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TLR4 gene expression and pro-inflammatory cytokines in Alzheimer's disease and in response to hippocampal deafferentation in rodents

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Running title: TLR4 and cytokines in Alzheimer's disease

Abstract

One important aspect in Alzheimer's disease pathology is the presence of chronic inflammation. Considering its role as a key receptor in the microglial innate immune system, TLR4 was shown to regulate the binding and phagocytosis of amyloid plaques by microglia in several mouse models of amyloidosis, as well as the production of pro-inflammatory cytokines. To our knowledge, TLR4 and its association with cytokines have not been thoroughly examined in the brains of subjects affected with Alzheimer's disease. Using quantitative reverse transcription polymerase chain reaction (qRT-PCR) in post-mortem human brains, we observed increased expression for the *TLR4* and *TNF* genes ($p = 0.001$ and $p = 0.025$ respectively), as well as a trend for higher *IL6* gene expression in the frontal cortex of AD subjects when compared to age-matched controls. Similarly, using a mouse model of hippocampal deafferentation without amyloidosis, (i.e. the entorhinal cortex lesioned mouse), we observed significant increases in the expression of both the *Tlr4* ($p = 0.0367$ and $p = 0.0193$ compared to sham-lesioned mice or to the contralateral side respectively) and *Il1b* ($p = 0.0055$ and $p = 0.0066$ compared to sham-lesioned mice or to the contralateral side respectively) genes in the deafferentation phase, but not during the ensuing reinnervation process. In conclusion, we suggest that the modulation of cytokines by TLR4 is differentially regulated whether by the presence of amyloid plaques or by the ongoing deafferentation process.

Keywords: Alzheimer, inflammation, TLR4, cytokines

Introduction

Alzheimer's disease (AD) is the most common form of dementia, representing between 50 and 56% of all cases.[1] Age being the major risk factor for this disease, the incidence of AD doubles every 5 years starting at the age of 65 to reach one third of people aged 85 years and older.[1] In addition to extracellular amyloid plaques,[2] neuronal and synaptic loss,[3, 4] as well as accumulation of neurofibrillary tangles in neurons,[5] AD pathology is also characterized by chronic inflammation.[6] Recent evidence has shown that increased inflammatory markers at midlife were associated, later in life, with a poorer performance in a word-recall test, as well as with lower volumes in AD-related brain regions.[7]

Toll-like receptor 4 (TLR4), which is encoded by the *TLR4* gene in humans, is a transmembrane protein which belongs to the pattern recognition receptor (PRR) family and that plays an important role in the innate immune system.[8] The binding of pathogen-associated molecular patterns (PAMPs) to TLR4 leads to the activation of the NF-κB signaling pathway, which results in the synthesis and secretion of inflammatory cytokines.[9] TLR4 is also important in mediating the binding and subsequent phagocytosis of fibrillary amyloid by microglia.[10] The presence of amyloid is believed to be crucial in activating the TLR4-mediated NF-κB/MAPK inflammatory pathway, which in turn, promotes the excessive secretion of pro-inflammatory and neurotoxic cytokines, such as IL-1β, IL-6 and TNF-α.[11, 12]

Although there is a small body of literature concerning the role of TLR4 in Alzheimer's disease, *TLR4* gene expression and its relationship with cytokine mRNA levels have not, to our knowledge, been characterized in the brains of human subjects in the context of Alzheimer's disease. In addition, we decided to further investigate the relationship between *Tlr4* and pro-

inflammatory cytokines in a rodent model of hippocampal deafferentation in absence of any amyloid pathology. Therefore, *TLR4*, *IL1B*, *IL6* and *TNF* mRNA levels were assessed in the brains of autopsy-confirmed control and AD human subjects and in a wild-type mouse model of hippocampal deafferentation and reinnervation (i.e. the entorhinal cortex lesioned mice).

Material and methods:

Diagnosis

The diagnosis of autopsied brains was based on the histopathological NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria for a definite diagnosis of AD.[13] The post-mortem human brains were obtained from the Douglas Bell Canada Brain Bank. This study was conformed to the Code of Ethics of the World Medical Association and was approved by the Ethics Board of the Douglas Mental Health University Institute Research Center.

Density of amyloid plaques in the frontal cortex

The identification of amyloid plaques was consistent with the classification of Khachaturian.[13] Paraffin-embedded sections of the frontal cortex were stained with the Holmes' silver method, to observe dense core amyloid plaques, as well as with the hematoxylin-eosin protocol. All plaques with a diameter ranging from 30 to 200 µM were counted. The total volume surveyed was approximately 10 mm^3. Plaque aggregates were counted as single plaques unless a plaque participating in the aggregate overlapped it by less than 25%. The surface of all the sections was measured with a digitizer after having outlined their boundaries at a magnification of 10X

with a Leitz drawing tube attached to a Wild M75 microscope. All the counts were performed by the same neuropathologist.[14]

