Full title: **Lymphocyte activation gene 3 (Lag3) is upregulated by prions but does not contribute to disease**

Short title: **Lag3 in prion diseases**

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Abstract

Prion diseases (PrDs), Alzheimer's disease and Parkinson's disease (PD) are fatal neurodegenerative disorders that share neuropathological and biochemical features, including the aggregation of pathological protein conformers. Lymphocyte activation gene 3 (Lag3, also known as CD223) is a member of the immunoglobulin superfamily of receptors expressed on immune cells and neurons, which serves as a receptor for α-synuclein aggregates in PD. Here we examined the possible role of Lag3 in the pathogenesis of PrDs. Through quantitative real-time PCR and RNA-sequencing, we found that the expression levels of Lag3 were relatively low in adult mouse brains, yet its expression was significantly upregulated after prion infection. However, we failed finding significant differences regarding the incubation time, PrP^{Sc} load, neurodegeneration and inflammatory gene expression profiles between the Lag3 knockout mice and wild-type littermate controls after prion infection. We conclude that loss of Lag3 has no significant influence on PrD pathogenesis. Considering that Lag3 is an immune checkpoint receptor, our results also suggest that immune checkpoint inhibition (an increasingly prevalent therapeutic modality against many types of cancer) might not exert positive or negative effects on the progression of PrDs.

Author Summary

Prion diseases (PrDs) are fatal neurological disorders that can be transmitted between individuals. Their causative agent is called the prion, and is a conformationally changed, aggregated form of a normal cellular protein called PrP^{C}. How prions spread from one cell to another is still largely unknown, but this spreading behavior is common to several additional proteins including α-synuclein. In this study, we test the hypothesis that lymphocyte activation gene 3 (Lag3), a neuronal receptor for α-synuclein aggregates, may serve as a prion receptor and facilitate its cell-to-cell transmission. We found that prion-infected Lag3-deficient mice progressed to terminal disease with the same kinetics and attack rates as wild-type mice, and exhibited the same type of CNS pathology. Therefore, Lag3 is not a mediator of prion transmission during PrD pathogenesis. Because Lag3 is an immune checkpoint receptor, our

study also suggests that immune checkpoint inhibition, a promising therapeutic intervention to combat neurodegenerative disorders, might not be an effective way to halt PrD progression. Currently, several phase II/III clinical trials are exploring Lag3 inhibition as an anticancer strategy. Our results suggest that it may be safe to administer Lag3 antibodies (and perhaps also other checkpoint inhibitors) to those patients who suffer from pathogenic prion mutations and may need to undergo cancer immunotherapy.

Introduction

Prion diseases (PrDs), also known as transmissible spongiform encephalopathies (TSEs), are progressive neurodegenerative disorders affecting many mammalian species. PrDs share common neuropathological features like protein aggregation, neuronal and synaptic degeneration and glial cell activation with other neurological conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Soto and Satani 2011). The replication, transmission and aggregation of pathological prion protein, PrP^{Sc}, play central roles in PrD pathogenesis (Aguzzi and Liu 2017, Aguzzi, Lakkaraju et al. 2018). Despite extensive research, the molecular mechanisms underlying the events driving disease progression are only partially known. What is more, validated targets regulating cell-to-cell spreading of prions, prion-induced neurotoxicity and the pathogenesis of PrDs are still waiting to be identified. Consequently, interventional therapies for PrDs are still lacking (Aguzzi, Lakkaraju et al. 2018).

Lymphocyte activation gene 3 (Lag3, also known as CD223) is a member of the immunoglobulin superfamily of receptors identified on activated human natural killer cells and T cells with diverse biologic effects (Triebel, Jitsukawa et al. 1990, Andrews, Marciscano et al. 2017). Additionally, it is expressed by other immune cells including B cells and dendritic cells (Kisielow, Kisielow et al. 2005, Workman, Wang et al. 2009). Lag3 is a 498-amino acid type-I transmembrane protein with four extracellular immunoglobulin superfamily-like domains. As an immune checkpoint receptor, Lag3 negatively regulates T cell proliferation, activation and homeostasis similarly to CTLA-4 and PD-1 (Workman and Vignali 2003, Workman, Cauley et al. 2004) and represents a promising cancer immunotherapeutic targets (Andrews, Marciscano

et al. 2017). Lag3 is associated with the T-cell receptor:CD3 complex (Hannier and Triebel 1999) and binds to major histocompatibility complex class-II molecules expressed on antigen-presenting cells (Baixeras, Huard et al. 1992, Huard, Mastrangeli et al. 1997). Lag3 also binds other molecules that may be expressed on tumor cells, including Galectin-3 and liver sinusoidal endothelial cell lectin (LSECtin) (Baixeras, Huard et al. 1992, Liu, Tang et al. 2004).

