Loss of Interleukin Receptor-Associated Kinase 4 Signaling Suppresses Amyloid Pathology and Alters Microglial Phenotype in a Mouse Model of Alzheimer's Disease

Brent Cameron,¹ Wayne Tse,¹ Raza Lamb,¹ Xiaoxia Li,² Bruce T. Lamb,^{1,3,4} and Gary E. Landreth¹

¹Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio 44106; Departments of ²Immunology and ³Neurosciences, Cleveland Clinic Foundation, Cleveland, Ohio 44195; and ⁴Department of Genetics, Case Western Reserve University, Cleveland, Ohio 44106

Alzheimer's disease (AD) is typified by the deposition of amyloid in the brain, which elicits a robust microglial-mediated inflammatory response that is associated with disease exacerbation and accelerated progression. Microglia are the principal immune effector cells in the brain and interact with fibrillar forms of A β (fA β) through a receptor complex that includes Toll-like receptors (TLR) 2/4/6 and their coreceptors. Interleukin receptor-associated kinases (IRAKs) are essential intracellular signaling molecules for transduction of TLR signals. Studies of mouse models of AD in which the individual TLRs are knocked out have produced conflicting results on roles of TLR signaling in amyloid homeostasis. Therefore, we disrupted a common downstream TLR signaling element, IRAK4. We report that microglial IRAK4 is necessary *in vitro* for fA β to activate the canonical pro-inflammatory signaling pathways leading to activation of p38, JNK, and ERK MAP kinases and to generate reactive oxygen species. *In vivo* the loss of IRAK4 function results in decreased A β levels in a murine model of AD. This was associated with diminished microgliosis and astrogliosis in aged mice. Analysis of microglia isolated from the adult mouse brain revealed an altered pattern of gene expression associated with changes in microglial phenotype that were associated with expression of IRF transcription factors that govern microglial phenotype. Further, loss of IRAK4 function also promoted amyloid clearance mechanisms, including elevated expression of insulin-degrading enzyme. Finally, blocking IRAK function restored olfactory behavior. These data demonstrate that IRAK4 activation acts normally to regulate microglial activation status and influence amyloid homeostasis in the brain.

Introduction

Alzheimer's disease (AD) is typified by loss of memory and cognition and ultimately by extensive death of neurons. It is pathologically defined by the presence of plaques within the brain, composed of β -amyloid (A β) peptides, and the hyperphosphorylated protein tau, which forms neurofibrillary tangles (Selkoe, 2000; Querfurth and LaFerla, 2010). A β deposition results in the migration of microglia, the brain's tissue macrophage, to plaques where they form stable associations (Meyer-Luehmann et al., 2008). The role of microglia in the pathogenesis of AD has been described as the archetypal two-edged sword (Wyss-

Coray, 2006). On one hand, the phagocytic capabilities of microglia help to clear amyloid deposits from the brain parenchyma. Conversely, plaque-associated microglia become phenotypically activated into an inflammatory state and express canonical pro-inflammatory cytokines that lead to bystander damage of surrounding neural tissue and exacerbation of disease pathogenesis (Akiyama et al., 2000).

It has been of great interest to identify the mechanisms by which microglia perform their discrete functions in response to amyloid deposits. Microglia employ a cell-surface receptor complex composed of innate immune receptors including Toll-like receptors TLR4, TLR2, TLR6, their coreceptors CD36 and CD14, and SRA, $\alpha_6\beta_1$ integrin, and CD47. This complex directly interacts with amyloid fibrils and activates downstream signaling events (Bamberger et al., 2003; El Khoury et al., 2003; Reed-Geaghan et al., 2009).

TLRs are pattern recognition receptors used by the innate immune system to mount an immediate, cell autonomous immune response to pathogens or danger signals (Kawai and Akira, 2011). There are 12 different murine TLRs and all (except TLR3) are reliant on the interleukin receptor-associated kinase IRAK4 to perform their inflammatory functions. IRAK4 is the first kinase activated upon TLR ligation (or in response to IL-1 β and IL-18) and is a gatekeeper of TLR signaling (Li et al., 2002; Suzuki et al., 2002).

Studies of TLR actions in murine AD models have led to conflicting results as to their exact role in AD pathogenesis. We re-

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Correspondence should be addressed to either of the following: Gary E. Landreth, Department of Neuroscience, Case Western Reserve University, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, E-mail: gel2@case.edu; or Bruce T. Lamb, Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, NC30/9500 Euclid Avenue, Cleveland, OH 44195, E-mail: lambb@ccf.org.

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ported decreased A β plaque levels in a murine AD model lacking the TLR4 coreceptor CD14 (Reed-Geaghan et al., 2010). Another study using a mouse model with defective TLR4 signaling demonstrated increased amyloid burden at advanced ages (Tahara et al., 2006; Song et al., 2011). However, knock-out of TLR2 resulted in decreased amyloid burden at younger ages but no difference at older ages (Richard et al., 2008). The basis of these disparate results is unknown. To resolve the controversy over the function of TLRs in AD models we have generated an AD model that lacks any endogenous IRAK4 kinase activity (Kim et al., 2007). In this model a kinase-dead mutant *IRAK4* gene was knocked into the endogenous locus, leaving the receptor complex intact but silencing all IRAK4-dependent signaling. This has allowed the dissection of signaling pathways that microglia employ to respond to fibrillar A β (fA β). We report that loss of IRAK4 function blocks microglial inflammatory responses *in vitro*. *In vivo*, loss of IRAK4 function reduced amyloid burden at later ages, reduced gliosis, altered microglial phenotype including altered expression of interferon regulator factor (IRF) transcription factors, and restored normal behavior.

Materials and Methods

Animals. APPPS1–21, referred to as APPPS1, mice carrying the KM670/671NL mutation in human amyloid precursor protein and L166P mutation in presenilin 1 on a C57BL/6J background have previously been described (Radde et al., 2006) and were obtained from Dr. Mathias Jucker (University of Tübingen, Germany). Mice possessing a mutant, kinase-dead *IRAK4* gene that was knocked into the endogenous locus (*IRAK4*^{KI/KI}) on a C57BL/6J background have been previously described (Kim et al., 2007). *IRAK4*^{KI/KI} mice were crossed to APPPS1 to generate *IRAK4*^{KI/WT}; APPPS1 or *IRAK4*^{KI/WT}. Next, *IRAK4*^{KI/WT}; APPPS1 were bred to *IRAK4*^{KI/KI} to generate *IRAK4*^{KI/KI}; APPPS1 or *IRAK4*^{KI/WT}; APPPS1 or *IRAK4*^{KI/KI} or *IRAK4*^{KI/WT}. Both males and females were used in this study. Animals were killed at 4 or 8 months of age. All animals were housed and cared for in accordance with Case Western Reserve University Institutional Animal Care and Use Committee guidelines.