TLR4 and cytokine gene expression levels

Total ribonucleic acid (RNA) was extracted from frontal cortex, temporal cortex or cerebellum tissues using either one of these devices: QIAsymphony® (Qiagen, Hilden, Germany) or Maxwell ® 16 (Promega, Madison, WI, USA). Purity of RNA was estimated using the absorbance values at 260 and 280 nm. To generate complementary DNA (cDNA), RNA samples were reverse-transcribed with SuperScript VILO Master Mix (Thermo Fisher, Waltham, MA, USA) according to the manufacturer's protocol. The TaqMan primers were purchased from Thermo Fisher (Waltham, MA, USA): Hs00370853_m1 for *TLR4*, Hs01555410_m1 for *IL1B*, Hs00985639_m1 for *IL6*, Hs01113624_g1 for *TNF* and 4326321.E for *HPRT1*. Using BestKeeper and NormFinder,[15, 16] *HPRT1* was chosen as the best housekeeping gene in the brain for Alzheimer's disease among 5 other genes: peptidylprolyl isomerase A (*PPIA*), ubiquitin conjugating enzyme E2 D2 (*UBE2D2*), cyclin-dependent kinase inhibitor 1B (*CDNK1B*), actin beta (*ACTB*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The Fast Taqman® qRT-PCR protocol was performed in the QuantStudio 12X Flex instrument (Thermo Fisher, Waltham, MA, USA). All qRT-PCR assays displayed efficiencies ranging from 94-115%. Data were analyzed using the ExpressionSuite software (Thermo Fisher, Waltham, MA, USA).

Statistical methods

For all analyses, extreme outliers in each diagnosis group (defined as any data point more than 3 interquartile ranges (IQRs) below the first quartile or above the third quartile) were removed. For human gene expression levels contrasted as a function of diagnosis, one-way

ANCOVAs corrected for age, gender and post-mortem intervals were performed. Associations between *TLR4* and cytokines mRNA levels were carried out using Pearson correlations corrected for age, sex, disease status and post-mortem intervals. Spearman correlation analyses were performed to assess the relationships between *TLR4* mRNA levels, amyloid plaque densities and age at onset to circumvent the slightly abnormal distributions.

Entorhinal cortex lesions

Animals

Two-to-three months old male C57BL/6J wild-type mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). All animals were housed individually in an enriched environment and fed *ad libitum* with a diet of standard laboratory chow. A 12-h-light-dark cycle was maintained with light onset at 07:00 and offset at 19:00, local time. All protocols were carried out in accordance with the Canadian Guidelines for Use and Care of Laboratory Animals and were approved by the McGill University Animal Care Committee.

Unilateral entorhinal cortex lesions (ECL)

Unilateral electrolytic lesions to the entorhinal cortex were conducted on 6 mice per time point according to the technique adapted for mice described by Blain *et al*.[17] Isoflurane-anaesthetized mice were placed in a stereotaxic apparatus in a flat skull position. Four lesion coordinates were determined from Lambda: 1) [AP: 0 mm], [L: - 3 mm], [DV: -3 mm, -4 mm]; 2) [AP: 0 mm], [L: -3.5 mm], [DV: -3 mm, -4 mm]; 3) [AP: + 0.5 mm], [L: -4 mm], [DV: -3 mm, -4 mm]; 4) [AP: +1 mm], [L: -4 mm], [DV: -3 mm, -4 mm]. A 1 mA current was applied for 10 seconds at each coordinate. Sham-operated animals (n = 6) were treated similarly except that the electrode was lowered only 1 mm and no current was applied. Following surgery, mice were given

a subcutaneous booster of physiological saline to prevent dehydration and nursed throughout their recovery. Lesioned mice were sacrificed 7 (deafferentation) or 21 (reinnervation) days after ECL, while sham-lesioned mice were all sacrificed on the same day. Mice were decapitated and their brain quickly removed. The contralateral and ipsilateral hippocampi were dissected out on dry ice and stored at -80ºC until use.

RNA extraction and quality assessment

Total RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). RNA samples were pooled in groups of 2. RNA quality was assessed at McGill University and Génome Québec Innovation Centre. RNA samples all had RIN > 7.8 and 260/280 ratios > 2.1.

Tlr4, Il1b, Il6 and Tnf mRNA levels

Tlr4, Il1b, Il6 and *Tnf* expression profiles were measured with the Mouse Clariom™ D Assay (Affymetrix, Santa Clara, CA, USA) by the McGill University and Génome Québec Innovation Centre. The resulting CEL files were analyzed by using the Transcriptome Analysis Console Version 4.0 (TAC 4.0) with the Mouse Transcriptome Assay (MTA) 1.0 Array. TAC 4.0 uses the Limma Bioconductor package for the analysis of expression data. Limma is performing an analysis of variance (ANOVA) with an empirical Bayesian correction.[18]

