5LO pathway affects key neuropathological features of the AD-like phenotype (behavior, A β , microgliosis, astrogliosis) but not others (tau pathology) in the LPS-dependent neuroinflammation model.

The opposite ways whereby 5LO influences the LPS-dependent effects in vivo supports the complex nature of the neuroinflammatory response in AD and its differential role in modulating amyloid and tau neuropathology.

Keywords

Alzheimer's disease; Transgenic mouse models; Amyloid beta; LPS; Behavior; Neuroinflammation

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose

AUTHOR CONTRIBUTIONS

Y. B. J. and D.P. designed the study, developed the experimental design, performed data analysis and wrote the paper.

Y.B.J., P.F.G., and J.C. performed the experiments.

All authors discussed the results and commented on the manuscript.

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1. INTRODUCTION

Alzheimer's disease (AD) is the most common aging-associated neurodegenerative dementia. While the hallmark pathology of AD includes accumulation of amyloid- β plaques and neurofibrillary tau tangles, inflammation is also a constant characteristic of the AD brain. Astrocytosis and microgliosis follow progression of AD pathology, and elevation of pro-inflammatory cytokines such as interleukin 1 (IL-1) is also seen in AD patients (Sheng et al. 1997a, Sheng et al. 1997b, Griffin et al. 1989). Several reports have shown significantly higher amounts of bacterial and viral genetic material in the brains of AD patients compared to controls, and infection with mouse hepatitis virus in the triple transgenic (3xTg) mouse model of AD results in acceleration of AD pathology (Hammond et al. 2010, Balin et al. 1998, Itzhaki et al. 2008, Kountouras et al. 2006, Sy et al. 2011). This evidence suggests that inflammatory states accelerate the development of the AD phenotype.

Several pro-inflammatory enzymes have been linked to AD, among which is the 5-lipoxygenase protein (5LO). 5LO uses arachidonic acid as substrate to produce inflammatory leukotriene metabolites, which then can modulate increased vascular permeability as well as immune cell adhesion and activation (Radmark and Samuelsson, 2010). 5LO is elevated in AD brains compared to age-matched controls, and aging increases 5LO expression in several areas of the brain including cortex and hippocampus (Firuzi et al., 2008; Chinnici et al., 2007; Ikonovic, et al. 2008). We have previously reported that 5LO is an endogenous modulator of both A β production and tau phosphorylation (Chu and Pratico, 2011; Chu and Pratico, 2013; Chu et al., 2012). Pharmacological inhibition of 5LO in an AD mouse model of amyloidosis results in fewer plaques as well as attenuation of learning and memory deficits (Chu and Pratico, 2011). However, it is not known whether 5LO plays a role in the inflammation-induced worsening of the AD-like phenotype. To address this question we challenged 3xTg and 3xTg mice genetically deficient for 5LO (3xTg/5LOKO) with a chronic course of lipopolysaccharide (LPS) injection. Chronic peripheral LPS injection in transgenic AD animals closely simulates inflammatory states caused by infection, which are able to modulate AD brain pathology (Sy et al. 2011). We found that 3xTg animals treated with LPS had impaired fear-conditioned contextual recall which was not seen in 3xTg/5LOKO animals. 3xTg animals treated with LPS did not have significantly greater amyloid β levels than controls, however we found that LPS significantly elevated the presenilin-1 and presenilin-enhancer 2 components of the γ -secretase, suggesting a misprocessing of APP. 3xTg animals without 5LO had lower A β levels than control 3xTg animals. Additionally, 3xTg animals lacking 5LO also resisted LPS-mediated elevations in γ -secretase protein levels. By contrast, compared with controls, knockout of 5LO did not protect 3xTg animals against LPS-mediated hyper-phosphorylation of tau. Finally, LPS induced astrocytosis and microgliosis and a significant up-regulation of inflammatory cytokines in 3xTg mice, but it failed to do so in 3xTg/5LOKO.