Tissue collection. Animals were lightly anesthetized with isoflurane and killed by cervical dislocation. The brain was bisected and one hemisphere was fixed in 4% paraformaldehyde (PFA) in phosphate buffer. The hippocampus and cortex were dissected from the opposite hemisphere and snap-frozen on dry ice. For biochemical assays, cortex and hippocampus were homogenized in tissue homogenization buffer (250 mM sucrose, 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 1 mM EGTA) containing Protease Inhibitor Cocktail (Sigma) using a glass on glass Dounce homogenizer. Sequential extraction of soluble A β using diethylamine followed by extraction of insoluble A β using formic acid was performed and A β _{3–40} and A β _{3–42} were analyzed by ELISA as previously described (Jiang et al., 2008).

Reagents. Antibodies for p38, phospho-p38, JNK, phospho-JNK, and ERK, and phospho-ERK were purchased from Cell Signaling Technology. IRF4 and IRF5 antibodies were purchased from Abcam. fA β _{1–42} was generated by dissolving lyophilized A β _{1–42} (American Peptide) in double-distilled, endotoxin-free water at a concentration of 1 mM and incubated at 37°C for 5 d and then stored at 4°C as previously described (Burdick et al., 1992; Lorenzo and Yankner, 1994). Immediately before use, A β was sonicated in a water-bath sonicator for 30 min. Lipopolysaccharide (LPS) (O111:B4) was purchased from List Biological Laboratories and sonicated for 30 min before use. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma.

Cell stimulation and Western blots. Neonatal primary microglia were plated at a density of 2×10^6 cells per 60 mm culture dish. Cells were changed to serum-free DMEM/F12 for 48–72 h before stimulation. Cells were then stimulated with fA β _{1–42} (5 μ M). After stimulation, the medium was aspirated and the cells washed with PBS. Cells were collected and sonicated in 1% Triton lysis buffer (1% Triton, 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 40 mM NaF, 1 mM EDTA, and 1 mM EGTA) containing Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktail 2 and

Cocktail 3 (Sigma-Aldrich). Protein concentration was determined by the BCA method. Equivalent amounts of protein were resolved by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, blocked in appropriate blocking solution, and probed with primary antibodies and secondary antibodies conjugated to horse radish peroxidase (GE Healthcare). Proteins were visualized by with enhanced chemiluminescence (Millipore). For loading controls, membranes were stripped and re-probed with appropriate nonphosphorylated antibodies.

Cell culture. Neonatal primary microglia from C57BL/6J or *IRAK4*^{KI/KI} neonatal mice were cultured from P0–P3 mouse pups as previously described (Reed-Geaghan et al., 2009). Briefly, mice were decapitated and the brain was removed and rinsed in PBS + 1 g/L glucose. The brain was then mechanically dissociated and digested in 0.5% trypsin-EDTA for 20–30 min at 37°C. Digestion was stopped by addition of DMEM/F12 media (Invitrogen) containing 10% heat-inactivated fetal bovine serum (FBS; Invitrogen) and 1% penicillin-streptomycin and trituration. The resultant homogeneous cell suspension was plated in 150 mm culture dish for 17–21 d at 37°C and 5% CO₂. Subsequently, loosely attached microglia were separated from the dish by shaking at room temperature. Astrocytes were removed by incubation in 0.25% trypsin/HBSS diluted 1:4 in serum-free DMEM/F12 for 1 h at 37°C. The remaining firmly attached microglia were removed by incubation in 0.25% trypsin/PBS. Both pools of microglia were combined, counted, and plated for subsequent experiments.

Reactive oxygen species analysis. Intracellular superoxide generation was measured as previously described (McDonald et al., 1997; Reed-Geaghan et al., 2009). Briefly, 1×10^5 *IRAK4*^{KI/KI} or C57BL/6J neonatal microglia were plated in a 24-well plate. Cells were switched to serum-free DMEM/F12 for 18+ h and then stimulated with either fA β _{1–42} (5 μ M) or LPS (100 ng/ml) for 30 min in the presence of nitroblue tetrazolium (NBT; 1 mg/ml; Roche). Afterward, cells were washed with PBS and fixed with 2% PFA. After fixation, >300 cells from three replicate wells per condition for each experiment were counted and the percentage of cells with a blue precipitate was quantified. Each experiment was repeated three times.

Immunohistochemistry. PFA-fixed hemispheres were embedded and frozen in Optimal Cutting Temperature compound (Tissue Tek). Coronal sections (10 μ m) were cut on a Leica 1950 Cryostat. For 6E10 staining, sections were treated with 70% formic acid, then blocked in 5% normal goat serum in PBS for 1 h before incubation with primary antibodies: 6E10 (Signet Laboratories); Iba1 (Wako); glial fibrillary acidic protein (GFAP; DAKO) overnight. For immunofluorescent staining, appropriate Alexa-conjugated secondary antibodies were added, nuclei were stained with DAPI, and slides were coverslipped in ProLong Gold (Invitrogen). Rehydrated sections were incubated with thioflavin S (Sigma), and rinsed with 70% ethanol. Nuclei were stained with propidium iodide (Roche) and coverslipped in ProLong Gold. Images were obtained on a Leica DM 5000B microscope. For quantification of thioS staining, three slides evenly spaced along the rostrocaudal axis containing the hippocampus for each mouse were selected and matched between genotypes, and one section per slide was analyzed for quantification. Plaque counts represent the average of the three sections per mouse. For quantification of Iba1, GFAP, and 6E10 staining, three nonoverlapping 10 $\times$ images of the cortex were obtained per section from three equally spaced sections per mouse per genotype. Cortical images were quantified using ImagePro Software (MediaCybernetics) for percentage area stained and plaque diameter (in pixel units). For each mouse, averages were calculated across images such that each animal only contributed a single value. For quantification of Iba1 staining and GFAP staining per 6E10 area, the Iba1 or GFAP area was divided by the corresponding 6E10 area for each image before calculating averages per mouse.