Results

Demographic characteristics of the studied cohort

Table 1 summarizes the demographic characteristics of the 44 autopsied control and 44 AD subjects included in this study. There is no significant difference for the age at death between the

two groups, the mean (standard error of the mean [SEM]) age being of 79.7 (1.1) and 80.7 (0.8) years for control and AD subjects respectively (t[86] = -0.746, p = 0.458). The mean (SEM) post-mortem interval (PMI) shows no statistical difference between the two groups (controls: 29.7 [3.3]; AD: 36.4 [7.3]; U[44, 44] = 936.5, p = 0.793]. As for sex, the proportion of women (50%) is exactly the same for both groups. The mean (SEM) age at onset in the AD group is 74.2 (1.1). As expected, there is a significant difference in the *APOE* ε4 allele frequencies between the control (13.6 %) and AD (70.5%) groups respectively (χ^2[1] = 29.147, p = 6.7 x 10^{-8}). As observed in the frontal cortex, no significant differences were observed between AD and control groups in the cerebellum except for *APOE* ε4 allele frequencies (data not shown).

TLR4 and cytokines mRNA levels as a function of disease status

Figure 1 illustrates the mRNA levels of the *TLR4*, *Il1B*, *IL6* and *TNF* genes in the cerebellum and frontal cortex of human control and AD subjects. In the frontal cortex, *TLR4* and *TNF* mRNA levels are significantly higher in AD cases compared to controls (F(1, 83) = 10.997, p = 0.001, fold change (FC) = 2.09 for *TLR4*; F(1, 79) = 5.209, p = 0.025, FC = 2.37 for *TNF*; Fig. 1a) in contrast to *IL1B* mRNA levels, which are not significantly different between the two groups (F(1, 81) = 1.324, p = 0.253, FC = 2.83; Fig. 1a). As for *IL6*, there is a trend for higher mRNA levels in the frontal cortex of AD subjects F(1, 81) = 2.977, p = 0.088, FC = 1.70). *IL1B* and *IL6* mRNA levels are still not significantly different between AD subjects and controls when using ANOVAs for contrasts (F(1, 84) = 1.082, p = 0.301 for *IL1B*; F(1, 84) = 2.721, p = 0.103 for *IL6*). In the cerebellum, *TLR4*, *IL1B*, *IL6* and *TNF* mRNA levels are unchanged in AD versus control subjects (F(1, 71) = 0.901, p = 0.346, FC = 1.23 for *TLR4*; F(1, 59) = 0.183, p = 0.670 for *IL1B*, FC = 1.19; F(1, 59) = 0.702, p = 0.406 for *IL6*, FC = 1.56; F(1, 55) = 0.080, p = 0.778 for *TNF*, FC = 0.66; Fig. 1b).

Association between *TLR4* mRNA levels, the extent of AD pathology and the gene expression of pro-inflammatory cytokines

In the frontal cortex, *TLR4* mRNA levels are positively correlated with amyloid plaques density ($r_S(52) = 0.348$, $p = 0.011$; Fig. 2a), but inversely correlated with the age at onset of AD ($r_S(40) = -0.389$, $p = 0.013$; Fig. 2b). This inverse relationship between age at onset and *TLR4* mRNA levels survives further correction for sex, post-mortem interval and amyloid load ($r(31) = -0.482$, $p = 0.009$), suggesting that the association of *TLR4* mRNA levels with the age at onset is not explained by the impact of these covariates on *TLR4* gene expression. In the frontal cortex of control and AD subjects, *TLR4* mRNA levels are positively correlated with *IL1B* ($r(75) = 0.299$, $p = 0.011$), *IL6* ($r(75) = 0.508$, $p = 6.1 \times 10^{-6}$) and *TNF* ($r(72) = 0.499$, $p = 1.5 \times 10^{-5}$) mRNA levels.

Tlr4, *Il1b*, *Il6* and *Tnf* mRNA levels in a mouse model of hippocampal deafferentation and reinnervation (ECL):

Figure 4 compares the mRNA levels of the *Tlr4*, *Il1b*, *Il6* and *Tnf* genes in the ipsilateral hippocampus of ECL mice at two timepoints: during deafferentation (at 7 days post-lesion) and throughout reinnervation (at 21 days post-lesion). Hippocampal *Tlr4* and *Il1b* mRNA levels during the active phase of deafferentation are significantly higher compared to sham-lesioned mice (FC = 1.66, $p = 0.0367$ for *Tlr4*; FC = 5.04, $p = 0.0055$ for *Il1b*; Fig. 4a). No change was observed for *Il6* and *Tnf* mRNA levels (FC = 0.98, $p = 0.2822$ for *Il6*; FC = 1.04, $p = 0.2164$ for *Tnf*; Fig. 4a) in the deafferented hippocampus. Consistent with the previous results, *Tlr4* and *Il1b* mRNA levels at 7 days post-lesions are significantly increased compared to the contralateral side (FC = 1.70, $p = 0.0193$ for *Tlr4*; FC = 4.80, $p = 0.0066$ for *Il1b*; Fig. 4b), while *Il6* and *Tnf* mRNA levels are similar (FC = 1.00, $p = 0.4596$ for *Il6*; FC = 1.13, $p = 0.1814$ for *Tnf*; Fig. 4b). During the