2. METHODS

2.1 Animals

All animal procedures were approved by the Institutional Animal Care and Usage Committee, in accordance with the U.S. National Institutes of Health guidelines and approved by the Temple University's Animal Care and Use Committee. The 3xTg mice used in this study contain a mutant amyloid precursor protein (APP; KM670/671NL), a human mutant PS1 (M146V) knockin, and tau (P301L) transgenes; 3xTg, and mice genetically deficient for 5LO (5LOKO) were crossed as previously described to create 3xTg/5LOKO mice (Oddo et al., 2003; Goulet et al., 1994). Naïve male 3xTg and 3xTg/5LO $^{-/-}$ mice aged 6 months were used for this study. All mice were housed on a 12 hours

light/dark cycle in the Medical Research Building at the Temple University Health Sciences Campus, which is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Standard mouse chow and water were provided *ad libitum*. Animals were intra-peritoneally either given phosphate-buffered saline (PBS) as control, or lipopolysaccharide (LPS; *Escherichia coli* 055:B5; Sigma, St. Louis, MO) at a dose of 0.5mg/kg, twice a week for 6 weeks (n=4, 3xTg (PBS), n = 5 3xTg (LPS), n = 4 3xTg/5LO-/- (PBS), n = 6 3xTg/5LO-/- (LPS)).

2.2 Behavioral paradigms

Following treatment with PBS or LPS, all animals were assayed in the Y-maze and fear conditioning paradigms. The Y-maze paradigm is a well-published instrument designed to assess working memory, while the fear conditioning paradigm has been used extensively to assess 24hr memory retention. All apparatuses were cleaned with 70% ethanol between animal trials and allowed to dry completely. The Y-maze (San Diego Instruments, La Jolla, CA, USA) and fear-conditioning paradigms (StartFear System, Harvard Apparatus; Holliston, MA, USA) were carried out as previously described (Chu et al., 2012; Joshi and Pratico, 2013). Testing was always performed in the same room and at the same time to ensure environmental consistency.

2.3 Biochemical analyses

Mice were sacrificed and brains were removed following behavioral assessment as previously described (Joshi et al. 2013). Cortices were dissected from one hemisphere and stored at -80° C while the other hemisphere was fixed in paraformaldehyde overnight, processed and paraffin-embedded, and used for immunohistochemistry analyses. Mouse brain cortical homogenates were sequentially extracted first in radio-immunoprecipitation assay buffer (RIPA) containing EDTA-free protease inhibitor (Roche) and phosphatase inhibitor (Thermo Fisher) for the soluble fractions and then in formic acid (FA) for insoluble fractions as previously described (Chu et al., 2012). A β 1–40 and A β 1–42 levels were assayed by a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) kit (Wako Chemicals, Richmond, VA, USA) in accordance to the manufacturer's protocols. Mouse IL-1 β levels were assayed by a sensitive sandwich ELISA kit following manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). Mouse IFN- γ levels were assayed using a protein array (RayBiotech, Norcross, GA, USA) in accordance to the manufacturer's protocols.

2.4 Immunoblotting

Brain homogenate RIPA or pH-neutralized FA samples were electrophoretically separated using 10% Bis-Tris gels or 3% to 8% Tris-acetate gel (Bio-Rad), according to the molecular weight of the target molecule, and then transferred onto nitrocellulose membranes (Bio-Rad). Membranes were blocked with Odyssey blocking buffer and incubated with primary antibodies overnight at 4° C. After 3 washing cycles in TBS-T, membranes were incubated with IRDye secondary antibodies (LI-COR) at 22° C for 1 hour. Signals were developed with Odyssey Infrared Imaging Systems (LI-COR). Actin was always used as an internal loading control. Antibodies and dilutions used for immunoblotting in this study can be found in Table 1.

2.5 Immunohistochemistry

Immunohistochemistry analysis was performed as previously described (Chu et al., 2012). Briefly, 6 μ m brain sections were deparaffinized, hydrated, and blocked with 2% serum. Citric acid used to retrieve antigen. Sections were incubated with primary antibody against HT7 (1:200), AT8 (1:100), AT180 (1:100), PHF1 (1:20), PHF13 (1:100) GFAP (1:100), or Iba1 (1:100) overnight at 4° C. Sections were washed, incubated with appropriate secondary

antibody and finally developed using the avidin-biotin complex method (Vector Laboratories) with 3,3'-diaminobenzidine as chromogen. Images were collected with a Olympus BX51 microscope (Olympus, Japan) with different objective lens connected to a digital color camera and processed to calculate the area occupied by immunoreactivity using the software Image-Pro plus for Windows version 5.0 (Media Cybernetics). The threshold optical density that discriminated staining from background was determined and kept constant for all quantifications. Images were taken at 100x magnification.

2.6 Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean. One-way analysis of variance (ANOVA), with Bonferroni multiple comparison tests were performed using Prism 5.0 (GraphPad Software, La Jolla, CA). All data are presented as mean $\pm$ standard error of the mean. Significance was set at $p < 0.05$.

