IL-10 Alters Immunoproteostasis in APP Mice, Increasing Plaque Burden and Worsening Cognitive Behavior

Highlights

- The anti-inflammatory cytokine, IL-10, increases Aβ accumulation in APP mouse brain
- IL-10 exacerbates memory impairment in APP mice and reduces synaptic proteins
- IL-10 increases ApoE, which, by binding aggregated Aβ, is sequestered in plaques
- IL-10 and ApoE suppress microglial Aβ phagocytosis in vitro

Authors

Paramita Chakrabarty, Andrew Li, ..., Pritam Das, Todd E. Golde

Correspondence

pchakrabarty@ufl.edu (P.C.), tgolde@ufl.edu (T.E.G.)

In Brief

Chakrabarty et al. show that Interleukin-10 increases Aβ plaque deposition and impairs cognition in APP mice. This is mechanistically linked to decreased microglial Aβ phagocytosis and increased ApoE expression and sequestration in plaques, consistent with ApoE’s role as a pathological chaperone.
IL-10 Alters Immunoproteostasis in APP Mice, Increasing Plaque Burden and Worsening Cognitive Behavior

Paramita Chakrabarty,1,* Andrew Li,1,4 Carolina Ceballos-Diaz,1 James A. Eddy,2 Cory C. Funk,2 Brenda Moore,1 Nadia DiNunno,1 Awilda M. Rosario,1 Pedro E. Cruz,1 Christophe Verbeeck,3 Amanda Sacino,1 Sarah Nix,3 Christopher Janus,1 Nathan D. Price,2 Pritam Das,3 and Todd E. Golde1,*

1Department of Neuroscience, Center for Translational Research in Neurodegenerative Disease, McKnight Brain Institute, University of Florida, Gainesville, FL 32610, USA
2Institute for Systems Biology, 401 Terry Avenue N, Seattle, WA 98109, USA
3Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL 32224, USA
4Present address: Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205, USA
*Correspondence: pchakrabarty@ufl.edu (P.C.), tgolde@ufl.edu (T.E.G.)
http://dx.doi.org/10.1016/j.neuron.2014.11.020

SUMMARY

Anti-inflammatory strategies are proposed to have beneficial effects in Alzheimer’s disease. To explore how anti-inflammatory cytokine signaling affects Aβ pathology, we investigated the effects of adenovirus (AAV2/1)-mediated expression of Interleukin (IL)-10 in the brains of APP transgenic mouse models. IL-10 expression resulted in increased Aβ accumulation and impaired memory in APP mice. A focused transcriptome analysis revealed changes consistent with enhanced IL-10 signaling and increased ApoE expression in IL-10-expressing APP mice. ApoE protein was selectively increased in the plaque-associated insoluble cellular fraction, likely because of direct interaction with aggregated Aβ in the IL-10-expressing APP mice. Ex vivo studies also show that IL-10 and ApoE can individually impair glial Aβ phagocytosis. Our observations that IL-10 has an unexpected negative effect on Aβ proteostasis and cognition in APP mouse models demonstrate the complex interplay between innate immunity and proteostasis in neurodegenerative diseases, an interaction we call immunoproteostasis.

INTRODUCTION

Altered central nervous system (CNS) proteostasis, characterized by accumulation of extracellular or intracellular proteinaceous deposits, is thought to be a key trigger of many neurodegenerative disorders (Golde et al., 2013). There is considerable evidence that various assemblies of the aggregated proteins that form these inclusions can activate the innate immune system which, in turn, can contribute to the degenerative cascade. There is also growing evidence that alterations in innate immune signaling can play a key role in regulating proteostasis of key pathogenic proteins linked to neurodegenerative disorders (reviewed in Czirr and Wyss-Coray, 2012). We term this complex interplay between the innate immune system and proteinopathy “immunoproteostasis.” In a contextually dependent fashion, immunoproteostasis can have positive or negative effects on the proteinopathy and degenerative phenotype. Because of these effects and the plethora of therapeutic targets in the innate immune system, there is considerable interest in manipulating immunoproteostasis for potential disease modification in neurodegenerative diseases.

Two long-standing and interrelated hypotheses in the Alzheimer’s disease (AD) field relevant to immunoproteostasis are that (1) proinflammatory activation of innate immunity can enhance Aβ accumulation and thereby initiate or accelerate pathological cascades in AD; and (2) anti-inflammatory strategies reduce Aβ accumulation and, through synergistic or independent mechanisms, could also be neuroprotective. We and others have directly tested the first of these hypotheses and found little experimental evidence to support it (Boissonneault et al., 2009; Chakrabarty et al., 2010a, 2010b, 2011; El Khoury et al., 2007; Herber et al., 2007; Naert and Rivest, 2011; Shaftel et al., 2007). These studies showed that manipulations that skew innate immunity toward a proinflammatory state consistently reduce Aβ accumulation in transgenic mouse models largely by enhanced microglial clearance of Aβ. Other studies using pharmacologic and genetic means to suppress innate immune activation in similar mouse models have revealed conflicting results. Some manipulations designed to suppress innate immune activation appear to decrease Aβ accumulation and improve AD-associated phenotypes in these models, whereas others have deleterious effects and promote Aβ accumulation and worsen AD phenotypes (Chakrabarty et al., 2012; El Khoury et al., 2007; Kiyota et al., 2010; Maier et al., 2008; Richard et al., 2008; Vom Berg et al., 2012). Furthermore, clinical trials with anti-inflammatory agents have failed to clearly show any evidence of beneficial effect in AD patients (Breitner et al., 2011; Leoutsakos et al., 2012).