The cellular expression pattern and function of Lag3 in the central nervous system (CNS) are still elusive. A recent study reported that Lag3 was expressed by neurons, serving as a cellular receptor for pathologic α-synuclein (Mao, Ou et al. 2016). Neuronal expressed Lag3 binds to α-synuclein fibrils, triggers their endocytosis into neuronal cell body, facilitates the spreading of α-synuclein pathology and plays a vital role in α-synuclein transmission and PD pathogenesis. However, whether and how Lag3 may contribute to the pathogenesis of other similar neurodegenerative diseases like PrDs are still not clear.

Here we examined the expression levels of Lag3 in the mouse brain after prion infection and investigated its possible role in the progression of PrDs by using Lag3 knockout (Lag3 KO) mice. We found that the expression levels of Lag3 were relatively low in the adult mouse CNS; however, its expression levels were significantly upregulated after prion infection. Loss of Lag3 had no significant influence on PrD pathogenesis. Lag3 knockout mice and the wildtype (WT) littermate controls showed similar incubation time after RML6 prion inoculation. Accordingly, we did not find any difference regarding the PrP^{Sc} load, neurodegeneration and inflammatory gene expression profiles in the brains of Lag3 WT and KO mice after prion infection. These results suggest that Lag3 plays a negligible role in PrD pathogenesis and immune checkpoint blockade might not be an effective way to halt the progression of PrDs.

Results

Upregulation of Lag3 in RML6 prion infected mouse brain

To investigate the possible involvement of Lag3 in the pathogenesis of PrDs, we examined the expression levels of Lag3 in the mouse brains inoculated with scrapie prions (Rocky Mountain

Laboratory, Passage 6; RML6) or with non-infectious brain homogenate (NBH) for control. Through quantitative real-time PCR (qRT-PCR), we found that the mRNA levels of Lag3 were significantly upregulated in mouse brains inoculated with RML6 prion at the terminal stage of the disease. RNA expression levels were ~ 8-fold higher than those of NBH-inoculated brains (**Fig 1A**).

To further characterize the changes in Lag3 during the course of disease progression, we measured the mRNA levels of Lag3 in two different brain regions (hippocampus and cerebellum) at different time points after prion inoculation by RNA sequencing (RNA-Seq). We found that the expression levels of Lag3 in both brain regions were relatively low in the mice inoculated with NBH, with 10-20 counts in the cerebellum and 70-110 counts in the hippocampus (**Fig 1B** and **1C**). However, in accordance with our qRT-PCR results, the mRNA levels of Lag3 were significantly higher (~ 10 fold) in both brain regions at the terminal stage of prion infection, compared with the NBH controls (**Fig 1B** and **1C**). Expression of Lag3 was already upregulated at 16 weeks after prion inoculation and increased continuously during the disease progression (**Fig 1B** and **1C**). These results indicate that Lag3 expression is drastically induced during prion infection and may be involved in the pathogenesis of PrDs.

Similar PrD pathogenesis in Lag3 WT and KO mice

Based on the gene expression analyses mentioned above, we hypothesized that loss of Lag3 might influence the course of PrDs. To test this, we inoculated the Lag3 WT and KO mice with either RML6 prion or NBH through the intracerebral route. The deletion of Lag3 gene in the Lag3 KO mice was confirmed by qRT-PCR (**S1 Fig**). In addition, by Western blotting we found that loss of Lag3 did not alter the PrP^{C} protein expression levels in the mouse CNS (**S2 Fig**). Nonetheless, we found that the Lag3 WT and KO mice showed similar incubation time after prion infection, with median survival time 173.5 days for Lag3 WT and 171 days for Lag3 KO mice (**Fig 2A**). These results suggest that Lag3 provides no significant contribution to the pathogenesis of PrDs even if its expression levels are dramatically upregulated during prion infection.

Similar levels of PrP^{Sc} and neurodegeneration in Lag3 WT and KO mice

Next, we set out to examine whether there were any neuropathological difference between the Lag3 WT and KO mice at the terminal stage of prion infection. Through western blot combining with proteinase K (PK) digestion, we found that there were similar levels of PK-resistant PrP^{Sc} in the brains of Lag3 WT and KO mice (**Fig 2B**), which indicate that loss of Lag3 does not affect the generation of pathologic PrP aggregates in PrDs. To examine whether loss of Lag3 affects prion-induced neurotoxicity, we quantified the NeuN positive area in the CA1 region of hippocampus, one of the most affected brain regions after prion infection, in Lag3 WT and KO mice inoculated with either RML6 prion or NBH. Indeed, we found that, after prion infection, the number of neurons in hippocampal CA1 were significantly decreased in both Lag3 WT and KO mice (**Fig 2C** and **2D**). However, the extents of neurodegeneration in Lag3 WT and KO mice were similar (**Fig 2C** and **2D**), suggesting that loss of Lag3 has no significant influence on prion-induced neurotoxicity. Taken together, these results indicate that Lag3 plays an insignificant role in the development of major neuropathological phenotypes induced by prion infection.