Adult microglial isolation. Isolation of adult microglia was performed as previously described (Hickman et al., 2008). Briefly, mice were anesthetized with Avertin and transcardially perfused with PBS/2.0 mM EDTA. The brains were then extracted (sans cerebelli) and mechanically dissociated using a sterile razor blade. Brains were then enzymatically digested in RPMI media (Invitrogen) for 45–60 min at 37°C/5% CO₂ containing Collagenase Type 3 (0.2%; Worthington) and Dispase (2.0 U/ml; Invitrogen). Digestion was stopped by addition of PBS/2.5 mM

EDTA/1% FBS and homogenates were triturated to obtain a homogeneous cell suspension. After centrifugation, brain homogenates were resuspended in an RPMI/Percoll mixture and centrifuged to isolate the microglia. After isolation, the Percoll was diluted with PBS and the microglia were pelleted and immediately frozen at -80°C until isolation of mRNA.

mRNA isolation, reverse transcription, quantitative PCR. Isolation of mRNA from adult microglia was performed using an RNeasy Mini kit (Qiagen). mRNA was quantified using a NanoDrop (Thermo Scientific). Equivalent amounts of RNA were reverse transcribed using a QuantiTect Reverse Transcription kit (Qiagen) including a genomic DNA elimination step. The cDNA was preamplified to allow detection of low copy number targets using TaqMan PreAmp master Mix (Applied Biosystems). Preamplified cDNA was detected using an Applied Biosystems StepOne Plus and the specified TaqMan assays and quantified using the $\Delta\Delta C_t$ method with GAPDH as the internal housekeeping gene control using the Applied Biosystems StepOne Software (v 2.1). All data presented represents relative quotient (RQ) values with 95% confidence interval indicated while the statistical analyses were performed on $\Delta C_t \pm \text{SEM}$ for each target gene as described by Yuan et al. (2006).

Behavior. Novel odor investigation and habituation were performed as described previously (Wesson et al., 2010). Briefly, mice were individually housed in clean cages 24–48 h before testing to eliminate background odors while still eliminating confounds from a novel environment. Mice were then presented with four odors (diluted in mineral oil; 2-heptanone, ethyl valerate, (R)-(+)-limonene, and isoamyl acetate; Sigma) in four separate blocks. Each block consisted of a single odor-laced cotton swab of an individual odor being presented through the air port in the cage, eliminating novel object confounds and prohibiting physical interaction with the odor delivery device, four consecutive times for each odor for 20 s periods separated by intertrial intervals of 30 s. Time spent investigating the novel odor in a snout-oriented manner within 1 cm of the port was recorded during testing by a single observer blinded to genotype.

Statistical analyses

Reported values are average $\pm$ SEM. Significance was determined using GraphPad Prism statistical software (GraphPad Software) to run either one-way ANOVA with Tukey's *post hoc* test or Student's *t* test where indicated in the figure legends.

Results

IRAK4 activity is necessary for $\text{A}\beta$ to initiate inflammatory signaling *in vitro*

The concomitant increase in neuroinflammation as amyloid pathology progresses during AD has been well documented and reflects the actions of many different but overlapping microglial signaling systems (Akiyama et al., 2000; Selkoe, 2000; Querfurth and LaFerla, 2010). We tested involvement of IRAK4 signaling in the inflammatory response to $\text{A}\beta$ by functional inactivation of IRAK4.

Increases in activated forms of the p38 MAP kinase have been found in the brains of both mouse models of AD and AD patients (Hensley et al., 1999; Zhu et al., 2000; Savage et al., 2002; Jin et al., 2005; Giovannini et al., 2008; Meyer-Luehmann et al., 2008) and it is activated following microglia exposure to $\text{fA}\beta$ (McDonald et al., 1998; Pyo et al., 1998; Wyss-Coray, 2006). We have previously shown that this activation is dependent upon a cell-surface receptor complex that includes TLR2, TLR4, and CD14 (Akiyama et al., 2000; Bamberger et al., 2003; Reed-Geaghan et al., 2009). Here we investigated if IRAK4 activity was necessary for $\text{A}\beta$ activation of p38, as assessed through its phosphorylation status. Microglia from wild-type (WT) animals were able to activate p38 in a time-dependent manner after exposure to $\text{fA}\beta$ (Fig. 1A), whereas the microglia isolated from IRAK4^{KI/KI} were not able to activate p38. This deficiency in p38 activation in the absence of IRAK4 activity

Figure 1. IRAK4 is required for $\text{fA}\beta$ stimulated pro-inflammatory signaling and ROS generation. **A**, Primary microglia were isolated from WT C57BL/6J or IRAK4 KI mice (P0–P2) and were treated with $5 \mu\text{M}$ $\text{fA}\beta_{1-42}$ for the indicated times. Cell lysates were collected and evaluated by Western blot for the indicated phosphorylated proteins. Blots were stripped and reprobed for nonphosphorylated proteins as loading controls. **B**, Microglia were treated with 100 ng/ml LPS, $2.5 \mu\text{M}$ $\text{fA}\beta_{1-42}$, or 390 nM PMA in the presence of NBT. Cells (>300 from 3 separate fields) were counted and the fraction blue formazan-positive cells determined. Data represent mean $\pm$ SEM for three independent experiments. $***p \leq 0.05$, one-way ANOVA with Tukey's *post hoc* analysis.

is similar to that reported in other chronic inflammatory disease models (Bamberger et al., 2003; El Khoury et al., 2003; Koziczak-Holbro et al., 2008; Reed-Geaghan et al., 2009). Thus IRAK4 is positioned upstream of p38 activation and downstream of the TLR receptors that are known to be involved in the recognition of $\text{fA}\beta$.

Other stress-activated kinase pathways such as the JNK pathway have been implicated in AD pathogenesis. Activation of JNK downstream of TLR7 ligands (Koziczak-Holbro et al., 2007; Kawai and Akira, 2011), TLR4 ligands (Li et al., 2002; Suzuki et al., 2002; Koziczak-Holbro et al., 2008), and IL-1 β (Lye et al., 2004; Reed-Geaghan et al., 2010) has been shown to be dependent on IRAK4 activity. We observed suppression of JNK activation in response to $\text{fA}\beta$ in microglia from IRAK4^{KI/KI} animals, as measured by pJNK levels, whereas the WT microglia were capable of activating JNK (Fig. 1A).

We also assessed activation of ERK kinases, which have been shown to be downstream of $\text{fA}\beta$ -induced inflammatory signaling (McDonald et al., 1998; Combs et al., 1999; Kim et al., 2004; Tahara et al., 2006; Giovannini et al., 2008; Song et al., 2011). We found that compared with WT microglia, IRAK4^{KI/KI} microglia are unable to activate the ERK kinases (Fig. 1A).