reinnervation phase, no significant difference was observed for *Tlr4*, *Il1b*, *Il6* and *Tnf* mRNA levels compared to sham-lesioned mice (FC = 1.05, p = 0.9957 for *Tlr4*; FC = 1.23, p = 0.6613 for *Il1b*; FC = 0.98, p = 0.5226 for *Il6*; FC = 0.9, p = 0.3974 for *Tnf*) or to the contralateral side (FC = 1.09, p = 0.6034 for *Tlr4*; FC = 1.36, p = 0.6000 for *Il1b*; FC = 1, p = 0.3023 for *Il6*; FC = 0.9, p = 0.5980 for *Tnf*). At 40 days post-lesion, *Tlr4*, *Il1b*, *Il6* and *Tnf* mRNA levels in the ipsilateral side of the hippocampus are not statistically different compared to sham-lesioned mice or to the contralateral side (data not shown).

Discussion

Inflammation is typically set off when innate immune cells detect infection, tissue injury or chronic tissue damage, such as in the case of AD. Tight control of the severity, duration, and location of inflammation is an absolute requirement for an appropriate balance between clearance of injured/degenerating tissue versus damage to healthy cells. Toll-like receptors, primarily known for their pathogen recognition and subsequent immune responses, have therefore been investigated for their possible pathogenic role in various chronic diseases.

Consistent with a small body of evidence in the literature, the results presented above suggest that the upregulation of the *TLR4* gene and the subsequent modulation of cytokines mRNA levels in AD play a significant role in the neuroinflammation process unfolding both, in the presence of amyloid plaques, as well in the deafferentation process. Indeed, *TLR4* mRNA levels are not only higher in the frontal cortex of post-mortem human AD brains compared to age-matched controls (Fig. 1a), but levels also correlate with the age at onset of AD (Fig. 2b). Moreover, *TLR4* gene upregulation is not present in the cerebellum, a control region with no or little pathology (Fig. 1b). Using an animal model of amyloid precursor protein overexpression (i.e.

the aged APP23 transgenic mice), Frank and collaborators reported that *Tlr4* gene expression parallels that of amyloid deposition,[19] a phenomenon similar to our observation linking frontocortical amyloid plaques density to *TLR4* mRNA levels (Fig. 2a). This association between *TLR4* gene expression and amyloid deposition can be explained, at least in part, by the well-known role of TLR4 in the microglial-mediated phagocytosis of amyloid plaques *in vivo*. Song and colleagues described that a loss-of-function mutation in the *Tlr4* gene in a mouse model of amyloid deposition is accompanied by a significant increase in amyloid load, as well as a marked reduction of microglial activation in both the neocortex and the hippocampus.[20] Furthermore, Qin and colleagues reported that a chronic mild stimulation of TLR4 reduces phosphorylated Tau brain load and attenuates memory and synaptic impairments in young Tau-transgenic mice.[21]

TLR4 is a known modulator of the release of pro-inflammatory cytokines in the context of both neuronal injury and amyloidosis. Indeed, as shown by Tanga and collaborators, *Tlr4* downregulation, either with a gene knockout in mice or a treatment with an antisense oligodeoxynucleotide in rats, has been shown to reduce *Tnf*, *Il1b* and *Il6* mRNA levels 3 to 7 days following a L5 spinal cord nerve transection surgery.[22] Moreover, as evidenced by Jin and colleagues, a loss-of-function mutation in the *Tlr4* gene in a 13-15 months old mouse model of amyloidosis was shown, to restore brain IL-1β and TNF-α protein levels to the concentrations observed in mice without amyloidosis.[23] However, studies on CSF, plasma and brain IL-1β, IL-6 and TNF-α expression levels have shown conflicting results concerning the upregulation of these cytokines in AD subjects compared to controls. These discrepancies could be explained by the high inter-individual differences observed for cytokine levels or by the fact that these cytokines are only elevated in subgroups of patients or during certain disease stages.[24-32]

In the present study, *TNF* mRNA levels are significantly elevated in the frontal cortex of AD subjects, but not in the cerebellum, a control region with little or no pathology (Fig. 1). Several anti-TNF-α biologic medications have reduced Aβ deposition, memory impairments, neurodegeneration, as well as microglial activation in old 3xTg AD mice[33, 34], suggesting that the physiologically high concentrations of TNF-α observed in the later stages of AD may actually signal an ongoing pathological process. These findings certainly support the notion that TNF-α may serve as a potent therapeutic target for the treatment of symptomatic AD. However, hippocampal injection of TNF-α in APP TgCRND8 mice at an early stage of amyloid deposition induced robust glial activation that attenuated Aβ plaques without altering APP levels, presumably by facilitating local scavenging activity.[35]. In this case, the use of anti-TNF-α biologics as a prevention strategy for presymptomatic subjects may be counter indicated.