Similar astrocytic and microglial reactions in Lag3 WT and KO mice

In addition to its expression in neurons (Mao, Ou et al. 2016), several studies reported that Lag3 was highly expressed and enriched in microglia both in the human and mouse brains (Zhang, Chen et al. 2014, Tasic, Menon et al. 2016, Galatro, Holtman et al. 2017). To investigate whether loss of Lag3 may affect the glial responses after prion infection, we evaluated the astrocytic and microglial reactions by Western blotting and immunohistochemistry in the brains of Lag3 WT and KO mice at the terminal stage of prion infection. We found that the protein levels of GFAP and Iba1 were significantly upregulated in the brains inoculated with RML6 prion, compared with the ones inoculated with NBH, in both of the Lag3 WT and KO mice (**Fig 3A-3C**). However, the upregulation levels of both of these proteins were similar between the Lag3 WT and KO mice (**Fig 3A-3C**).

As expected, we found that the density of GFAP+ reactive astrocytes and CD68+ reactive microglia was significantly increased after prion infection. However, there was no difference detected between the Lag3 WT and KO mice (**Fig 3D-3G**). These results indicate that loss of Lag3 does not affect astrocytic and microglial responses in PrDs.

Similar expression levels of inflammatory genes in prion-infected Lag3 WT and KO mice

To compare the inflammatory gene expression levels in the brains of the Lag3 WT and KO mice at the terminal stage of prion infection, we examined the mRNA levels of major inflammatory genes like tumor necrosis factor alpha (TNFα), Interleukin 1 beta (IL1β), Interleukin 12 beta (IL12β), cyclooxygenase 2 (Cox2), Inducible nitric oxide synthase (iNOS) and Transforming growth factor beta 1 (Tgfb1) by qRT-PCR. We found that, the expression levels of TNFα, IL12β and Tgfb1 were significantly upregulated, while the expression levels of IL1β, Cox2 and iNOS were drastically downregulated after prion infection (**Fig 4**). However, the mRNA levels of all these factors were similar between the Lag3 WT and KO mice with or without prion infection, except that the mRNA level of Cox2 was slightly higher in the brains of Lag3 KO mice, compared with the Lag3 WT mice, after prion infection (**Fig 4**). Together, these results suggest that the presence or absence of Lag3 does not exert a discernible effect onto the regulation of inflammatory genes induced or repressed by prion infection.

Discussion

This study was primarily inspired by a study performed by the Dawson lab that showed that Lag3 acts as a receptor for α-synuclein aggregates (Mao, Ou et al. 2016). Although α-synuclein and PrP^{Sc} are molecularly and biogenetically distinct, they both form highly ordered aggregates which coalesce into amyloid fibrils. Since it appears likely that Lag3 interacts with the amyloid-specific pattern of aggregated α-synuclein, we reasoned that it might interact also with further amyloids, potentially including prions. A decisive advantage of the prion infection models is their precision: mice exposed to a defined prion inoculum succumb to scrapie after a highly

predictable incubation time; even minor shifts in the incubation period allow to detect genetic modifiers of pathogenesis. However, we found that prion-infected Lag3-deficient mice progressed to terminal disease with the same kinetics and attack rates as WT mice, and exhibited the same type of CNS pathology. Therefore, we can confidently conclude that Lag3 is not a mediator of prion ingress into cells relevant to disease.

Using various technologies such as qRT-PCR and next generation RNA-Seq, we found that Lag3 was conspicuously upregulated in the brains of prion-infected mice. Even then, however, the increase of Lag3 expression seemingly contributed nothing to the pathogenesis of PrDs. We failed finding any significant difference between the Lag3 WT and KO mice regarding the PrP^{Sc} load, prion-induced neurotoxicity, glial cell reactions and inflammatory gene expression in the brain at the terminal stage of PrDs.

Since Lag3 was reported to be expressed in peripheral immune cells (Triebel, Jitsukawa et al. 1990, Kisielow, Kisielow et al. 2005, Workman, Wang et al. 2009), neurons (Mao, Ou et al. 2016) and microglia (Zhang, Chen et al. 2014, Tasic, Menon et al. 2016, Galatro, Holtman et al. 2017), the cellular contribution(s) to the increased Lag3 levels in the prion affected brains still needs to be determined. A factor preventing us answering this important question is the dearth of anti-Lag3 antibodies for immunohistochemical examinations of Lag3 expression in mouse brain sections. Based on the available data in the literature, infiltrating T cells may be a source of the CNS Lag3 expression during prion infection. Infiltrating T cells were reported to be present in prion-infected mouse brains and also in brains of patients suffering from Creutzfeldt-Jakob disease (CJD) (Lewicki, Tishon et al. 2003). Additionally, Lag3 is highly expressed and enriched in microglia isolated from both human and mouse brains (Zhang, Chen et al. 2014, Tasic, Menon et al. 2016, Galatro, Holtman et al. 2017). In fact, since the expression of Lag3 was so specific to isolated microglia, some study even suggested using Lag3 as one of the markers for identifying microglia in the adult CNS (Galatro, Holtman et al. 2017) .