Given conflicting data on how suppressing immune activation can alter Aβ proteostasis, we explored the effects of Interleukin (IL)-10 expression in amyloid precursor protein (APP) transgenic mouse models. IL-10 is a key cytokine that represses excessive...
inflammatory responses and inhibits the effector functions in macrophages and myeloid cells by inhibiting inflammatory cytokine pathways (Banchereau et al., 2012). We tested the effect of recombinant adeno-associated virus (AAV2/1)-mediated intracranial expression of murine IL-10 in two transgenic APP models: TgCRND8 mice (Chishti et al., 2001) and Tg2576 mice (Hsiao et al., 1995). We find that IL-10 expression leads to increased amyloid loads, decreased levels of immediate early genes and synaptic markers, worsened cognitive behavior, reduced microglial Aβ phagocytosis, and increased ApoE expression and its sequestration within insoluble amyloid plaques.

RESULTS

IL-10 Exacerbates Aβ Plaque Burden in APP Transgenic Mice without Affecting APP Metabolism

We generated recombinant AAV vectors encoding murine IL-10. Transduction of human embryonic kidney 293T (HEK293T) cells with the IL-10 expression vector showed that the IL-10 expressed from the AAV vector was efficiently secreted (Figure S1A available online). When primary neuroglial cultures were transduced with AAV2/1-IL-10 and then subsequently treated with fibrillar Aβ42 (fAβ42) or lipopolysaccharide (LPS), IL-10 suppressed both fAβ42- and LPS-induced inflammatory immune activation (Figure S1B).

We next evaluated the effects of IL-10 expression in two different APP mouse models. Two independent cohorts of neonatal transgenic TgCRND8 (Tg) mice and nontransgenic (nTg) littermates were injected with AAV2/1-IL-10 or AAV2/1-GFP in the cerebral ventricles and then analyzed after 5 or 6 months (cohort A, Figure 1; cohort B, Figure S2). We have previously shown that AAV2/1-GFP serves as an appropriate control for these types of studies (Chakrabarty et al., 2010a, 2010b). Following delivery of AAV2/1-IL-10, IL-10 was significantly increased in the brains and plasma of TgCRND8 (IL-10/Tg) and nTg (IL-10/nTg) mice (Figures S1D and S1E). In the CRND8 cohort A, analysis of Aβ plaque burden showed that IL-10 significantly increased total plaque burden by greater than 50% in the hippocampus and cortex of CRND8 mice, while Thioflavin-S (ThioS)-positive dense core compact plaques increased by ~38% (Figures 1A and 1B). Biochemical analysis of Aβ levels in sequentially extracted RIPA-, SDS-, and formic-acid (FA)-solubilized mouse brain lysates showed significantly increased levels of SDS-solubilized Aβ and FA-solubilized Aβ in IL-10/Tg mice (Figure 1C). There was no change in RIPA-soluble Aβ (Figure 1C).

Both the RIPA-soluble and SDS-soluble mouse brain extracts were separated by PAGE and examined using the Aβ N-terminus-specific 82E1 monoclonal antibody (mAb). Levels of 82E1-immunoreactive, low-molecular-weight, RIPA-solubilized Aβ oligomers were unchanged in IL-10/Tg and control/Tg mice (data not shown). In the SDS fraction, an 82E1 immunoreactive band migrating at ~8 kDa was slightly increased in a select group of IL-10/Tg mice (Figure 1D, asterisk). Immunofluorescence analysis of Aβ in microglia surrounding plaques showed increased Aβ accumulation within microglial cells in IL-10/Tg mice (Figure 1E). Notably, in the second cohort of CRND8 mice tested, we observed similar results on Aβ (Figure S2). We further tested whether accumulated Aβ in IL-10-expressing mice lead to increased phosphorylated tau. However, we found no significant changes in endogenous mouse tau phosphorylation in IL-10/Tg mice compared to control/Tg mice (Figure S3).