We also observed a trend for higher *IL6* mRNA levels in the frontal cortex of AD subjects (Fig. 1a), that is not observed in the cerebellum (Fig 1b). In the APP TgCRDN8 mice, the cerebroventricular injection at birth of AAV1 viruses expressing the murine *Il6* gene reduced the amyloid burden in the hippocampus and the forebrain 5 months later, during early amyloid deposition. As evidenced in wild-type mouse microglia treated with recombinant mIL-6, the reduction in amyloid burden seems to be due to an IL-6-mediated microglial phagocytosis of amyloid plaques, suggesting a possible beneficial role for IL-6 in the early stage of AD.[36] However, in cultured rat cortical neurons, treatment with IL-6 exacerbated Aβ-modulated NMDA cell damage.[37]

Even though *IL1B* gene expression is not upregulated in the frontal cortex of AD subjects as opposed to *TNF* and *IL6* genes, the mRNA levels of all these cytokines are correlated with *TLR4* mRNA levels in control and AD subjects, although to a lesser extent for the *IL1B* gene (Fig. 3).

These associations suggest that, regardless of the indirect impact of the AD pathology on these cytokines' gene expression, TLR4 directly modulate their local mRNA levels.

Although the results described above in end-stage AD suggest an Aβ-induced TLR4 modulation of cytokines mRNA levels in pathologically affected brain areas, it is also quite conceivable that the deafferentation process per se may, in an amyloid-independent manner, upregulate directly *TLR4* gene expression and subsequently, the mRNA levels of cytokines. To address this specific question, we used the well-established entorhinal cortex lesion (ECL) paradigm. In this model, it is possible to examine, in a plaque-free environment, the impact on the gene expression of multiple inflammation-related biomarkers of a) the synaptic loss and the microglial activation present in the first 14 days following the lesion and b) the synaptic remodelling and the cholinergic sprouting in the 14-30 days post-lesion window.[38-40] Moreover, this model mimics one of the first neuronal losses observed in AD, namely the degeneration of the perforant path, which consists mostly of projections from layer II entorhinal cortex neurons to the dentate gyrus of the hippocampus.[41] Since synapse loss is the major correlate of cognitive impairment,[42] the ECL mouse model is of great use in monitoring the inflammatory responses that might be involved in AD.

During the deafferentation phase at 7 days post-ECL, mouse ipsilateral *Tlr4* and *Il1b* mRNA levels in the hippocampus are significantly elevated compared to sham-lesioned mice or to the contralateral control side (Fig. 4a), consistent with previous work by Tanga and colleagues in the degenerating peripheral nervous system.[22] In contrast, at the same timepoint, *Il6* and *Tnf* mRNA levels remain unchanged when compared to sham-lesioned mice or to the contralateral control side (Fig. 4a). As expected, during the reinnervation phase at 21 days post-ECL, *Tlr4*, *Tnf*, *Il1b* and *Il6* gene expression levels are similar to those observed in sham-lesioned mice or in the

contralateral control side (Fig. 4b), suggesting that the modulation of these pro-inflammatory cytokines by TLR4 occurs essentially during the deafferentation process when the hypertrophy of microglia and astrocytes peaks.[43-45] However, since every observation present in this article rely on a correlation or an association in time, it is worth mentioning that we can't imply causal relationships between *TLR4* mRNA levels and any of the variables present in our association analyses. One central question however remains: when exactly is TLR4 activated in sporadic Alzheimer's disease? The results presented above, combined with recent findings in the ADNI cohort indicating that the involvement of inflammation in AD slightly precedes that of amyloid-β (Aβ) and Tau,[46] suggest an early contribution of the TLR4/cytokines cascade in the presymptomatic phase of the disease, years before the onset of AD.

In summary, the significant contribution of TLR4 to the neuroinflammation process in Alzheimer's disease may not only be triggered by amyloidosis, but also by an amyloid-independent deafferentation process that is taking place in the early phases of the disease. However, the cytokines, which are downstream in the TLR4 pathway, seems to behave differently in response to TLR4 activation whether it is due to amyloid deposition or to the deafferentation process. These observations provide us with new insights into the role of TLR4 in the inflammation process that is existing in both the presymptomatic and symptomatic phases of sporadic Alzheimer's disease.

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Conflict of interest/disclosure statement

The authors have no conflict of interest to report.

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Table 1

	CTL *N*=44	AD *N*=44
Age at death		
Mean ± SEM, years	79.7 ± 1.1	80.7 ± 0.8
Age at onset		
Mean ± SEM, years		74.2 ± 1.1
PMI		
Mean ± SEM, hours	29.7 ± 3.3	36.4 ± 7.3
Sex		
Nb (%) women	22 (50%)	22 (50%)
Apolipoprotein E4 allele****		
Nb (%)	6 (13.6 %)	31 (70.5 %)

Demographic characteristics of the studied human cohort ****$p \leq 0.001$. CTL = control; AD = Alzheimer's disease; PMI = post-mortem interval; SEM = standard error of the mean; Nb = number.