IRAK4 function is required for $\text{A}\beta$ to stimulate reactive oxygen species production

Microglia exposed to $\text{fA}\beta$ have been shown to stimulate NADPH oxidase assembly leading to generation of reactive oxygen species (ROS) (McDonald et al., 1997; Wilkinson and Landreth, 2006;

Figure 2. Loss of IRAK4 kinase activity reduces Aβ levels *in vivo*. Serial extraction of soluble (DEA extract) (**A**, **B**) and insoluble (formic acid extract) Aβ (**C**, **D**) was performed on brain homogenates of 4-month-old (**A**, **C**) or 8-month-old (**B**, **D**) APPPS1 or APPPS1;IRAK4^{KI/KI}. Aβ_{x-40} and Aβ_{x-42} were detected using sandwich ELISA. At 4 months mean total Aβ (ng Aβ/mg total protein) for APPPS1 mice were DEA soluble Aβ₄₀ = 3.05 and Aβ₄₂ = 46.26 and formic acid (FA) soluble Aβ₄₀ = 67.67 and Aβ₄₂ = 637.39. At 8 months mean total Aβ values for APPPS1 mice were DEA soluble Aβ₄₀ = 3.22 and Aβ₄₂ = 44.95 and FA soluble Aβ₄₀ = 88.96 and Aβ₄₂ = 1500.00. These data represent normalized mean ± SEM. *N* ≥ 5 animals/genotype/age. **p* ≤ 0.05, Student's two-tailed *t* test.

Wilkinson et al., 2006; Richard et al., 2008). This response has been shown to be dependent on the microglial cell-surface receptor complex including TLR2, TLR4, and CD14 (Bamberger et al., 2003; Kim et al., 2007; Reed-Geaghan et al., 2009). We sought to determine whether generation of ROS was dependent on upstream IRAK4 signaling. Microglia from WT C57BL/6J or IRAK4^{KI/KI} animals were exposed to fAβ. Microglia from WT animals generated a robust ROS response in response to either fAβ, LPS, or PMA (Fig. 1*B*). LPS served as a positive control for TLR4 activation, which we have previously shown is necessary for microglia to generate a ROS response (Radde et al., 2006; Reed-Geaghan et al., 2009). PMA, acting through a protein kinase C-dependent, TLR-independent pathway, acts as a positive control demonstrating the functional integrity of the signaling pathway in the absence of IRAK4 activity (McDonald et al., 1997; Bianca et al., 1999; Bamberger et al., 2003; Kim et al., 2007). However, microglia from IRAK4^{KI/KI} mice were unable to generate ROS when exposed to either fAβ or LPS (Fig. 1*B*). These data demonstrate that IRAK4 activity is required for the ability of microglia to generate ROS in response to the stimulation of the fAβ cell surface receptor complex.

Loss of IRAK4 kinase activity reduces Aβ burden *in vivo*

The APPPS1 mouse model expresses the KM670/671NL mutation in amyloid precursor protein (APP) and the L166P mutation in presenilin 1 driven by the Thy1 promoter that results in early and rapid deposition of amyloid beginning at 6 weeks of age (Radde et al., 2006; Jiang et al., 2008). This mouse was crossed to the IRAK4^{KI/KI} mice to generate AD mice that lacked IRAK4 activity. We assessed amyloid levels at 4 and 8 months of age, representing intermediate and later stages of disease progression, allowing us to evaluate the longitudinal effects of microglial TLR signaling on amyloid homeostasis.

We did not find significant changes in either soluble (Fig. 2*A*) or insoluble (Fig. 2*C*) levels of either Aβ₄₀ or Aβ₄₂ at 4 months of age. This indicates that at a moderate stage of plaque deposition that IRAK4-dependent TLR signaling plays a minor role in Aβ clearance or maintenance. However, by 8 months of age, when

extensive Aβ deposition has occurred, loss of IRAK4 activity significantly reduces levels of soluble Aβ₄₂ by 43% (Fig. 2*B*) and insoluble Aβ₄₂ by 30% (Fig. 2*D*). These data suggest that activation of IRAK4 in later stages of disease impairs Aβ clearance mechanisms. *In vitro* studies demonstrated that there was no difference between WT and IRAK4^{KI/KI} microglia in their capacity for fluid phase uptake of soluble Aβ (data not shown) (Burdick et al., 1992; Lorenzo and Yankner, 1994; Mandrekar et al., 2009), or for Aβ stimulated phagocytic uptake of beads (data not shown) (Reed-Geaghan et al., 2009, 2010). Also, there is no difference in APP expression or its processing as monitored by levels of α or β C-terminal fragments between the APPPS1 and the APPPS1;IRAK4^{KI/KI} mice as assessed by Western blot indicating that they express and process equivalent amounts of the mutant APP transgene (data not shown). Analysis of mice lacking IRAK4 activity at 2 months of age demonstrated a paradoxical and unexplained increase in APP processing and subsequently soluble and insoluble Aβ levels; however, this effect was not observed in older mice. These data demonstrate dynamic responses of microglia during the course of disease progression and its alteration by inactivation of IRAK4 signaling.

Loss of IRAK4 kinase activity reduces plaque burden

Deposition of amyloid into dense core plaques has been correlated with the accumulation and activation of microglia (Perlmuter et al., 1990; Hsiao et al., 1996; McDonald et al., 1997; Frautschy et al., 1998; Reed-Geaghan et al., 2009). Furthermore, plaque formation is associated with dysmorphic neurites (Hickman et al., 2008; Meyer-Luehmann et al., 2008). Therefore we analyzed dense core, thioS+ plaques. There was no significant difference in plaque number, area, or size at 4 months between the APPPS1 and APPPS1;IRAK4^{KI/KI} mice in the hippocampus (3*C*, *D*, *L–N*). We observed a small yet significant increase in plaque area in the cortex at 4 months of age in the APPPS1;IRAK4 KI animals compared with the APPPS1 animals (Fig. 3*J*) that was not explained by plaques number (Fig. 3*I*), but rather by larger plaques at 4 months of age (Fig. 3*K*). At 8 months, however, we found a significant decrease in thioS+ plaque number, area, and size in the APPPS1;IRAK4^{KI/KI} animals compared with the APPPS1 animals in the cortex (Fig. 3*F*, *I–K*) and hippocampus (Fig. 3*H*, *L–N*), consistent with the ELISA data (Fig. 2*D*). The total plaque burden, as evaluated by staining with 6E10 (Fig. 4), revealed no significant differences in the cortex at 4 months of age. Analysis at 8 months of age, however, demonstrated an approximate 50% reduction in plaque burden (Fig. 4*O*), due largely to a significant reduction in plaque size (Fig. 4*P*). These data demonstrate that loss of IRAK4 signaling diminishes Aβ deposition and enhances clearance that is related to age and disease progression.