We next examined whether IL-10 affects plaque deposition in another APP transgenic mouse model in a different experimental paradigm. Eight-month-old Tg2576 mice were injected in the hippocampus with AAV2/1-IL-10 or AAV2/1-GFP and analyzed at 13 months of age. Soluble IL-10 was elevated in the brains of AAV2/1-IL-10 injected mice (14.2 ± 4.7 ng/ml; 4.3× over control). Immunohistochemical analysis of Aβ plaque burden showed an overall increase of 43% in the brains of IL-10-expressing Tg2576 mice compared to GFP-expressing mice without any change in ThioS-positive plaques (Figures 2A and 2B). ELISA analysis of Aβ showed increased guanidine-hydrochloride (GN-HCl)-extractable insoluble Aβ42 but no changes in Aβ40 levels and unchanged levels of TBSx-soluble Aβ (Figure 2C).

We performed an initial series of studies to evaluate IL-10-induced changes in APP gene expression, its cleavage products, or Aβ-degrading enzymes. No significant changes in full-length APP, CTFRx, CTFFβ, or PrPC were noted in CRND8 and Tg2576 cohorts (Figures S4A and S4C–S4E). Endogenous mouse APP and Aβ levels were not altered in nTg mice expressing IL-10 or GFP (Figures S4B, S4C, and S4F). Quantitative real-time PCR of mouse APP, beta-secretase 1 (BACE1), insulin-degrading enzyme, and human APP transcript levels did not reveal any significant alterations in IL-10-expressing mice (Figure S4G). Although neprilysin (NEP) mRNA appeared to be lowered in IL-10/Tg mice (Figure S4G), NEP protein levels were not altered (Figure S4H).

IL-10 Exacerbates Context and Fear Tone Memory in TgCRND8 Mice

We assessed hippocampus-dependent contextual and amygdala-dependent tone fear-conditioned memory in CRND8 mice (cohort A) (Figure 3A) (Hanna et al., 2012). All mice actively explored the novel environment of the training chamber and spent <3% of the total exploration time on pauses or immobility, with no significant differences between the groups (Figure 3B). The immediate freezing response to the foot shock significantly differentiated the groups and was lower in both IL-10/Tg (13.6%) and control/Tg (25.2%) mice compared to IL-10/nTg (40.3%) and control/nTg (34.9%) mice, respectively (Figure 3B). The freezing response of males and females was comparable, with no significant interactions involving gender (p = 0.377, Gender x Group interaction effect). The IL-10/Tg mice showed decreased freezing during contextual memory test compared to the control/nTg and control/Tg groups, which indicates the decline in their contextual fear memory (Figure 3C). Control/Tg mice showed comparable context memory to the memory of nTg mice (Figure 3C). During the tone test, the groups did not differ in their exploration of the modified chamber during the phase preceding the presentation of the tone (Figure 3D). IL-10/Tg mice froze significantly less than control/nTg and IL-10/nTg mice during the presentation of the CS tone, while control/Tg mice showed a trend of lower freezing response than control/nTg mice (Figure 3D).
IL-10 Reduces c-FOS and zif268 Transcripts as well as Protein Levels of Synaptophysin and Total PSD95

RNA analysis showed that IL-10/Tg mice displayed 62.8% reduction in the c-Fos transcript compared to control/Tg mice (nCounter GX Mouse Inflammation array; log ratio = −1.315; p = 0.0250; q value = 0.00913). No significant change in c-fos transcript was detected in IL-10/nTg mice compared to control/nTg mice. RNA levels of zif268, a key player in neuronal plasticity and learning, is also decreased in IL-10/Tg mice compared to control/Tg mice (log ratio = −0.97;
p = 0.00038; q value = 0.00029). We further examined levels of the presynaptic vesicle protein, synaptophysin, by immunohistochemistry and immunoblotting and found that IL-10/Tg mice have 27.5% less synaptophysin than control/Tg (Figures 4A–4C). In addition, though phospho-PSD95 protein is unchanged in IL-10/Tg, total levels of PSD95 are reduced by 50% in IL-10/Tg mice compared to control/Tg mice (Figures 4A and 4B).

IL-10 Expression Alters Innate Immune Homeostasis in CRND8 Mice and Has Modest Effects on Primary Microglial Phagocytosis

We investigated how IL-10 alters the immune milieu and Aβ phenotypes using (1) immunophenotyping and immunohistochemical techniques and (2) functional assays to evaluate the phagocytic potential of murine microglia and astrocytes. We analyzed how IL-10 affects the expression of M1 and M2 phenotypic markers in the CNS (Gordon and Taylor, 2005) (Figure 5A). Quantitative real-time PCR of IL-10/Tg or control/Tg mice and their nTg littermates demonstrate a significant increase in Ym-1 in both IL-10/Tg and IL-10/nTg mice compared to genotype-matched control mice. MRC-1, arginase, or transforming growth factor β (TGF-β) transcript levels did not reach statistical significance. None of the M1-specific markers (IL-1β, IL-6, iNOS) showed significant alterations.