Considering the dramatic microglial activation in the prion infected brain (Aguzzi and Zhu 2017), the increased Lag3 levels may primarily result from the increased microglia number induced by prion infection. Indeed, by using RNA-Seq, we found that mRNA levels of Lag3 were significantly upregulated as early as 16 weeks after prion inoculation (**Fig 1B** and **1C**), which is consistent with the time-course of microglia activation in prion infected mouse brain (Sandberg, Al-Doujaily et al. 2014). However, Lag3 seemed to play no role in regulating microglial activation and inflammatory gene expression (**Fig 3 and Fig 4**).

In recent years, accumulating evidence have indicated that peripheral immune system is implicated in the pathogenesis of neurodegenerative diseases such as AD and PD (Heneka, Golenbock et al. 2015, Wang, Liu et al. 2015, Prinz and Priller 2017). It was even suggested that boosting the systemic immunity through immune checkpoint inhibition might be a promising therapeutic intervention to combat these devastating disorders (Schwartz 2017). Indeed, previous studies indicated that blocking the checkpoint signaling on peripheral immune cells with anti-PD1 antibodies enhanced immune cell infiltration into the AD-affected mouse brain from choroid plexus through an interferon-γ dependent mechanism, resulting in amyloid clearance and cognitive improvement (Baruch, Rosenzweig et al. 2015, Baruch, Deczkowska et al. 2016). However, similar results were not observed in another study conducted by the joint force of three different institutions with several amyloid transgenic mouse models (Latta-Mahieu, Elmer et al. 2018).

Just like PD1, Lag3 was reported to be one of the key immune checkpoint molecules expressed on major peripheral immune cells, negatively regulating T cell proliferation, activation and homeostasis (Workman and Vignali 2003, Workman, Cauley et al. 2004). Therefore, our results not only imply that Lag3 may not be a functionally relevant prion receptor, but they also suggest that immune checkpoint blockade might not be an effective way to halt the progression of PrDs.

On a slightly more positive note, we have found that Lag3 deficiency does not worsen prion pathology. This may be comforting to those patients who harbor pathogenic mutations in the

prion gene and are at risk of developing genetic prion diseases (such as CJD, Fatal Familial Insomnia, and Gerstmann-Sträussler-Scheinker disease). These patients develop cancer with the same incidence as everybody else, and the need will inevitably arise to treat them with checkpoint inhibitors whose use is becoming extremely prevalent in both solid and hematological malignancies (Nguyen and Ohashi 2015, Andrews, Marciscano et al. 2017, Ribas and Wolchok 2018). The present study suggests that the use of such agents in patients with *PRNP* mutations is unlikely to have any prion-specific deleterious effect.

Materials and methods

Ethics statement

All animal experiments were performed according to Swiss federal guidelines ('Ethical Principles and Guidelines for Experiments on Animals' 3rd edition, 2005) and were approved by the Animal Experimentation Committee of the Canton of Zurich (permit 040/2015). All efforts were made to minimize animal discomfort and suffering.

Prion inoculation

Lag3 KO (a gift from Prof. Ted Dawson, Johns Hopkins University, School of Medicine, Baltimore, USA) and WT littermate control mice were intracerebrally inoculated with 30 µl of 0.1% normal brain homogenate (NBH) or brain homogenate from RML6 prion infected mice (RML6). Diagnosis of scrapie was undertaken according to appropriate clinical criteria, namely ataxia, limb weakness, front leg paresis and rolling. Mice were sacrificed at the terminal stage of prion disease. For RNA-sequencing experiments, C57/BL6 mice purchased from the Jackson laboratory were used and sacrificed at different time points after prion infection.

Western blot

Western blot was performed as previously described (Liu, Zhou et al. 2015), with small modifications. The primary antibodies used were mouse monoclonal antibody against actin (1:10000, Merck Millipore, MAB1501R), PrP^{C} (1:5000, POM1, homemade), GFAP (Sigma,

AMAB91033); rabbit polyclonal antibody against Iba1 (1:1000, Wako, 019-19741). Appropriate secondary antibodies (1:10000, Jackson ImmunoResearch Laboratories) were incubated according to the primary antibodies. Membranes were visualized and digitized with ImageQuant (LAS-4000; Fujifilm). Optical densities of bands were analyzed by using ImageJ. For the western blot examining PrP^{Sc}, protein samples were digested with proteinase K (60 μg/ml) for 30 minutes at 37 °C.