IRAK4 kinase activity contributes to reactive gliosis

Phenotypic activation of microglia and astrocytes in AD is associated with increases in inflammatory mediators and is correlated with the

Figure 3. Loss of IRAK4 kinase activity reduces dense core plaques. APPPS1 (**A**, **C**, **E**, **G**) or APPPS1;IRAK4^{KI/KI} (**B**, **D**, **F**, **H**) mice were killed at the indicated age and coronal sections were stained with thioflavin S. Cortical (**I**) and hippocampal (**L**) thioS-positive deposits from matching sections were counted and averaged. Plaque area in the cortex (**J**) and hippocampus (Hpc) (**M**) was quantified using ImagePro software. Plaque diameter in the cortex (**K**) and in the hippocampus (**N**) was quantified using ImagePro software. Graphs represent mean $\pm$ SEM at each age. $N \geq 5$ animals/genotype/age. $**p \leq 0.01$, Student's two-tailed t test for APPPS1 vs. APPPS1;IRAK4 KI.

deposition of amyloid (Akiyama et al., 2000; Cameron and Landreth, 2010) and with cognitive decline (Edison et al., 2008; Wesson et al., 2010). Given the central role of IRAK4 in TLR signaling we sought to determine whether there were alterations in microglial or astrocyte activation in our animal model that were correlated with the differences in amyloid levels. We found that IRAK4 protein levels were elevated in the APPPS1 mice compared with the WT mice at 8 months of age as assessed by Western blot of whole-brain homogenates (data not shown). This indicates that IRAK4 is responsive to the deposition of amyloid, likely the result of microgliosis in the transgenic mice. At 4 months of age, moderate microglial (Fig. 4*A,B*) and astrocyte (Fig. 4*C,D*) activation was detected as assessed by Iba1 and GFAP staining, respectively. Quantification of cortical area occupied by Iba1 (Fig. 4*M*) or GFAP (Fig. 4*N*) did not reveal differences between APPPS1 and APPPS1;IRAK4^{KI/KI} mice at this age. However, at 8 months of age there was extensive, plaque-associated microgliosis in the APPPS1 mice (Fig. 4*E,I,M*) that was significantly reduced in the APPPS1;IRAK4^{KI/KI} mice (Fig. 4*F,J,M*).

Indeed, there was also marked reduction in astrocytosis in the APPPS1;IRAK4^{KI/KI} mice (Fig. 4*H,L,N*) compared with the APPPS1 mice (Fig. 4*G,K,N*). However, the abundance of microglia and astrocytes was proportional to plaque burden and did not differ between genotypes (Fig. 4*Q,R*). These data suggest that loss of IRAK4 activity does not abolish the ability of glia to respond to deposited A β and glia in the mice of both genotypes respond equivalently. Therefore reductions in A β are sufficient to decrease the glial response. It should be noted that we observed reduction in both microglial and astroglial activation. While IRAK4 is known to primarily function in myeloid lineage cells, we cannot exclude a direct effect of IRAK4 KI in astrocytes since the transgene is a germline knock-in.

Loss of IRAK4 activity alters the inflammatory response of microglia

Microglial activation status has historically been classified either as resting or activated. More recently the recognition of the phenotypic complexity and heterogeneity of microglia has resulted in

Figure 4. IRAK4 kinase activity is necessary for reactive gliosis. APPPS1 or APPPS1;IRAK4^{KI/KI} mice were killed at 4 months (**A–D**) or 8 months (**E–L**) and coronal sections were stained for microglia (Iba1, green; **A, B, E, F, I, J**) or astrocytes (GFAP, green; **C, D, G, H, K, L**) and total amyloid (6e10, red; all images). **A–H**, 10 $\times$ representative cortical images are shown. High-magnification representative images at 8 months of age are shown (**I–L**). Cortical area occupied by microglia (Iba1, **M**), astrocytes (GFAP, **N**), and A β (6E10, **O**) was quantified. 6E10-positive plaque diameter (**P**) was calculated. Cortical area occupied by microglia (Iba1, **Q**) or astrocytes (GFAP, **R**) was normalized to the corresponding A β plaque area (6E10). All image analysis (**M–R**) was performed with ImagePro software. Graphs represent mean $\pm$ SEM at each age. $N \geq 5$ animals/genotype/age. * $p \leq 0.05$, *** $p \leq 0.001$, Student's two-tailed t test for APPPS1 vs. APPPS1;IRAK4 KI.

their classification as surveillant, M1 (or pro-inflammatory), or M2 (alternative activated), a state associated with tissue repair and anti-inflammatory action (Anderson and Mosser, 2002; Gordon, 2003; Mosser and Edwards, 2008). M1 and M2 macrophages, or microglia, are functionally distinct yet are plastic and transition between the various states (Gordon and Taylor, 2005; Varin and Gordon, 2009; Gordon and Martinez, 2010). The M1/M2 states are distinguished by their signature expression of a panel of genes.

We assessed microglial phenotype by evaluation of gene expression profiles in microglial-enriched isolates from the adult

brain through quantitative reverse transcription PCR (Hickman et al., 2008). In the APPPS1 animals at 4 months of age we observed elevated expression of IL1 β and TNF α (Fig. 5A). Significantly, at 4 months we report upregulation of IFN γ expression in the APPPS1;IRAK4^{KI/KI} compared with the APPPS1 mice. The elevated IFN γ expression was correlated with downregulation of IL-10 in the APPPS1;IRAK4^{KI/KI} mice (Fig. 5B). The most striking results were observed at 8 months of age where we observed nearly a fourfold increase in IFN γ in the APPPS1;IRAK4^{KI/KI} compared with the APPPS1 animals (Fig. 5C) as well as relative increases in the IFN γ -responsive genes iNOS (Fig. 5C), and

SOCS1 (Fig. 5D). Notably, we observed a dramatic upregulation in IL-4 expression in the APPPS1;IRAK4^{KI/KI} animals compared with the APPPS1 mice (Fig. 5D). Together these data indicate that IRAK4 activity is involved in the acquisition of a M1 state by microglia as disease progresses and that loss of IRAK4 activity leads to enhanced expression of both IFN γ and IL-4, a combination that has previously been shown to be neuroprotective in AD (Butovsky et al., 2005). Suppression of canonical M2 marker expression at later stages of amyloid deposition is independent of IRAK4 activation. The discordant expression of M1 and M2 markers suggests that loss of IRAK4 activity results in dysregulation of coordinated patterns of gene expression that typify these phenotypic states. Moreover, the gene expression profile represents the composite of the expression of genes within a heterogeneous population of microglia, only a fraction of which are plaque associated, and the latter may be transcriptionally distinct from nonplaque associated cells.