3. RESULTS

3.1 LPS induces fear-conditioned contextual recall deficits in 3xTg but not 3xTg/5LOKO animals

We administered either saline (PBS) or LPS intraperitoneally (0.5 mg/kg) to 3xTg mice twice a week for six weeks. At the end of treatment, we observed that this treatment did not affect Y-maze behavior, consisting of spontaneous alternation percentage and total entries in all animal groups (Fig 1.A, B). By contrast, in the fear conditioning paradigm, while no differences were detectable in the training phase (data not shown) or cued recall among all the different groups, LPS-injected 3xTg animals demonstrated a significant impairment in the contextual recall compared to saline-injected 3xTg, saline-injected 3xTg/5LOKO animals and LPS-injected 3xTg/5LOKO animals (Fig.1C, D).

3.2 APP misprocessing induced by LPS is not seen in 3xTg/5LOKO animals

We then investigated if A β levels and metabolism were modulated by LPS in the brains of the randomized mice. As shown in Fig. 2A, B, we found that though soluble A β 40 and A β 42 levels tended to increase with LPS in the 3xTg animals, no statistical significance was reached. Similarly, we observed that LPS did not modify A β levels in 3xTg/5LOKO animals, although we found that animals lacking 5LO had lower overall A β levels, a finding which we have previously described (Giannopoulos et al., 2013). Several reports have indicated that LPS modulates A β metabolism (Eriksson *et al*, 2012; Jaeger *et al* 2009; Lee *et al*, 2008; Bitting *et al*, 1996). To see if this was occurring in our system, we probed for components of the A β processing pathway including the A β precursor protein (APP), the alpha-secretase (ADAM10), β -secretase-1 (BACE1) and the γ -secretase complex (PS1, PEN2, APOE, NCT) (22). We found no changes among the different groups in steady-state levels of APP, ADAM10, or BACE1 as shown in Fig. 2C. However, we found PS1 and PEN2 levels significantly increased in 3xTg animals treated with LPS when compared with controls as shown in Fig. 2C, D. By contrast, 3xTg/5LOKO animals treated with LPS did not show any γ -secretase protein elevation. We also probed for levels of apolipoprotein (APOE) and neprilysin (NEP) which putatively clear or degrade A β *in vivo*. As shown in Fig 2C, LPS stimulation or absence of 5LO failed to modulate either target.

3.3 LPS-mediated tau hyperphosphorylation is not affected by the absence of 5LO

In contrast with the rather small effect on A β levels, we found that LPS treatment significantly exacerbated the tau pathology in the 3xTg mice. Steady-state levels of total human tau protein as recognized by the specific antibody HT7 on western blot analysis were comparable among all groups of animals and were not modulated either as a function of LPS

treatment or 5LO knockout status (Fig. 3A, B). However, we observed that immunoreactivity of AT8, which recognizes tau phosphorylated at Ser 202 and Thr 205, and AT180, which recognizes tau phosphorylated at Thr 231 and Ser 235 were significantly higher in mice receiving LPS (Fig. 3A, B). This elevation of AT8 and AT180 by LPS was unaltered by unavailability of 5LO. AT8 and AT180 epitopes were also elevated when the ratio with HT7 total tau immunoreactivity was taken into consideration (AT8: 3xTg+LPS: 1.5, 3xTg/5LOKO+LPS:1.7; AT180: 3xTg+LPS:1.4, 3xTg/5LOKO+LPS: 1.4). Nevertheless, we could not detect any differences between genotype or treatment groups in the steady-state levels of PHF1 immunoreactivity, which recognizes Ser396 and Ser404 tau, or PHF13, which recognizes Ser404 tau (Fig. 3A, B). To investigate whether LPS-mediated increase in tau phosphorylation lead to insoluble tau deposition in our system, we probed the neutralized formic-acid extracted fraction of brain homogenates with the human-specific HT7 antibody. We found that there were no differences in insoluble tau levels among the different animal groups (Fig. 3C). We also probed for HT7, AT8, and AT180 by immunohistochemistry and, as with our immunoblotting results, found increased immunostaining for AT8 and AT180 epitopes in both animals, 3xTg and 3xTg/5LOKO, treated with LPS but not for HT7 (Fig. 3D).

LPS-mediated phosphorylation of tau has been shown to occur through cyclin-dependent kinase 5 (cdk5) in the 3xTg mouse, with LPS causing an increase in the steady-state level of its p25 coactivator. As shown in Fig. 3E, F, LPS elevated levels of p25 in both 3xTg and 3xTg/5LOKO animals without altering either cdk5 or p35 levels. By contrast, LPS did not modulate levels of total or phosphorylated GSK3 β , another important AD tau kinase.