Immunofluorescence

Immunofluorescent staining was performed according to the procedure published previously (Liu, Zhou et al. 2015). For mouse brain tissue staining, 25 μm-thickness sections were cut by using Cryostat (Leica). The following antibodies were used, anti-GFAP (1:300, Agilent Technologies, Z0334), anti-NeuN (1:500, Abcam, ab177487) and anti-CD68 (1:200, BioRad, MCA1957). Brain sections were incubated in the primary antibody overnight at 4°C followed by incubation in the secondary antibody for 2 hours at room temperature. The images were captured by using the FLUOVIEW FV10i confocal microscope (Olympus Life Science) and quantified with ImageJ.

Quantitative real-time PCR (qRT-PCR)

Total RNA from mouse brain was extracted by using TRIzol (Invitrogen) according to the manufacturer's instruction and cDNA were synthesized by using the QuantiTect Reverse Transcription kit (QIAGEN). qRT-PCR was performed using the SYBR Green PCR Master Mix (Roche) on a ViiA7 Real-Time PCR system (Applied Biosystems). The following primers were used: Mouse actin: sense 5′-AGATCAAGATCATTGCTCCTCCT-3′; antisense, 5′-ACGCAGCTCAGTAACAGTCC-3′. Mouse Lag3: sense, 5′-CCAGGCCTCGATGATTGCTA-3′; antisense, 5′-CAGCAGCGTACACTGTCAGA -3′. Mouse TNFα: sense, 5′-ACGTCGTAGCAAACCACCAA-3′; antisense, 5′-ATAGCAAATCGGCTGACGGT -3′. Mouse IL1β: sense, 5′-TGCAGCTGGAGAGTGTGGATCCC-3′; antisense, 5′-TGTGCTCTGCTTGTGAGGTGCTG -3′. Mouse IL12β: sense, 5′-

TGGTTTGCCATCGTTTTGCTG-3′; antisense, 5′-ACAGGTGAGGTTCACTGTTTCT-3′. Mouse Cox2: sense, 5′-GGGCCATGGAGTGGACTTAAA-3′; antisense, 5′-ACTCTGTTGTGCTCCCGAAG -3′. Mouse iNos: sense, 5′-TCCTGGACATTACGACCCCT-3′; antisense, 5′-CTCTGAGGGCTGACACAAGG -3′. Mouse Tgfb1: sense, 5′-GTGGACCGCAACAACGCCATCT-3′; antisense, 5′-CAGCAATGGGGGTTCGGGCA-3′.

RNA sequencing

RNA sequencing on hippocampal and cerebellar tissues of mice intracerebrally injected with RML6 or NBH was performed as previously described (Nuvolone, Hermann et al. 2016, Nuvolone, Schmid et al. 2017). Briefly, total RNA was extracted using the RNeasy Plus universal mini kit (QIAGEN) and subjected to quality control using Bioanalyzer 2100 (Agilent Technologies) and Qubit (1.0) Fluorometer (Life Technologies). Libraries were prepared after poly A–enrichment with the TruSeq RNA Sample Prep kit v2 (Illumina) and sequencing was performed using the TruSeq SBS kit v4-HS (Illumina) on Illumina HiSeq 2500 at 1 × 100 bp. Data analysis was performed as previously described (Robinson, McCarthy et al. 2010, Nuvolone, Kana et al. 2013) using the R packages GenomicRanges (Lawrence, Huber et al. 2013) and edgeR from Bioconductor Version 3.0.

Statistical analyses

Unless otherwise mentioned, unpaired, two-tailed student's t-tests were used for comparing data from two groups. All data was presented as mean ± SEM. Statistical analysis and data visualization was done by using GraphPad Prism 7 (GraphPad). P-values <0.05 were considered statistically significant.

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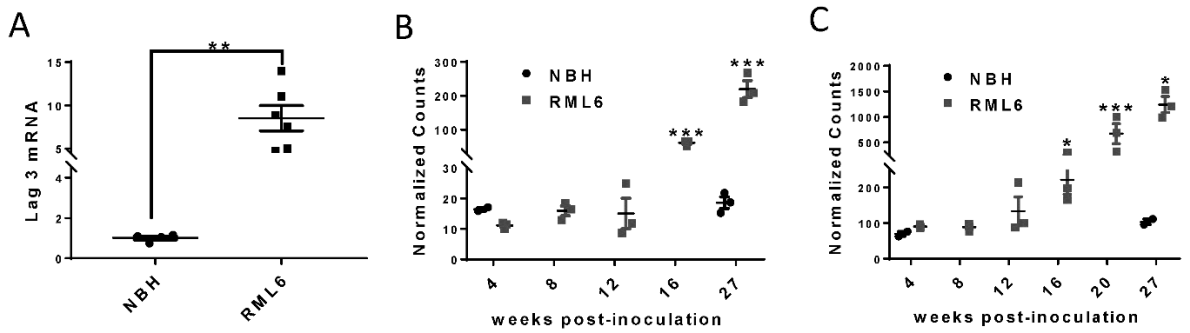