IRF transcriptional machinery shifts toward an M2 state with loss of IRAK4 activity

Much of the transcriptional response of macrophages following TLR stimulation is regulated through the IRF transcription factor family (Honda and Taniguchi, 2006). Importantly, IRF4 and IRF5 have been shown to have opposing effects. IRF4 is associated with an M2 state (Ahyi et al., 2009) whereas IRF5 is associated with activation of an M1 state and suppression of an M2 state (Takaoka et al., 2005; Krausgruber et al., 2011). We analyzed whole-brain homogenates via Western blot to determine protein levels of IRF4 and IRF5. At 4 months of age, APPPS1 and APPPS1;IRAK4 KI show no significant differences in IRF4 levels (Fig. 6A,C), but IRF5 is significantly reduced by 75% in the APPPS1;IRAK4 KI animals compared with APPPS1 animals (Fig. 6A,D). At 8 months of age, APPPS1;IRAK4 KI animals express 40% more IRF4 protein (Fig. 6B,C) and 55% less IRF5 protein (Fig. 6B,D).

IRF3 and IRF7 are also critical to the TLR response. IRF3 expression has been shown to be induced by IFN γ and be neuroprotective in the CNS (Koziczak-Holbro et al., 2008; Piya et al., 2011; Tarassishin et al., 2011). IRF7 is also crucial to the interferon response (Honda et al., 2005b; Nakajima et al., 2009; Ning et al., 2011), and upregulation of IRF7 *in vivo* has been shown to play a protective role in the CNS (Honda and Taniguchi, 2006; Salem et al., 2011). Therefore we determined the transcript levels of IRF3, IRF4, IRF5, and IRF7 from microglial-enriched isolates. At 4 months of age, we found significant increases in IRF4 and IRF7 transcription in the APPPS1;IRAK4^{KI/KI} microglia compared with the APPPS1 mice (Fig. 6E). At 8 months of age we observed significant increases in IRF3, IRF4, and IRF7 transcription in microglia from APPPS1;IRAK4^{KI/KI} mice (Fig. 6F). IRF5 is known to have both constitutive and inducible expression and have up to six different splice variants that are under complex cell type and promoter-specific regulation (Mancl et al., 2005; An et al., 2008). This makes direct comparison of whole-brain homogenates and adult microglial isolates difficult and likely accounts

Figure 5. Loss of IRAK4 activity alters the inflammatory response of microglia. Microglia from the brain of 4-month-old (**A, B**) or 8-month-old (**C, D**) C57BL/6J, IRAK4^{KI/KI}, APPPS1, or APPPS1;IRAK4^{KI/KI} were isolated. mRNA was isolated, converted to cDNA, and analyzed by quantitative PCR. The data are expressed as RQ values with 95% confidence interval indicated. Representative M1 cytokines and markers (**A, C**) and M2 cytokines and markers (**B, D**) were assessed. All genotypes were normalized to expression of the individual genes in C57BL/6J (dotted line) using the $\Delta\Delta C_t$ method. All statistics were done at the ΔC_t level using the $\Delta C_t \pm$ SEM as indicated by Yuan et al. (2006). Samples were compared using one-way ANOVA with Tukey's *post hoc* test; $n \geq 8$ animals/genotype/age. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, for APPPS1 vs. APPPS1;IRAK4^{KI/KI} or Student's *t* test, # $p \leq 0.05$ for APPPS1 vs. APPPS1-21;IRAK4^{KI/KI}.

for discrepancies between protein and mRNA levels. These data suggest A β signaling through an IRAK4-dependent pathway is necessary for inflammatory transcriptional programming of microglia. Upregulation in IRF3, IRF4, and IRF7 transcription in the IRAK4^{KI/KI} animals (Fig. 6) is consistent with evidence where IRAKs may play a role in regulating the balance between inflammatory cytokine and interferon production in response to TLR ligands (An et al., 2008; Salem et al., 2011).

Loss of IRAK4 activity preserves amyloid clearance pathways

To further investigate how IRAK4 activation may influence clearance of amyloid we analyzed several A β clearance pathways. Importantly, our data show differences in microglial transcript levels of PPAR γ , a type II nuclear receptor, whose activation has been shown to be beneficial in ameliorating AD pathology (Landreth, 2007). At 4 months, when the APPPS1 and APPPS1;IRAK4^{KI/KI} animals display equivalent A β loads (Figs. 2–4), there are no differences in expression of genes associated with A β clearance pathways by microglia (Fig. 7A). At 8 months of age, PPAR γ expression in the APPPS1 mice was suppressed; however, loss of IRAK4 activity was associated with a reversal of this effect (Fig. 7B). The microglia from APPPS1 mice also demonstrated an approximate 50% reduction in the expression of transcripts for the extracellular A β degrading enzyme insulin degrading enzyme (IDE), which was not observed in microglia from the APPPS1;IRAK4^{KI/KI} mice. This finding is consistent with evidence from other AD mouse models where transgene expression depresses amyloid clearance pathways and that PPAR γ activation can drive IDE expression (Hickman et al., 2008; El Chartouni et al., 2010). A β deposition is associated with activation of pathways that suppress endogenous clearance mechanisms of A β . In our animal model, this suppression is mediated in part by IRAK4 and loss of IRAK4 activity helps to maintain clearance mechanisms and explain the decreased A β deposition that we observed (Figs. 2–4). Given the marked increase in IFN γ transcripts at 8 months of age in the APPPS1;IRAK4^{KI/KI} mice (Fig. 5C), we also investigated the complement pathway based on evidence that IFN γ can in-