No gross morphological changes in the microglial or astrocyte processes were observed (Figure 5B). Immunoblotting with cd11b and glial fibrillary acidic protein (GFAP) showed a trend toward elevation in the IL-10/Tg and IL-10/nTg mice compared to control/Tg mice (Figures 4A and 4B).
Next, we investigated the effect of recombinant IL-10 following fAβ42 treatment of primary murine microglial cells. In contrast to the broad immunosuppressive effect of IL-10 on mixed neuroglial cultures, IL-10 showed mixed effects on fAβ42-treated microglia. For example, IL-10 attenuated fAβ42 induced CCL5, CXCL10, TLR1, and TNFα expression but augmented CCL2 and CCL8 expression (Figure 6A). We then examined functional effects of IL-10 on the phagocytic potential of primary murine microglia and astrocytes cultured in vitro (Figures 6B and 6C; Figure S6). Wild-type murine microglia or astrocytes were treated with recombinant cytokines, followed by the addition of preaggregated fluorescent fAβ40 or fAβ42. Flow cytometric and microscopic analysis showed that IL-10 treatment leads to decreased internalized fAβ40 in IL-10-treated microglia compared to vehicle-treated control microglia (Figures 6B and 6C). Following 1 hr of fAβ40 incubation, microglial cells were chased for 24 hr in fresh medium containing no fAβ. IL-10-treated cells showed a trend toward decreased clearance of internalized fAβ40 at 1 hr (p = 0.0533, one-tailed t test) and 3 hr (p = 0.13, one-tailed t test) following “chase” in fresh medium (Figure S6A). Simultaneously, IL-6 treatment resulted in increased fAβ40 internalization compared to vehicle-treated control after 1 hr incubation (Figure S6B). In these studies, IL-10 did not affect fAβ42 uptake by microglia at the time points tested (Figure 6B). Astrocytes are relatively resistant to fAβ phagocytosis under similar experimental conditions (Chakrabarty et al., 2012). Flow cytometric analysis of fAβ40 or fAβ42 phagocytosis by murine astrocytes showed that IL-10 treatment does not alter fAβ internalization (Figure S6C), whereas IL-6 treatment leads to a significant increase in astrocytic fAβ internalization after 1 hr incubation (Figure S6B).

Integrated Systems Approach Identifies Inflammatory Pathway Changes in Response to IL-10 Expression in APP Mice

We used NanoString nCounter GX mouse inflammation gene expression arrays to perform expression profiling of RNAs that are altered by IL-10 in APP mice brain. Of the 179 mRNAs initially tested, 21 transcripts were significantly altered, including chemokines and complement pathway genes (q value < 0.05; Figure 7A). In a follow-up study, we constructed a Neurodegenerative custom array (manufactured by NanoString) composed of key inflammatory and proteostasis mediators, including genes most upregulated in the GX Mouse Inflammation array and other known mediators of neurodegenerative pathways (Table S1). Of the 240 genes tested in this array, we found that 140 genes were significantly differentially expressed in IL-10/Tg mice (99 upregulated, 41 downregulated, q value < 0.05; Figures 7B and 7C; Table S2), and 47 genes were differentially expressed in IL-10/nTg mice (45 upregulated, 2 downregulated, q value < 0.05;
Figures S7A and S7B; Table S3) compared to genotype-matched controls. While the magnitude of change for common differentially expressed genes upon IL-10 expression is often greater in the nTg mice, the number of differentially expressed genes in Tg mice is significantly greater ($p < 0.0002$, two-tailed Z test), indicating a more diverse effect of IL-10 in the Tg mice. Overall, the gene classes and pathway components altered most significantly in Tg and nTg mice were similar: chemokines (Tg: Ccl8, Ccl5; nTg: Ccl2, Ccl8), complements (Tg: C4a/4b, c3ar1, c1qa, c1qa; nTg: C4a/4b, c1qb), FcγRs (Tg: FcγR3a; nTg: FcγR2b), and immune signaling mediators (Tg: HLA-DRB1; nTg: Ly86, Ms4a6a, Ptpn6, Ctsc) (Figures 7C and S7B).

We used the Ingenuity Pathway Analysis (IPA) tool to identify biological pathways that are affected by overexpression of IL-10 in Tg and nTg mice. Differentially expressed genes were mapped to canonical pathways, which highlight individual immune and inflammatory signaling pathways that are most enriched for up- or downregulated genes in both the Tg and nTg mice (Figures 7D and S7C; for a full list of altered pathways in Tg mice, see Table S4). Seven of the nine most significantly altered pathways that we examined in the Tg mice included at least one directly overlapping gene with the IL-10 signaling pathway, supporting a role between IL-10 expression and downstream activation of these pathways. In the IL-10/Tg mice, pattern recognition and acute phase response pathways were the most significantly altered. In the IL-10/nTg mice, all the pathways affected have been classically defined as part of IL-10 signaling by multiple groups (Sabat, 2010; Shouval et al., 2014). We further applied the differential rank conservation (DIRAC) method to quantitatively measure how network expression ordering differs within and between phenotypes (Eddy et al., 2010); in this case, we included all measured genes in the analysis, as opposed to restricting the focus on differentially expressed genes as in IPA. Using DIRAC, we were able to identify multiple pathways in both Tg and nTg mice that were consistently reordered between IL-10 and control cohorts, so that these pathways could be used as statistically significant and accurate molecular classifiers (Figure 7E). Notably, the changes captured in these pathway signatures, especially among nTg mice, do not necessarily reflect pathway-level activation or repression in response to IL-10 overexpression but often more subtle changes that manifest as relative changes in the expression levels of different components within a single pathway. In summary, we identified eight pathways that accurately distinguished between IL-10/Tg mice and control/Tg mice but were not significantly changed in nTg mice (100% of samples classified correctly by DIRAC) (Figure 7E; Table S5). The motivation of this study was not to develop...
Figure 5. IL-10 Expression Leads to an M2 Phenotype and Does Not Affect Astrogliosis or Plaque Engagement of Astroglia in TgCRND8 Mice