3.4 Knockout of 5LO prevents the LPS-induced neuroinflammatory phenotype

Because LPS produces potent activation of immune cells in the brain and 5LO is a pro-inflammatory enzyme, we next asked how knockout of 5LO modulates LPS-driven brain inflammation in the 3xTg mouse. We found that chronic LPS treatment increased protein levels of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1(Iba1) in the brain, markers of astrocytes and microglia, respectively in 3xTg animals (Fig. 4A,B). GFAP- and Iba1-positive cells were also elevated in the brain parenchyma in 3xTg animals as assessed by immunohistochemistry (Fig. 4C). By contrast, in the brains of animals lacking 5LO, LPS did not change GFAP or Iba1 steady-state protein levels, nor was there an increased astrocytic or microglial burden as seen in 3xTg animals. We also assessed brain levels of interleukin-1 β (IL-1 β) and interferon γ (IFN γ), two major inflammatory cytokines found to be up-regulated in AD patients. As shown in Fig. 4D, we found that while LPS elevated IL-1 β and IFN γ levels in 3xTg animals, it failed to do so in 3xTg/5LOKO animals.

4. DISCUSSION

In the present study we investigated the biological effects of chronic LPS exposure in a transgenic model of AD with plaques and tangle, 3xTg mice, and compared them with the 3xTg mice genetically deficient for 5LO. We found that LPS impaired fear-conditioned contextual recall in 3xTg but not 3xTg/5LOKO mice. While LPS did not significantly change A β levels in either 3xTg or 3xTg/5LOKO, animals without 5LO had lower A β levels than those with 5LO. Additionally, knockout of 5LO prevented LPS-mediated elevation of steady-state levels of PS1 and PEN2 proteins of the γ -secretase complex. Regardless of the availability of 5LO, LPS induced tau hyper-phosphorylation at the AT8 and AT180 epitopes, which was secondary to a selective activation of the cdk5 kinase pathway. However, this phosphorylation did not result in a greater level of PHF1 or PHF13 tau phosphorylation or elevation in the formic acid-soluble tau fraction. Finally, we found that LPS injection induced a substantial neuroinflammatory response characterized by

astrocytosis, microgliosis and elevation of IL1- β and IFN γ in the 3xTg mice, which was blunted in the 3xTg/5LOKO animals.

Taken together, our findings support the idea of a differential regulation of the main features of the AD-like phenotype secondary to LPS-induced neuroinflammation. In recent years it has become more and more evident that neuroinflammation is an important component of the AD neuropathology, even though its precise role in the pathogenesis of the disease is still under debate. Thus, while reactive microglia cells presumably have a beneficial role by clearing amyloid deposits, anti-inflammatory drugs significantly reduce the risk of AD (Bard et al., 2000; Breitner et al., 2011). In addition, other studies have shown that inflammation worsens the development of tau pathology (Sy et al., 2011, Kitazawa et al., 2005; Ghosh et al., 2013).

In the current paper, we observed that while 5LO genetic absence significantly blunted the effect of LPS on neuroinflammatory biomarkers, behavior and APP processing, it failed to protect against LPS-mediated tau hyper-phosphorylation. Considering the tight connection reported by others between inflammatory stimuli and tau phosphorylation, our results are somewhat surprising and unexpected. However, they clearly indicate that nature of the neuroinflammatory response in AD is rather a complex one, and suggest that in this particular experimental model of LPS-induced neuroinflammation, the biological interrelationship between amyloid and tau is probably not one of co-dependency, and that, at least in this model, the evolution of the two main pathological features can be seen as separate events in the neurobiology of AD.

Additionally, the relationship between inflammatory stimuli and tau phosphorylation has been thought to involve non-neuronal cells in the brain, particularly immune cells such as microglia, and an inflammatory cytokine signature. However in our system, 5LO knockout reduced both astrocytosis and microgliosis as well as brain levels of IL1- β and IFN- γ . Our results therefore suggest that inflammatory stimuli such as LPS may directly modulate tau phosphorylation in neurons, even in the absence of an ongoing classical inflammatory state.