Figure 6. IRF transcriptional machinery shifts to an M2 state with loss of IRAK4 activity. Whole-brain homogenates were analyzed by Western blots for IRF4 and IRF5 at 4 months (**A**) and 8 months (**B**). Densitometric quantification of IRF4 (**C**) and IRF5 (**D**) of Western blots, normalized to β -actin, was performed. Graphs represent mean $\pm$ SEM; $n \geq 6$ animals/genotype/age. Samples were compared with Student's *t* test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Microglia from the brain of 4-month-old (**E**) or 8-month-old (**F**) C57BL/6J, IRAK4^{KI/KI}, APPPS1, or APPPS1; IRAK4^{KI/KI} were isolated. mRNA was isolated, converted to cDNA, and analyzed via quantitative PCR. The data are expressed as RQ values with 95% confidence interval indicated. All genotypes were normalized to expression of the individual genes in C57BL/6J (dotted line) using the $\Delta\Delta$ Ct method. All statistics were done at the Δ Ct level using the Δ Ct $\pm$ SEM as indicated by Yuan et al. (2006). Samples were compared using one-way ANOVA with Tukey's *post hoc* test; $n \geq 8$ animals/genotype/age. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, for APPPS1 vs. APPPS1; IRAK4^{KI/KI} or Student's *t* test, and # $p \leq 0.01$ for APPPS1 vs. APPPS1; IRAK4^{KI/KI}.

duce complement and drive clearance of amyloid (Negishi et al., 2005; Chakrabarty et al., 2010). However, we did not find any difference in either C1qa or C3 transcript levels between the microglia from APPPS1 and the APPPS1; IRAK4^{KI/KI} mice at any age (data not shown).

Loss of IRAK4 activity preserves olfactory behavior in APP transgenic mice

Finally, we tested if loss of IRAK4-linked changes in amyloidosis is sufficient to rescue transgene-associated behavioral impairment in APPPS1 mice. We used a simple olfactory-based task, which has been shown to be robust and sensitive to amyloidosis in several mouse models and in repeated measures testing has provided similar (but more significant) results as found in a fear-learning assay (Wesson et al., 2010; Yang et al., 2011). In this task, measures of novel odor investigation duration positively correlate with levels of soluble and insoluble CNS amyloid, reflecting perturbations in CNS processing of odor information (Wesson et al., 2011). We observed prolonged novel odor investigation in the APPPS1 mice at 4 months of age and no significant difference between the APPPS1 and the APPPS1; IRAK4^{KI/KI} at this age (Fig. 8A). This is indicative of disruptions in early olfactory information processing. At 8 months of age, a significant impairment was still observed in the APPPS1 mice, but the APPPS1; IRAK4^{KI/KI} animals were indistinguishable from WT animals and significantly different from the APPPS1 mice (Fig. 8B). There was no significant effect of aging on novel odor investigation (WT 4 month vs WT 8 month, $p \geq 0.05$).

We evaluated whether the behavioral preservation was correlated with plaque burden in the piriform cortex. Analysis of thioS staining in the piriform cortex, an area important in olfactory information processing, demonstrated that amyloid plaques were not significantly different at 4 months of age correlating with the similarity in behavior. However, at 8 months the APPPS1; IRAK4^{KI/KI} mice had significantly fewer thioS plaques in the piriform cortex compared with the APPPS1 mice (Fig. 8C). These data demonstrate that inactivation of IRAK4 signaling in APPPS1 transgenic mice serves to restore behavioral function at later stages of disease pathogenesis.

Discussion

The TLRs are critical elements in the innate immune response through their ability to detect pathogens. These receptors act in concert with other cell-surface receptors to detect a broad array of molecules through which they subserve their tissue maintenance functions. Microglia detect fibrillar forms of A β through a complex of receptors that include integrins, scavenger receptors, TLR2/4/6, and their coreceptors CD14 and CD36 (Bamberger et al., 2003; El Khoury et al., 2003; Reed-Geaghan et al., 2009). The conversion of microglia from surveillant into an M1, pro-inflammatory phenotype is reliant upon classical TLR signaling pathways (Landreth and Reed-Geaghan, 2009; Takeuchi and Akira, 2010). TLR signaling is mediated through two distinct pathways, one that is dependent on the adaptor protein MyD88 and one that is independent (Takeuchi and Akira, 2010). IRAK4 is selectively activated through the MyD88-dependent pathway and transduces signals for all TLRs (except TLR3). To date, fA β has been shown to drive signaling only through this pathway.

Studies designed to evaluate the roles of the individual TLRs in AD pathogenesis have produced conflicting results. Rivest et al. (Richard et al., 2008) found that loss of TLR2 led to reduced plaque load early in disease but was no different at later stages. A loss of function mutation of TLR4 resulted in diminished cytokine expression, but increased levels in both soluble and insoluble A β levels at an advanced age (Tahara et al., 2006) that was associated with behavioral deficits (Song et al., 2011). Moreover, we reported that loss of CD14, the coreceptor for both TLR2 and TLR4, resulted in decreased plaque load at an intermediate stage of plaque deposition (Reed-Geaghan et al., 2010).

Confusion has also arisen from studies involving direct activation of the individual TLRs by injection of their specific ligands. Acute TLR4 activation by LPS suppressed amyloid pathology (DiCarlo et al., 2001); however, sustained TLR4 activation resulted in increased amyloid deposition (Qiao et al., 2001; Sheng et al., 2003). Perhaps most relevant to our findings was the report that chronic administration of the TLR9 ligand, methylated CpG, to an AD mouse model from 1.5 months until 17 months of age resulted in extensive reduc-

Figure 7. Loss of IRAK4 activity preserves amyloid clearance pathways. Microglia from the brain of 4-month-old (**A**) or 8-month-old (**B**) C57BL/6J, IRAK4^{KI/KI}, APPPS1, or APPPS1; IRAK4^{KI/KI} were isolated. mRNA was isolated, converted to cDNA, and analyzed via quantitative PCR. Graphs represent RQ values with 95% confidence interval indicated. All genotypes were normalized to expression of the individual genes in C57BL/6J (dotted line) using the $\Delta\Delta Ct$. All statistics were done at the ΔCt level using the $\Delta Ct \pm SEM$ as indicated by Yuan et al. (2006). Samples were compared using one-way ANOVA with Tukey's *post hoc* test; $n \geq 8$ animals/genotype/age. * $p \leq 0.05$, or ## $p \leq 0.01$ (via Student's *t* test) for APPPS1 vs. APPPS1;IRAK4^{KI/KI}.

tions in amyloid burden and normalized the behavior of these animals. (Scholtzova et al., 2009).

The recognition that more than one TLR is involved in the microglial response to A β has led to attempts to broadly inhibit signaling through genetic inactivation of their common adapter MyD88. These studies, too, have led to conflicting outcomes. Knock-out of MyD88 resulted in decreased in soluble, oligomeric, and fA β levels and decreased macrophage activation but without any changes in behavior (Hao et al., 2011; Lim et al., 2011, 2012). In contrast, MyD88 haploinsufficiency delayed amyloid deposition, but impaired spatial memory and was associated with increased oligomeric A β species (Michaud et al., 2011). The basis of the varied experimental outcomes is unknown. These studies are all confounded by the integral structural role that MyD88 plays in the assembly of the intracellular signaling platform for TLR signaling (Motshwene et al., 2009; Lin et al., 2010), which would be disrupted by knocking out MyD88. Furthermore, MyD88 enhances IFN γ -mediated stabilization of pro-inflammatory gene products (Sun and Ding, 2006).