Fig 1. Upregulation of Lag3 in prion-infected mouse brain. A, qRT-PCR results of Lag3 expression in the mouse brains inoculated with either NBH or RML6 prion. ** P< 0.01 (n=6). **B-C**, RNA-Seq data of Lag3 expression in hippocampus (**B**) and cerebellum (**C**) at different time points after prion infection. * P<0.05; *** P< 0.001 (n=3 at each time points). NBH, Normal Brain Homogenate; RML6, Rocky Mountain Laboratory, Passage 6.

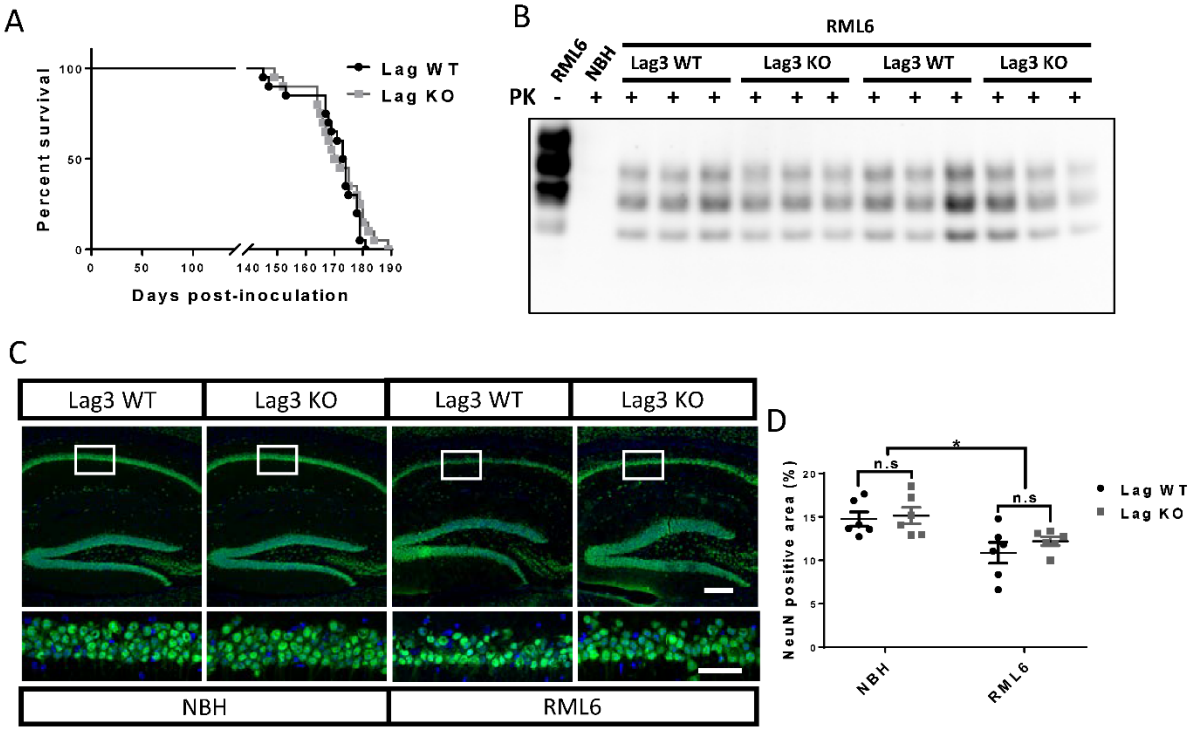


Fig 2. Incubation times, PrP^{Sc} levels and neurodegeneration in Lag3 WT and KO mice after prion infection. A, survival curve of Lag3 WT and KO mice after prion infection. Median incubation time: 173.5 days for Lag3 WT mice and 171 days for Lag3 KO mice. Twenty mice containing both genders were pooled together for both of the Lag3 WT and KO mice. **B**, representative image of a Western blot examining PK-resistant PrP^{Sc} levels in the brains of Lag3 WT and KO mice. **C**, representative images of NeuN staining in the hippocampus of the Lag3 WT and Lag3 KO mice. Scale bars: 100 μm for the upper images and 50 μm for the lower images. **D**, quantitative data of the NeuN positive areas shown in **C**. * P< 0.05 (n=6). n.s, not significant. NBH, Normal Brain Homogenate; RML6, Rocky Mountain Laboratory, Passage 6.