(A) Sustained IL-10 production leads to a partial M2 phenotype in 6-month-old Tg and nTg littermate brains in CRND8 mice. Quantitative real-time PCR demonstrates that IL-10/Tg mice have elevated levels of M2 phenotype marker, Ym-1, but show no significant changes in MRC1 or arginase (Arg). Analysis of three M1 phenotype markers, IL-6, iNOS, or IL-1β, showed no changes. n = 2–3 mice per group; each sample was tested in triplicate. ***p < 0.001, one-way ANOVA with post hoc Tukey's test. Data represent mean ± SEM.

(B) Representative images of Iba-1 (microglia) and GFAP (astrocyte) immunoreactivity in intact hippocampus (top) and higher magnification of selected area of interest (bottom) from control/Tg and IL-10/Tg mice is shown. Insets depict individual cells (high magnification) from corresponding low-magnification panels. Scale bars, 125 μm (top), 25 μm (bottom), and 12.5 μm (insets, bottom). n = 6 mice per group.

(C) Representative immunoblot and densitometric analysis of normalized levels of GFAP and cd11b obtained from 6-month-old IL-10/Tg and control/Tg mice. Molecular weight markers are indicated on the left (in kilodaltons). The lower panels represent blots reprobed with anti-actin antibody to depict loading amount. n = 5 mice per group; p > 0.05, one-way ANOVA with Tukey’s multiple comparison test. Data represent mean ± SEM.

(D) IL-10 expression does not alter astrocytic or microglial engagement around cored Aβ plaques. The number of astrocytes (GFAP-Cy3) and microglia (Dylight 594 conjugated tomato lectin) engaged closely with the ThioS reactive plaques were quantified by counting the number of DAPI-positive nuclei (restricted by a circular area of 100 μm diameter around each plaque core, as depicted by the white circle). Data represent mean ± SD. Scale bar, 25 μm. n = 6–7 mice per group, ten plaques per mouse.

See also Figure S5.
Figure 6. Recombinant IL-10 Attenuates fAβ40 Uptake by Primary Glia

(A) List representing log ratio changes of differentially expressing genes in primary murine microglia treated with 10 μM fAβ42 or IL-10, alone or in combination, following analysis by NanoString Inflammation GX array (q = 0.05). n = 3 per group. A selected set of altered transcripts is shown as fold ratio change over naive glia. n = 2–3 per treatment.

(B) IL-10 treatment decreases microglial phagocytosis of fAβ40 but does not affect fAβ42 uptake. Flow cytometric analysis for the presence of Aβ42-Hilyte555 or fAβ40-Hilyte488 in primary mouse glia cells was conducted following exposure to Aβ or vehicle control for various times (15 min to 1 hr). Data represent percent of microglial population positive for 555 nm or 488 nm fluorescence (mean ± SEM). *p < 0.05, **p < 0.01, unpaired two-tailed t test.

(C) Representative pictograms depicting flow cytometric (fluorescence-activated cell sorting; FACS) and microscopic analysis of IL-10- or vehicle (Control)-treated primary microglia internalizing Aβ40-488 nm at different time points.

See also Figure S6.
classifiers; however, gene expression patterns within these pathways that accurately distinguish between IL-10 and control groups provide good measures of confidence for network-level differences. Moreover, these pathways represent networks that are potentially altered in APP mice uniquely in response to IL-10 overexpression.

IL-10 Increases ApoE Expression and Results in ApoE Redistribution within Amyloid-Plaque-Associated Insoluble Brain Homogenate

Analysis of transcriptome changes in IL-10-expressing mice using the custom NanoString array showed that IL-10 expression increased ApoE RNA levels in both Tg (\(1.7 \times p = 1.43 \times 10^{-5}\))
Figure 8. IL-10-Induced ApoE Is Redistributed to Insoluble Plaque-Associated Cell Fraction and Impairs Microglial Uptake of Aβ
(A and B) Representative anti-ApoE immunoblots from sequentially extracted brain lysates of TgCRND8 mice. In an intensity analysis, immunoreactive bands of interest for the RIPA and SDS lysates were normalized to β-actin, and those for FA lysates were normalized to dry weight of the hemibrain. Molecular weight markers are indicated (in kilodaltons). Data represent mean ± SEM. n = 6–7 per group. ***p < 0.001, one-way ANOVA with Tukey’s multiple comparison test.