Our approach to evaluating TLR actions in mouse models of AD was to functionally inactivate the common signaling element IRAK4 in APPPS1 mice. The use of the kinase dead IRAK4 allows the assembly of TLR signaling complexes, but arrests downstream signaling. A primary outcome of this study was the observation of dysregulated gene expression with aberrant expression of both M1 and M2 marker genes. This finding is similar to that found upon knock-out of the TLR coreceptor CD14 (Reed-Geaghan et al., 2010). An unexpected result of this study was the elevation in IFN γ in the APPPS1;IRAK4 KI mice (Fig. 5). There is no consensus on the effect of IFN γ in AD models as it has been shown to both worsen A β pathology

Figure 8. Loss of IRAK4 activity restores novel odor investigation. C57BL/6J, APPPS1; IRAK4^{KI/KI}, and APPPS1;IRAK4^{KI/KI} animals were subjected to a novel odor habituation protocol. Investigation times for Trial 1 odors are presented for 4-month-old mice (**A**) and 8-month-old mice (**B**). Four different odor presentations for Trial 1 for each animal were averaged such that each animal only contributed a single data point. Data represent mean $\pm$ SEM; $n \geq 5$ animals/genotype/age. * $p \leq 0.05$, one-way ANOVA with Tukey's *post hoc* test. APPPS1 or APPPS1; IRAK4^{KI/KI} mice were killed at 4 or 8 months and coronal sections were stained with thioflavin S. Piriform cortex thioS-positive deposits from matching sections were counted and averaged (**C**). Data represent mean $\pm$ SEM; $n \geq 5$ animals/genotype/age. ** $p \leq 0.01$, Student's *t* test within each age group.

(Yamamoto et al., 2007), improve A β pathology (Chakrabarty et al., 2010), and to be neuroprotective when combined with IL-4 (Butovsky et al., 2005). In this study, significant decreases in total A β levels in the APPPS1;IRAK4^{KI/KI} mice at 8 months of age were correlated with elevated IFN γ . This finding is consistent with our observation of elevated IFN γ in our CD14^{-/-}xAPP/PS1 animals compared with APP/PS1 mice in which we also found decreased fA β burden (Reed-Geaghan et al., 2010). These studies are congruent with those of Scholtzova et al. (2009) who reported that TLR9 activation was associated with reduced amyloid deposition, a treatment that is known to drive a strong interferon response (Kawai and Akira, 2011).

The recent appreciation of the phenotypic heterogeneity of microglia has resulted in their classification as surveillant, M1 or M2, analogous to phenotypic states observed in peripheral mac-

rophages (Mosser and Edwards, 2008; Gordon and Martinez, 2010). Controversial findings from Jimenez et al. (2008) argue that as AD pathology progresses there is a switch from M2 toward an M1 state. Conversely, Colton et al. (2006) found an initial induction of a pro-inflammatory M1 phenotype was followed by a predominant shift toward M2 phenotype among a more heterogeneous population of microglia. Microglial heterogeneity may be reflective of different phenotypic states of plaque-associated and nonplaque-associated microglia. The present study supports a central role of the TLRs in governing the phenotypic status of microglia. Indeed, this and our previous study demonstrated a heterogeneous gene expression profile in the brains of mice lacking CD14 or IRAK4 function, reflecting dysregulation of expression of genes associated with the inflammatory response upon loss of these TLR signaling elements (Reed-Geaghan et al., 2010). It is important to appreciate that activation or inactivation of single receptors may not only disrupt their direct signaling pathways, but also relieve repression of other pathways, and this may account for the aberrant gene expression profiles in the mutant mice (An et al., 2008; Negishi et al., 2012). TLR responses are also spatially and temporally regulated whereby manipulation of signaling at the plasma membrane can alter endosome/lysosome trafficking and influence the signaling pathways that are activated. In addition, stimulus duration impacts the final gene products that are generated from activation of signaling pathways (Honda et al., 2005a; Kagan et al., 2008; Zanoni et al., 2011).

Recently, the appreciation that the IRFs are critical regulators of TLR responses has allowed for a reevaluation of transcriptional control of microglial phenotypic states (Honda and Taniguchi, 2006). Specifically, the ratio of IRF4 to IRF5 has been shown to be critical in regulating the anti-inflammatory and pro-inflammatory gene programs that are engaged (Satoh et al., 2010; Krausgruber et al., 2011). Here we have documented that TLRs act to regulate the microglia expression of IRFs in a mouse model of AD. We found that transcript levels of IRF3, IRF4, IRF5, and IRF7 and protein levels of IRF4 and IRF5 favored conversion into an anti-inflammatory state in the APPPS1;IRAK4^{KI/KI} mice compared with the APPPS1 mice (Fig. 6). We conclude that loss of IRAK4 signaling resulted in broadly dysregulated expression of M1/M2 phenotypic markers, but the IRFs suggest an overall shift to an anti-inflammatory transcriptional state.

In conclusion, these experiments revealed that later in disease pathogenesis IRAK4-dependent signaling influences A β homeostasis and deposition. We argue that these effects reflect the action of TLR signaling pathways on microglia and influence the phenotype of these cells. (Honda and Taniguchi, 2006). How amyloid deposition, and the immune response to it, influences the clearance of amyloid is of importance in light of recent evidence that sporadic forms of AD arise from impaired clearance of A β from the brain (Mawuenyega et al., 2010). Moreover, our data demonstrate that activation of IRAK4 in microglia polarizes microglia toward a more inflammatory M1 state, one that is less favorable for clearance of amyloid (Koenigsknecht-Talboo and Landreth, 2005). Thus, suppression of IRAK4 signaling could be of therapeutic utility (Holtzman et al., 2011). The action of IRAK4 in the macrophage/microglial response to complex ligands is not unique to A β and our findings build upon a growing body of literature demonstrating that blocking IRAK4 function in complex disease models may prove to be beneficial (Koziczak-Holbro et al., 2009; Kim et al., 2011). These data demonstrate the critical role of IRAK4-dependent signaling in the AD brain and its impact on A β homeostasis and microglial phenotype.

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