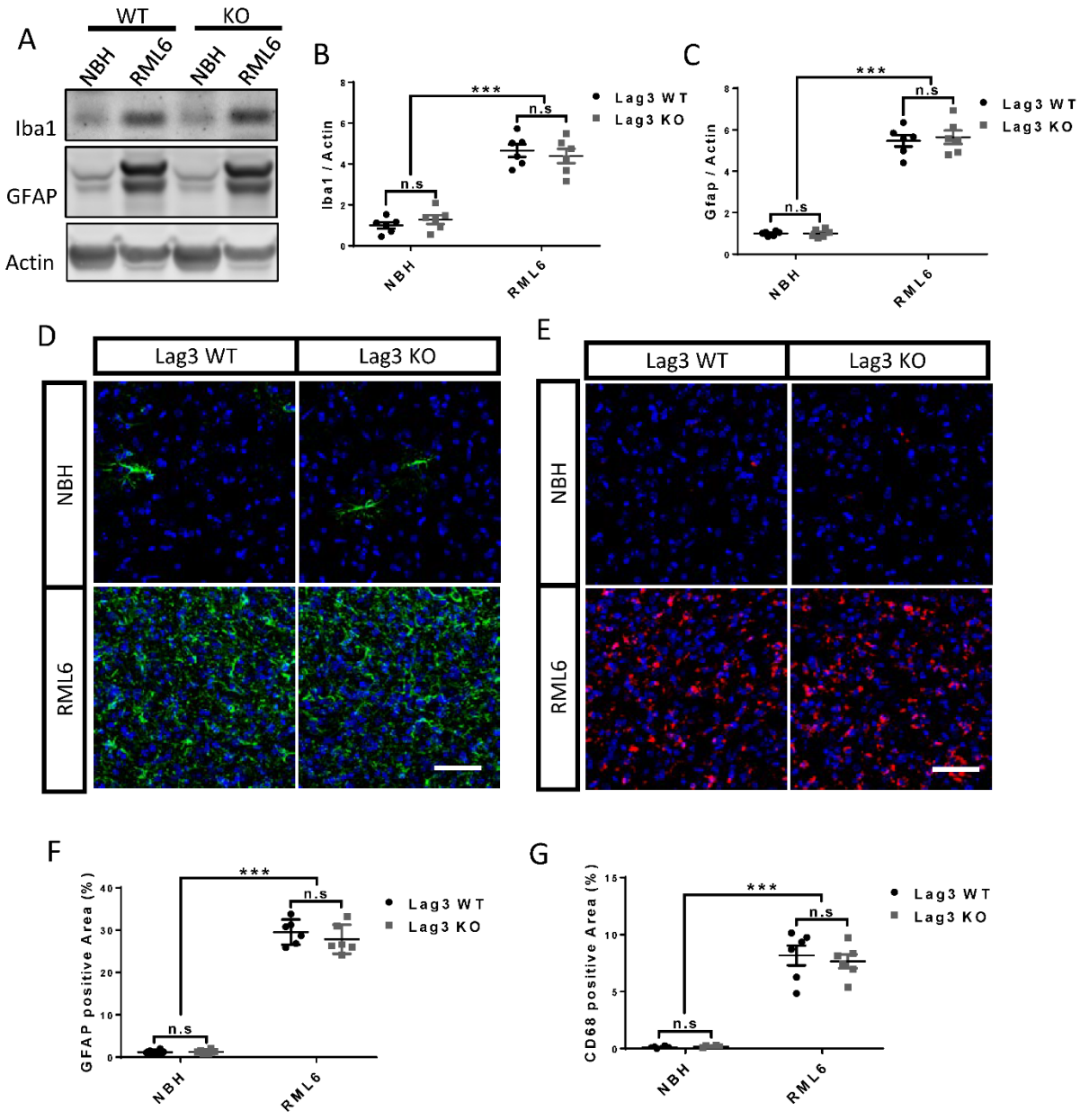


Fig 3. Astrocytic and microglial activation in the brains of terminal-sick Lag3 WT and KO mice. A, representative images of GFAP and Iba1 western blot in the brains of Lag3 WT and KO mice inoculated with NBH or RML6. **B-C**, quantitative data of GFAP (**B**) and Iba1 (**C**) protein levels shown in a. *** P< 0.001(n=6). n.s, not significant. **D-E**: representative images of immunofluorescent staining of GFAP (**D**) and CD68 (**E**) in the brains of Lag3 WT and KO mice inoculated with NBH or RML6. Scale bar: 50 μm. **F-G**, quantitative data of GFAP (**F**) and CD68 (**G**) positive areas shown in **D** and **E**, respectively. *** P< 0.001 (n=6). n.s, not significant. NBH, Normal Brain Homogenate; RML6, Rocky Mountain Laboratory, Passage 6.