(C and D) Representative ThioS-stained Aβ plaque shows increased plaque-associated ApoE in IL-10/Tg mice cortex (C). Representative GFAP-stained sections also demonstrate selective increase in plaque-associated ApoE in IL-10/Tg mice (D). DAPI represents cellular nuclei. n = 3–6 per group.

(legend continued on next page)
and nTg (1.5 x, p < 0.05) mice (Tables S2 and S3). We observed a similar effect on ApoE transcription in AAV2/1-IL-10-transduced primary mixed neuronal-glialextrapolated derived from wild-type mice (Figure S8A). We did not find any significant changes in ApoE protein in sequentially extracted RIPA and SDS brain lysates of IL-10/Tg and control/Tg mice (Figure S8A). However, increased levels of ApoE and cleavage products were found in the insoluble formic-acid-extracted IL-10/Tg mice brain lysates (1.5 x compared to control/Tg, p < 0.005), suggesting that IL-10 increases ApoE expression and alters its compartmentalization in Tg mice (Figure S8B). Immunofluorescence demonstrated that, as expected, ApoE was localized within astrocytes in both IL-10/Tg and control/Tg mice (Figure S8B). In the IL-10/ Tg mice, ApoE immunostaining was also selectively increased within Aβ plaques, which is consistent with the increased ApoE sequestration in the formic acid biochemical fraction (Figures 8C and 8D).

To investigate whether ApoE can increase amyloidosis by directly affecting glial phagocytosis, we explored how ApoE-conditioned media affects glial uptake of fluorescent Aβ. To recapitulate in vivo conditions, we transduced the astrocytes in mixed glial culture with either rAAV2/1-GFP or rAAV2/1-ApoE and maintained the mixed culture for 3 days (Figures S8C and S8D). Robust overexpression of ApoE was confirmed by western blot analysis of conditioned media (Figure S8C). Microglia were then isolated from the mixed glial culture, and phagocytosis of preformed fluorescent Aβ was examined by immunofluorescence and flow cytometry. Microglial cells isolated from the ApoE-treated culture and maintained in ApoE-conditioned media showed a striking attenuation in internalization of Aβ compared to control microglia grown in GFP-conditioned media (p = 0.0027) (Figures 8E and S8D). Given the evidence for high-affinity binding of ApoE to Aβ, we explored whether a direct interaction between Aβ aggregates and ApoE in the glial-conditioned media might account for the dramatic effect on phagocytosis (Figure 8F). A pull-down assay using control aggregated amyloids (Iconomidou et al., 2010a; Wilkins et al., 2000), aggregated Aβ42, and aggregated reverse Aβ42 showed that ApoE binds selectively to aggregated Aβ42 but not to the other amyloids (Figure 8F).

### DISCUSSION

In this study, we have directly tested the hypothesis that expression of the anti-inflammatory cytokine IL-10 would have beneficial effects in two APP mouse models. Rather than finding beneficial effects, we found that IL-10 worsens multiple AD-relevant phenotypes in APP mice, including amyloid plaque pathology and memory and learning. There was no evidence that the pro-amyloidogenic effect was due to altered APP expression or processing, nor were levels of key Aβ-degrading enzymes altered. IL-10 had a complex effect on innate immune activation status in the brain. IL-10 altered the innate immune gene expression toward a M2-like activation state. Simultaneously, a network-based approach identified several inflammatory pathways consisting of chemokines and acute phase reactants to be upregulated in IL-10-expressing Tg and nTg mice.