Figures

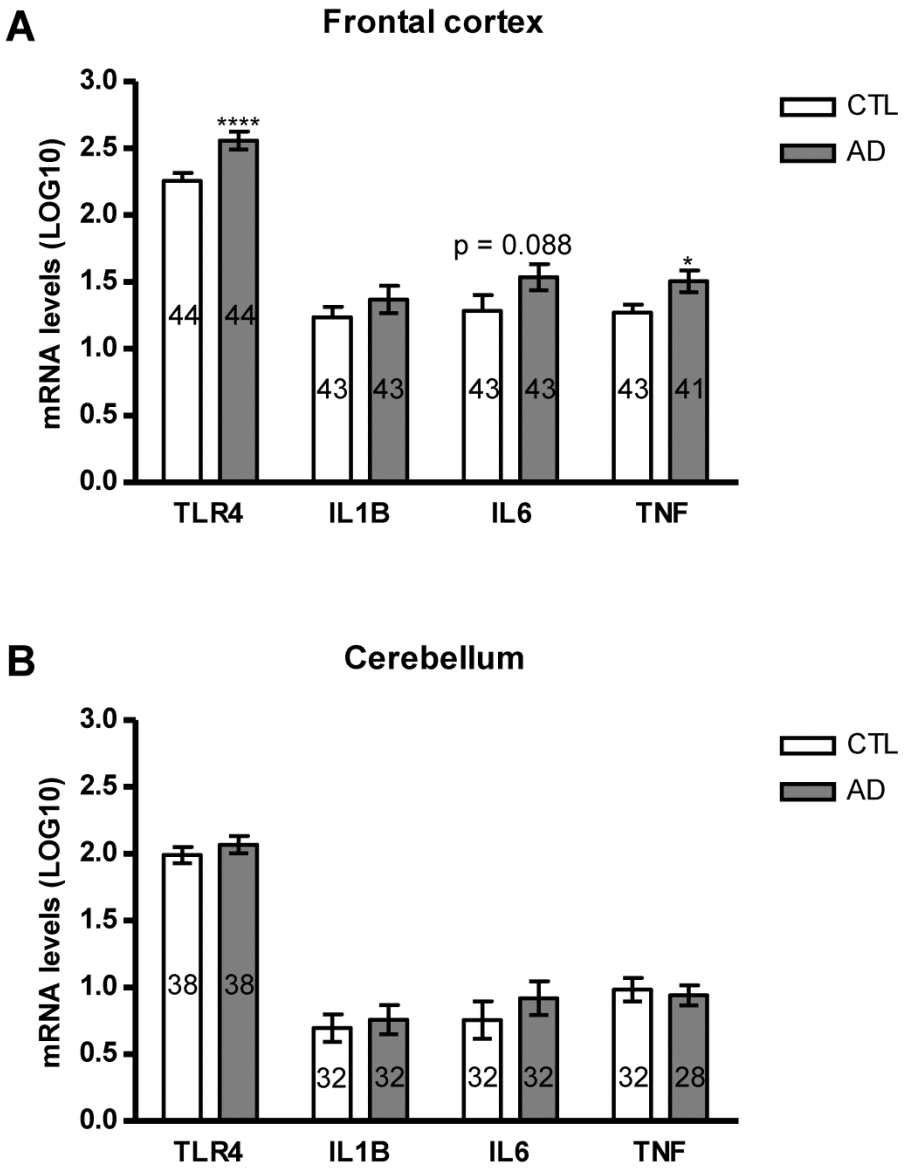

Fig. 1 Gene expression of TLR4 and pro-inflammatory cytokines as a function of disease status in the frontal cortex and the cerebellum of post-mortem human subjects Each bar represents a mean $\log_{10}$RQ+1 value ± standard of the mean (SEM). CTL = control subjects; AD = Alzheimer's disease subjects. n numbers are indicated on each bar. *$p \leq 0.05$, **** $p \leq 0.001$. RQ = relative quantification.