These inflammation-independent in vivo effects of LPS lead to a marked increase in AT8- and AT180-immunoreactivity in the brains of the mice treated but not in the steady-state levels of other tau epitopes. This selective phosphorylation of tau was mediated by a specific activation of cdk-5, but not GSK-3 β kinase. Interestingly, cdk-5 activation is thought to be an early tau-associated event in AD, since its activation and AT8 immunoreactivity have been coincidentally reported at the early stages and not the late ones in the development of AD neuropathology (Augustinack et al., 2002). However, despite the up-regulation of the cdk5 pathway we observed that level of PHF1 and PHF13 immuno-positive tau or the insoluble form of this protein did not change significantly after the chronic course of LPS. This finding could be explained by the relatively young age of the mice used in our study. Thus, both phosphorylation of these specific epitopes as well the formation and accumulation of insoluble tau fraction are considered late events in the development of the classical tau neuropathology. Our results are in agreement with other previous work by Parachikova et al., (2010). Thus, in that paper they found that 8 month old 3xTg animals treated for 4 months with the anti-inflammatory minocycline had improvements in cognition, a reduction in A β load and less microgliosis. However, when tau phosphorylation was assessed, minocycline treatment was associated with lowering phosphorylation of early/intermediate-stage AT100 tau and increasing levels of PHF-1 tau immunoreactivity, indicating that the advancement of tau pathology was unperturbed.

This biological disconnect between inflammation and some of the AD pathological feature implies that tau phosphorylation is sensitive to “other” types of LPS-dependent effect.

Reports by Kitazawa et al., (2005) and Gosh et al., (2013) when taken together also support our current data, as well as this general concept. Thus, the first paper found that 6-week LPS treatment did not modulate A β in 4 month old 3xTg animals, but increased tau phosphorylation. The second one, reported that inducing a proinflammatory state by overexpressing IL1- β in aged 3xTg mice leads to reduction in A β but an exacerbation of tau pathology.

In our model, we found that genetic absence of 5LO was protective against LPS-mediated effects on APP processing and behavior. Thus, LPS increased brain levels of PS1 and PEN2, two major components of the γ -secretase complex, in 3xTg but not in the 3xTg/5LOKO mice without altering A β levels. The proposed stoichiometry of the γ -secretase has been shown to require all four proteins in a 1:1:1:1 ratio, and we speculate that additional insult that elevates NCT and/or APH1 would be required to significantly elevate A β levels (Kimberly, et al., 2003). On the other hand, since astrocytes and microglia have been shown to increase expression of γ -secretase components upon LPS exposure *in vivo*, it is possible that the changes we observed in γ -secretase expression stem from glia cells (Nadler et al., 2008). In a similar way, we hypothesize that the beneficial effect on behavioral impairments we observed is secondary to the significant reduction in the neuroinflammatory response to LPS of the 3xTg/5LOKO. For both cases, further studies investigating specific role of LPS and 5LO in neuronal and glia, separately, and in combination, would be instructive.

In summary, we found that while knockout of 5LO is able to abrogate behavioral, γ -secretase up-regulation and cellular and humoral inflammatory insults caused by LPS exposure, it failed to alter LPS-induced tau hyper-phosphorylation. These findings suggest that the dysregulated tau metabolism secondary to LPS occurs independently of the known pro-inflammatory reactions of this stimulus. This novel effect is particularly intriguing because it provides experimental evidence showing that brain inflammation does not necessarily associate and/or result in modulation of some of the pathological features of the AD brain. Because in this particular experimental model our data question the link between A β and tau pathology, they further support the concept that targeting amyloid alone is not sufficient to rescue tau neuropathology and therefore significantly benefit the outcome of AD.

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Figure 1. Effect of LPS on Y-maze and fear conditioning behavior in 3xTg and 3xTg/KO animals
A) Spontaneous alternating behavior and **B)** total entries in the Y-maze of 3xTg mice treated with PBS/saline (n=4), 3xTg mice treated with LPS 0.5 mg/kg twice weekly for 6 weeks (n=5), 3xTg/5LOKO mice (n=4) treated with PBS/saline and 3xTg/5LOKO mice (n=6) treated with LPS. **C)** Contextual and **D)** cued recall freezing responses in the fear conditioning behavioral paradigm. (*p<0.05).

Figure 2. Modulation of Aβ and APP processing by LPS in 3xTg and 3xTg/5LOKO animals
 Levels of **A)** Aβ₄₀ and **B)** Aβ₄₂ assayed in the cortical RIPA lysate using sensitive and specific ELISA in 3xTg (n=4), 3xTg/LPS (n=5), 3xTg/5LOKO (n=4) and 3xTg/5LOKO/LPS (n=6) animals. (*p<0.05; between 3xTg and 3xTg/5LOKO animals) **C)** Representative immunoblots assaying APP metabolism in the same animals. **D)** quantification of proteins composing the γ-secretase protein complex, PS1, PEN2, NCT, and APH1. (*p<0.05; between the 3xTg/LPS group and all other groups).