Mechanistically, we can attribute the negative effects of IL-10 on Aβ proteostasis to synergistic effects of decreased Aβ phagocytosis by microglia, increased endogenous ApoE expression, and enhanced accumulation of ApoE in insoluble amyloid plaques (summarized in Figure 8G). Our Aβ phagocytosis data, in combination with previous data with inflammatory cytokines (Chakrabarty et al., 2010a), indicate that IL-10-mediated alterations of innate immunity dampen microglial phagocytosis of Aβ in vitro. Additionally, ApoE by binding with Aβ aggregates, may negatively regulate Aβ aggregate clearance, thereby promoting further plaque deposition. Human APOE has been shown to have complex effects on Aβ. It promotes deposition of fibrillar Aβ and, in humans, can regulate clearance of soluble Aβ in an isoform-dependent manner (reviewed in Liu et al., 2013). Not only are human APOE carriers at risk for developing AD, but APOE4 may also have negative impact on cognition in normal aging and AD (Corder et al., 1993; Deary et al., 2002; Honet et al., 2009; Liu et al., 2014). Mouse ApoE is comparable to human APOE4 at residues 112 and 158, and numerous studies suggest that the mouse protein may have enhanced amyloidogenic properties (Bales et al., 1997; DeMattos et al., 2004; Fagan et al., 2002; Holtzman et al., 2000a). Knocking out both endogenous mouse ApoE alleles reduces Aβ plaque deposition in APP mice (Holtzman et al., 2000a), and even haploinsufficiency reduces amyloid loads (Kim et al., 2011). Similarly, overexpression of APOE4 in predepositing APP mice exacerbates Aβ pathology, whereas overexpression of APOE2 can enhance clearance of preexisting deposits (Dodart et al., 2005; Hudry et al., 2013). In our study, the increased level of insoluble ApoE is plaque associated, which is consistent with mouse ApoE functioning as a pathological chaperone promoting Aβ fibrillogensis (Bales et al., 1999; Holtzman et al., 1999, 2000b; Wisniewski et al., 1994). This is consistent with our immunofluorescence and amyloid pull-down data that mouse ApoE preferentially associates with fibrillar Aβ, suggesting that the behavioral and amyloidogenic phenotype induced by IL-10 can result from the accumulation of Aβ bound to ApoE and, possibly, neurotoxic cleavage fragments (Cho et al., 2001; Jones et al., 2011; reviewed in Mahley and Huang, 2012). However, it remains to be seen whether increasing the “protective” forms of ApoE (ApoE2) can result in an opposite phenotype in the presence of IL-10, i.e., binding...
and clearing Aβ. Thus, the present study demonstrates that a key anti-inflammatory cytokine exacerbated Aβ proteostasis and brain function by altering Aβ clearance and/or deposition. These observations are highly consistent with unpublished data showing that IL-10 deficiency in APP mice dramatically reduces Aβ load and other AD-related phenotypes, including lowered ApoE levels by RNA-seq analysis (log2 fold change = −0.6, FDR = 4 × 10⁻⁷) (Guillot-Sestier et al., 2015).

These data are also highly consistent with our previous but relatively limited study, showing that direct intracranial expression of IL-4, another key anti-inflammatory cytokine, results in Aβ plaque accumulation in TgCRND8 mice (Chakrabarty et al., 2012). Furthermore, the data also agree with our previously published findings that proinflammatory cytokines (IL-6, TNF-α, and IFN-γ) attenuated Aβ plaque deposition and increased microglial Aβ phagocytosis (Chakrabarty et al., 2010a, 2010b, 2011). Thus, in contrast to the long-standing hypothesis that proinflammatory stimuli promote Aβ deposition, we find the opposite: anti-inflammatory stimuli promote amyloid deposition.

In two recent studies, AAV-mediated hippocampal targeted expression of IL-10 and IL-4 were reported to decrease gliosis and improve spatial memory in APP/PS1 mice (Kiyota et al., 2010, 2012). Moreover, IL-4, but not IL-10, was reported to attenuate plaque deposition in these mice. Given that our IL-10 data were reproduced in two independent CRND8 cohorts and in a hippocampal paradigm in the Tg2576 mouse model, and given that a robust proamyloidogenic effect of IL-4 was observed both histochemically and confirmed biochemically in TgCRND8 mice (Chakrabarty et al., 2012), it is not easy to reconcile our observations with these previous reports. We note that the group sizes in the Kiyota study are low, especially for behavioral analyses. Moreover, they do not specify gender of the mice, which can influence both behavioral effects and plaque loads in the bigenic APP/PS1 mice (Wang et al., 2003). It is also possible that the source of the discrepant observations could be attributed to the use of familial AD-linked mutant PSEN1 transgenic mice, as PSEN1 has been reported to have immunomodulatory actions in the brain (Choi et al., 2008).

Although our own studies in this area have been internally consistent, a broader survey of published studies regarding alterations of Aβ and other phenotypes in APP mice via manipulation of chemokines, cytokines, and other innate immune modulators suggests that a unified view of immunoproteostasis mechanisms in AD is not feasible at this time (Czirr and Wyss-Coray, 2012). Manipulations such as LPS, TLR agonists, or astrocytic overexpression of inflammatory cytokine IL-1β, which induce inflammatory glial activation, reduce plaque load (Herber et al., 2007; Scholtzova et al., 2009; Shaftel et al., 2007). Thus, coupled with our previous observations in IL-6, IFN-γ, and TNF-α overexpression paradigms in CRND8 mice (Chakrabarty et al., 2010a, 2010b, 2011), these studies suggest that, at least with respect to Aβ-related phenotypes, a proinflammatory environment may have beneficial outcomes. However, other manipulations that can have an anti-inflammatory effect have also been shown to reduce plaques and improve cognition. Notable recent examples include genetic deficiency of inflammasome (Nlrp3 or Casp1 knockouts), deficiency of Mrp14, and loss of IL-12/IL-23 signaling (Heneka et al., 2013; Vom Berg et al., 2012). Further additional manipulations (for example, loss of CD14, CD40L, Myd88, fractalkine signaling, TGF-β signaling, or overexpression of TGFβ1) can have complex, and sometimes unexpected, effects on AD-relevant phenotypes in mouse models that can be challenging to reconcile with data from similar paradigms (Lee et al., 2010; Lim et al., 2011; Reed-Geaghan et al., 2010; Tan et al., 1999; Town et al., 2008; Wyss-Coray et al., 2000).