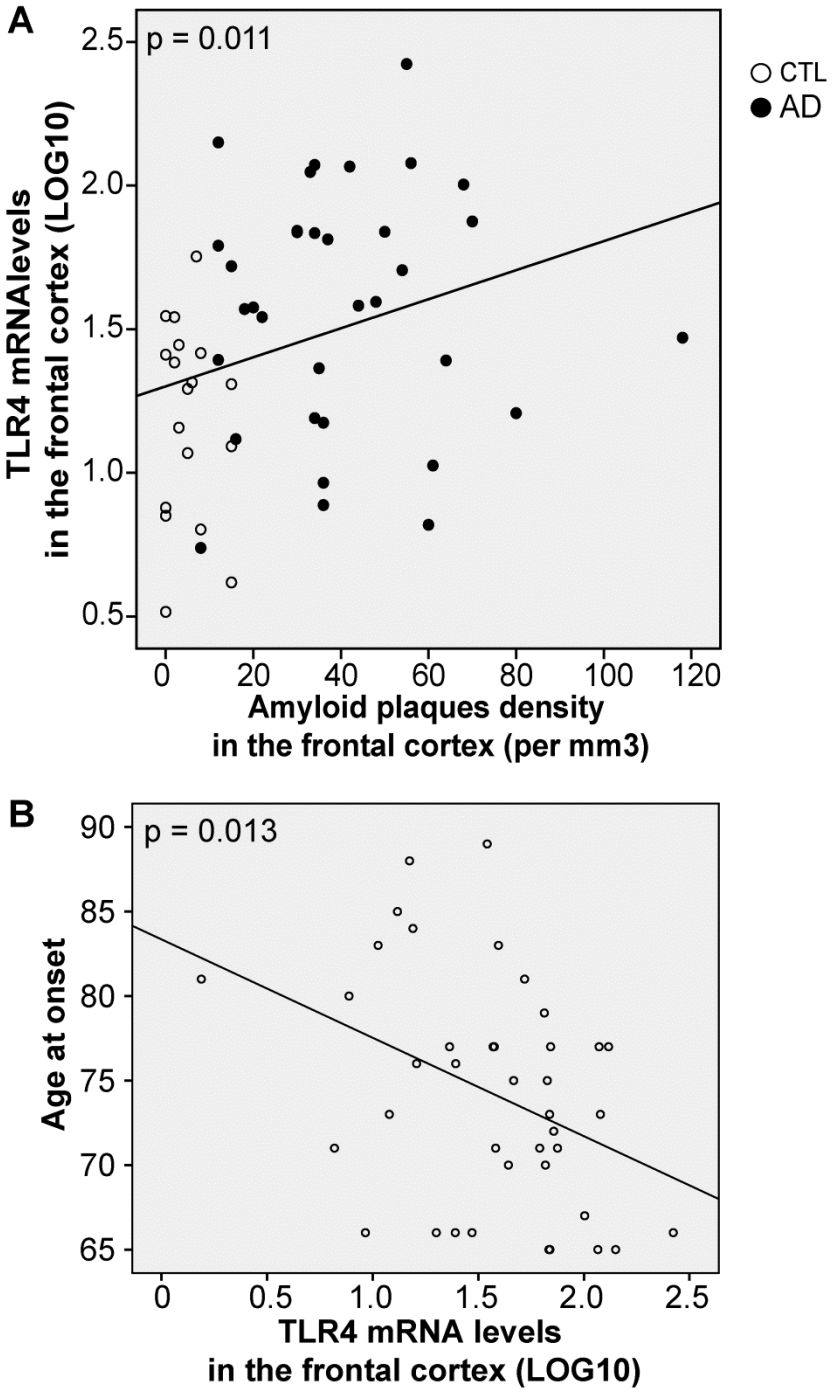

Fig. 2 Frontal cortex TLR4 gene expression as a function of AD pathology measures in post-mortem human subjects Frontocortical TLR4 mRNA levels in correlation with a) amyloid plaques density and b) age at onset. mRNA levels are reported in $\log_{10}$RQ values. CTL = control subjects; AD = Alzheimer's disease subjects; RQ = relative quantification.