Figure 3. LPS-induced tau hyperphosphorylation in 3xTg and 3xTg/5LOKO animals

A) Representative immunoblots probing for HT7, AT8, AT180, PHF1 and PHF13 in the cortical RIPA lysate of 3xTg (n=4), 3xTg/LPS (n=5), 3xTg/5LO (n=4), 3xTg/5LO/LPS (n=6) animals. **B)** Densitometry of immunoblots shown in panel A. **C)** Formic acid-soluble HT7 tau assayed in cortical brain homogenate of animals. **D)** Representative immunohistochemistry of hippocampal sections probing for HT7 (DaDd), AT8 (De-Dh), and AT180 (Di-Dl); scale bar 100μM. **E)** Representative immunoblots of cortical RIPA lysates probing for GSK3α, GSK3β, pSer 21 GSK3α, pSer9 GSK3β, cdk5, p35, and p25. **F)** Densitometry of immunoblots shown in panel E. (*p<0.05; between control animals and those treated with LPS).

Figure 4. LPS-induced neuroinflammation in 3xTg is prevented in the 3xTg/5LOKO animals
A) Representative immunoblots probing for GFAP and Iba1 in the cortical RIPA lysate of 3xTg (n=4), 3xTg/LP (n=5), 3xTg/5LO (n=4), 3xTg/5LO/LPS (n=6) animals. **B)** Densitometry of immunoblots shown in panel A. **C)** Representative immunohistochemistry of cortical sections probing for GFAP (Ca-Cd) and Iba1 (Ce-Ch); scale bar 100μM. **D)** Representative quantification of GFAP-positive and Iba1-positive cells in brain sections show in panel C. **E)** Selective and specific ELISA probing for IL-1β and protein array assaying for IFN-γ in the RIPA cortical lysate of animals. (*p<0.05; between the 3xTg/LPS group and all other groups).

Table 1

Antibodies used in the study.

Antibody	Immunogen	Host	Application/Dilution	Source
APP	Amino acid 66–81 of APP {N-terminus }	Mouse	WB, 1:1 000	Millipore
BACE-1	Human BACE sequence (CLRQQHDDFADDISLLK)	Rabbit	WB, 1:200	IBL
ADAM10	Amino acid 732–748 of human ADAM 10	Rabbit	WB, 1:500	Millipore
PS-1	Valine 293 of human presenilin 1	Rabbit	WB, 1:200	Cell Signaling
Nicastrin	carboxy-terminus of human nicastrin	Rabbit	WB, 1:200	Cell Signaling
APH-1	Synthetic peptide from hAPH-1a	Rabbit	WB, 1:200	Millipore
Pen-2	N-terminal of mouse Pen-2	Rabbit	WB, 1:200	Invitrogen
HT-7	Amino acid 159–163 of human tau	Mouse	WB, 1:750 IHC, 1:200	Pierce
AT-8	Partially purified phospho-S202/T205 tau	Mouse	WB, 1:200 IHC, 1:100	Pierce
AT-180	Partially purified phospho-T231/S235 tau	Mouse	WB, 1:200 IHC, 1:100	Pierce
PHF-13	Partially purified phospho-Ser396 tau	Mouse	WB, 1:200 IHC, 1:100	Cell Signaling
PHF-1	Purified phospho-Ser396/S404 tau	Mouse	WB, 1:200 IHC, 1:20	Dr. P. Davies
GFAP	C-terminus of GFAP of human origin	Mouse	WB, 1:200 IHC, 1:100	Santa Cruz
Iba1	Linear peptide corresponding to human Iba1	Mouse	WB, 1:200 IHC, 1:100	Millipore
GSK3 α/β	Amino acid 1-420 full length GSK-3 β of Xenopus origin	Mouse	WB, 1:200	Santa Cruz
p-GSK3 α/β	Amino acid Ser21 of human GSK-3 α .	Rabbit	WB, 1:200	Cell Signaling
Cdk5	C-terminus of Cdk5 of human origin	Rabbit	WB, 1:200	Santa Cruz
P35/25	C-terminus of p35 /25 of human origin	Rabbit	WB, 1:200	Santa Cruz
Actin	C-terminus of Actin of human origin	Goat	WB, 1:1 000	Santa Cruz