Though genetic association studies have not reproducibly established the association of SNPs within the IL-10 gene with AD risk, the recent and unequivocal associations of SNPs within other genetic loci that encode innate immunity genes bolster the preclinical data that innate immunity has a significant role in AD (Depboylu et al., 2003; Griciuc et al., 2013; Guerreiro et al., 2013; Kamboh et al., 2012; Lambert et al., 2013). In spite of a clear contribution of immunoproteostasis in AD, epidemiologic and clinical data largely focused on NSAID use and AD risk reveal a fairly conflicted literature related to anti-inflammatory strategies. Long-term NSAID use has been repeatedly shown to confer protection in epidemiologic studies (Szekely et al., 2004), but subsequent clinical trials with celecoxib and naproxen have not shown any benefits in patients (Martin et al., 2008), and different clinical trials with celecoxib and naproxen have been shown to have potential therapeutic benefit in AD preclinical models, to date, none have shown efficacy in clinical trials, nor has the effect in preclinical studies been unequivocally linked to effects on immunoproteostasis (Gold et al., 2010; Sano et al., 2011).

A striking feature of our study is the synergistic effect of IL-10 induced Aβ accumulation on fear-conditioned memory and synaptic protein levels. As noted, IL-10 had minimal effect on learning and memory in the nTg littermates, but in the TgCRND8 transgenic mice, it significantly exacerbated context and tone fear memory, concurrent with marked loss of synaptic proteins. Although many studies suggest that soluble oligomeric species of Aβ are most often associated with various memory impairments in APP mice and humans (Zahs and Ashe, 2013), in TgCRND8 mice, we have seen a strong correlation between total Aβ loads and impairments in fear-conditioned memory (Hanna et al., 2012). Notably, multiple studies also show that increased insoluble APOE, both in humans and mice, is associated with worse cognitive function, which is consistent with our observations (Bennett et al., 2005; Nilsson et al., 2004; Raber et al., 2000).

The observation that IL-10 can increase ApoE expression and promote its codeposition with Aβ has implications for AD therapy. There is ample evidence that promoting expression of the protective APOE2 isoform would have beneficial effects and APOE4 harmful effects; thus, we might expect that increased IL-10 or any factor that promotes APOE expression...
in humans may have genotype-dependent effects. Indeed, in the context of APOE2, one would propose that IL-10 could be beneficial if its effect on APOE is dominant. In contrast, given the intermediate amyloid-promoting effects of APOE3, empirical studies would be needed to determine effects of IL-10 in the context of APOE3.

In conclusion, we have demonstrated that IL-10 expression has a proamyloidogenic effect in APP mice leading to dysfunctional immunoproteostasis, impaired memory, and reduction of synaptic markers. These data further highlight the complex role of innate immune activation in AD and other neurodegenerative diseases where specific innate manipulations can have unexpected positive or negative effects on proteostasis and neurodegeneration.

EXPERIMENTAL PROCEDURES

**Animal Models and AAV2/1 Injection**
All animal procedures were approved by the Institutional Animal Care and Use Committee and conducted as described elsewhere (Chakrabarty et al., 2010a). See Supplemental Experimental Procedures for details.

**Western Blot, Immunohistochemistry, ELISA, and RNA Analysis**
See Supplemental Experimental Procedures for details.

**Contextual Fear Conditioning**
See Supplemental Experimental Procedures and Figure 3A for details.

**β-Phagocytosis**
Wild-type murine microglia or astrocyte cultures were evaluated for uptake of fluorescently labeled IA42 or IA40 in the presence of cytokines. See Supplemental Experimental Procedures for details.

**RNA Analysis**
Transcriptome data obtained from the NanoString array were analyzed using NanoStringNorm R, IPA, and DIRAC as described in Supplemental Experimental Procedures. The q values were obtained as described elsewhere (Storrey and Tibshirani, 2003). The raw data have been deposited at https://www.synapse.org/#!Synapse:syn2866151.

**Statistical Analysis**
A one-way or two-way ANOVA with Tukey’s multiple comparison test was used for statistical comparison unless otherwise stated (SimaStat 3.0 version). For t tests, multiple comparison test parameters were applied, controlling for a false discovery rate of 5%. Graphical analyses were conducted using Prism 5 (GraphPad Software), and final images were created using Photoshop CS2 (Adobe).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, eight figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.11.020.

**ACKNOWLEDGMENTS**

This work was supported by NIH grants RO1AG32991 (P.D.) and R01AG018454 (T.E.G.) and NIA grant AG046139-01 (T.E.G., N.P.). We thank Drs. Terrence Town and Marie-Victoire Guillot-Seistier (University of Southern California) for helpful discussion.

Received: September 15, 2013
Revised: October 24, 2014
Accepted: November 20, 2014
Published: January 22, 2015

**REFERENCES**