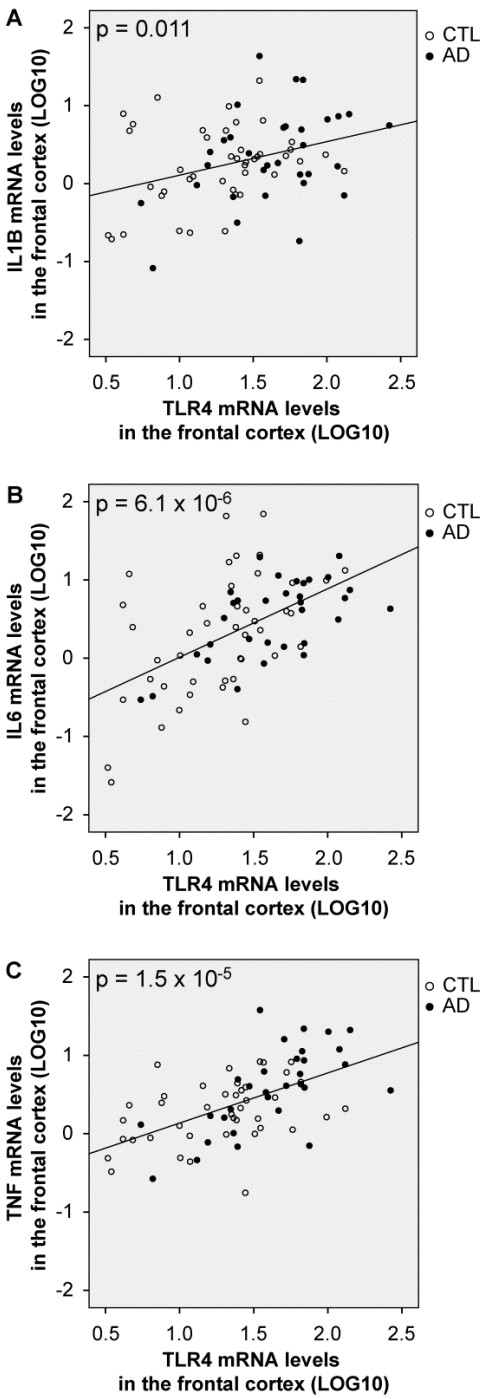

Fig. 3 Correlation, in the frontal cortex of post-mortem human subjects, between the gene expression of TLR4 and pro-inflammatory cytokines mRNA levels are reported in $\log_{10}$RQ values. Pro-inflammatory cytokines are the following: a) IL1B; b) IL6; c) TNF. CTL = control subjects; AD = Alzheimer's disease subjects; RQ = relative quantification.

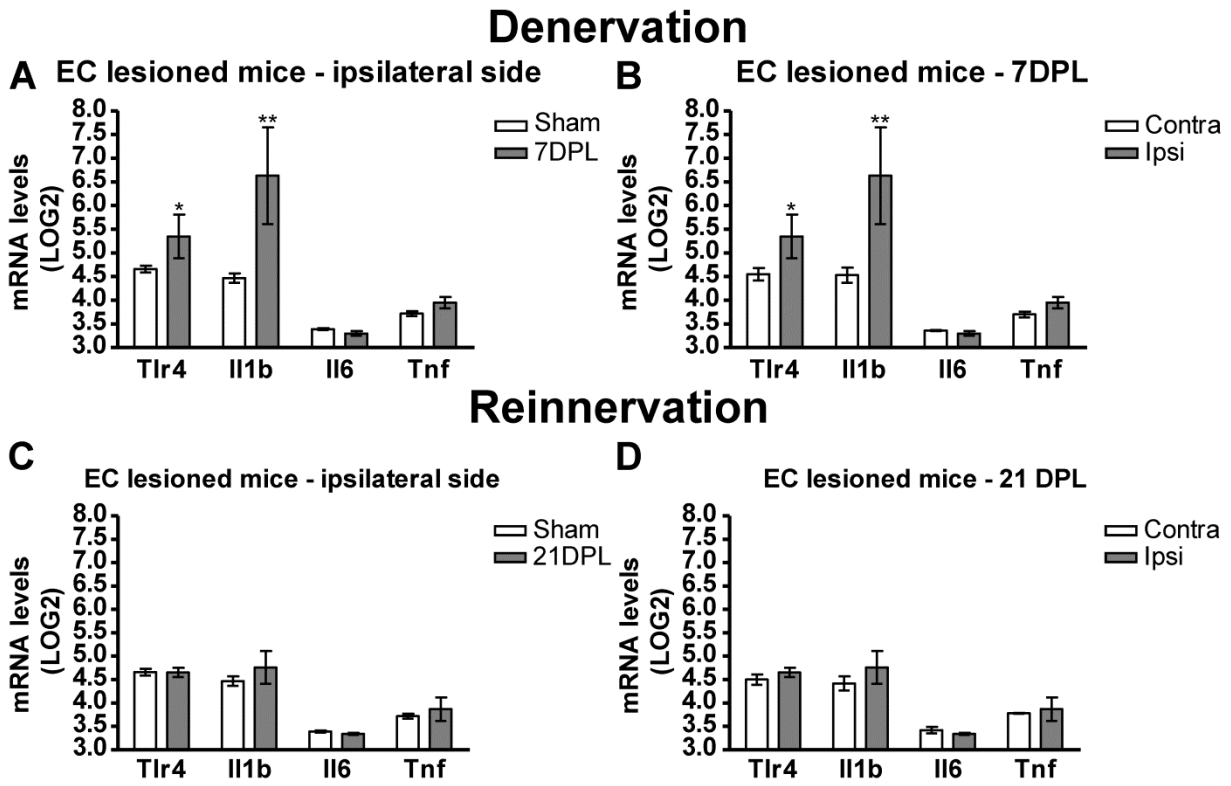

Fig 4 Gene expression of TLR4 and pro-inflammatory cytokines in a mouse model of entorhinal cortex lesion (ECL) Each bar represents a mean $\log_2$ expression value ± SEM. Ipsilateral mRNA levels were compared to sham-lesioned mice or to the contralateral side for both a) the deafferentation phase 7 days after the lesion; b) the reinnervation phase 21 days after the lesion. n = 2 mice per pool x 3 pools per group. *$p \leq 0.05$, ** $p \leq 0.01$ or n.s. = not significant. Sham = sham-lesioned mice; 7DPL = 7 days post-lesion; 21DPL = 21 days post-lesion; ipsi = ipsilateral side; contra = contralateral side.