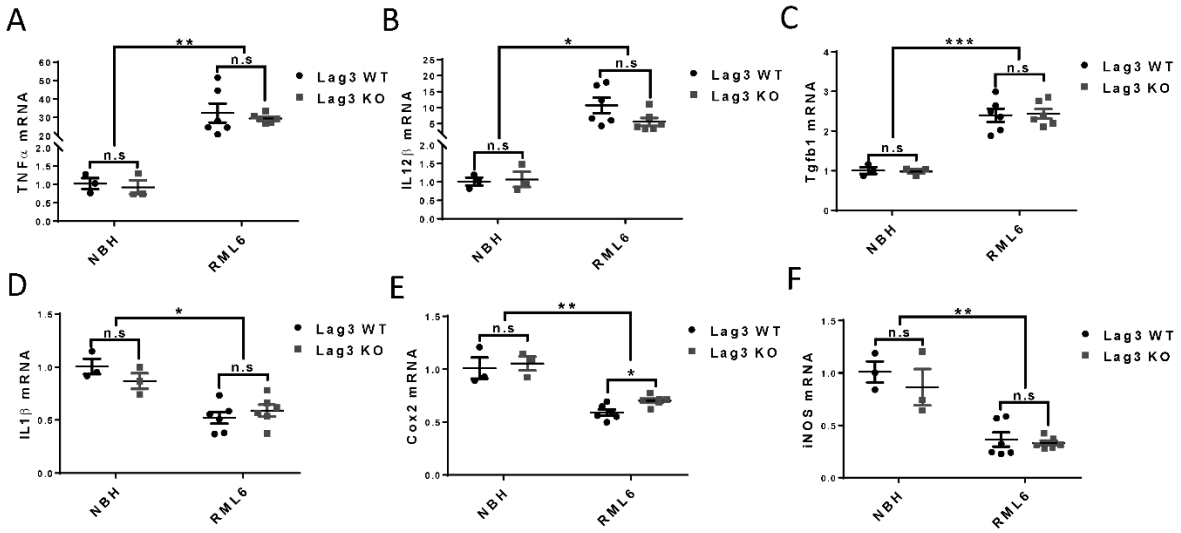
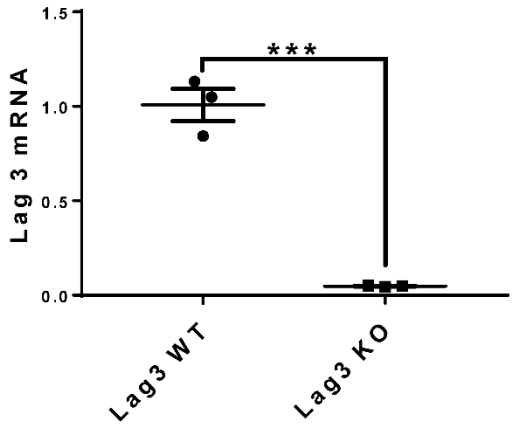
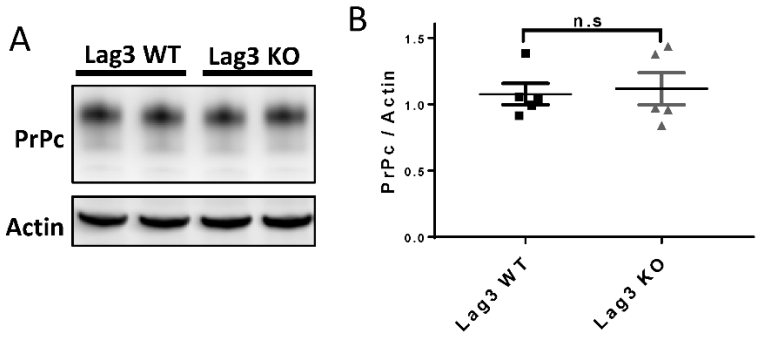


Fig 4. Expression levels of inflammatory genes in the brains of terminal-sick Lag3 WT and KO mice. qRT-PCR results of TNFα (**A**), IL12β (**B**) and Tgfb1 (**C**), IL1β (**D**), Cox2 (**E**) and iNOS (**F**) expression in the brains of Lag3 WT and KO mice inoculated with NBH or RML6. * P<0.05; ** P< 0.01; *** P< 0.001 (n=3 for NBH groups and n=6 for RML6 groups). n.s, not significant. NBH, Normal Brain Homogenate; RML6, Rocky Mountain Laboratory, Passage 6.

Supplementary figure



S1 Fig. Depletion of Lag3 in the brain of Lag3 KO mice. qRT-PCR results of Lag3 expression in the brains of Lag3 WT and KO mice. *** P< 0.001 (n=3).



S2 Fig. PrP^{C} protein levels in the brains of Lag3 WT and KO mice. A, representative images of PrP^{C} western blot in the brains of Lag3 WT and KO mice. **B**, quantitative data of PrP^{C} protein levels shown in **A**. n=5. n.s, not significant.