The E3 ubiquitin ligase Idol controls brain LDL receptor expression, ApoE clearance, and Aβ amyloidosis

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Apolipoprotein E (ApoE) is an important modifier of Alzheimer’s disease (AD) pathogenesis, and its abundance has been linked to the clearance of β-amyloid (Aβ) in the brain. The pathways that control the clearance of ApoE in the brain are incompletely understood. We report that Idol, an E3 ubiquitin ligase that targets the low-density lipoprotein receptor (LDLR) for degradation, is a critical determinant of brain ApoE metabolism and Aβ plaque biogenesis. Previous work has shown that Idol contributes minimally to the regulation of hepatic LDLR expression in mice. By contrast, we demonstrate that Idol is a primary physiological regulator of LDLR protein in the brain, controlling the clearance of both ApoE-containing high-density lipoprotein (HDL) particles and Aβ. We studied the consequences of loss of Idol expression in a transgenic mouse model of Aβ amyloidosis. Idol deficiency increased brain LDLR, decreased ApoE, decreased soluble and insoluble Aβ, reduced amyloid plaque burden, and ameliorated neuroinflammation. These findings identify Idol as a gatekeeper of LDLR-dependent ApoE and Aβ clearance in the brain and a potential enzyme target for therapeutic intervention in AD.

INTRODUCTION

Alzheimer’s disease (AD) is a major form of dementia characterized by progressive loss of neurons and neuronal processes and impaired brain function (1, 2). Key pathologic features of AD include the accumulation of extracellular amyloid plaques and the formation of intraneuronal neurofibrillary tangles, consisting of β-amyloid (Aβ) peptides and hyperphosphorylated tau proteins, respectively (3). Decades of research suggest that the pathogenesis of AD is linked to interactions between multiple factors, including Aβ, apolipoprotein E (ApoE), tau, and aging (4). However, our understanding is still incomplete, and there are no effective treatments for AD.

ApoE is a major cholesterol carrier in the brain (5). Although several genetic risk factors for AD have been identified, the APOE genotype is the strongest (6). Mounting evidence suggests that ApoE influences AD risk through effects on Aβ metabolism. Genetic deletion of ApoE, anti-ApoE immunotherapy, and increasing ApoE lipization have all been shown to reduce Aβ aggregation in mouse models (7–10). Thus, expanding our understanding of ApoE and Aβ metabolism and their mechanistic links may identify new opportunities for intervention in AD.

The low-density lipoprotein receptor (LDLR) is a cell surface receptor that facilitates the endocytosis of plasma lipoprotein particles containing ApoB100 or ApoE (11). We identified Idol as a modulator of LDLR function and cholesterol homeostasis (12–15). Idol is an E3 ubiquitin ligase that is transcriptionally regulated by the cholesterol-responsive liver X receptors (LXRs). The Idol-UBE2D complex ubiquitinates the LDLR on its cytoplasmic domain, thereby targeting it for lysosomal degradation (16, 17). Previous studies have suggested that ectopic overexpression of LDLR decreases ApoE and inhibits Aβ deposition (18, 19). However, the question of whether increasing endogenous LDLR expression would affect AD pathogenesis remains to be addressed.

RESULTS

The LXR-Idol pathway regulates LDLR protein expression in the mouse brain

To address the function of the LXR-Idol pathway in the mouse brain, we first characterized Idol expression. Idol mRNA was readily detected in all examined subregions of the mouse central nervous system (CNS) (fig. S1A). Analysis of different cell types present in the CNS revealed abundant Idol mRNA in primary mouse microglia and neurons and lower amounts in astrocytes (Fig. 1A). We targeted the mouse Idol gene with a knockout cassette containing a LacZ reporter (13). Immunohistochemistry confirmed coexpression of β-galactosidase (β-Gal) protein with the microglia marker Iba1 and the neuronal marker NeuN in Idol Cre cells (Fig. 1B).

Microglia can exert context-specific effects on AD pathogenesis (20, 21). We tested the ability of Idol to regulate LDLR expression in these cells on the basis of its prominent expression in microglia. Treatment with an LXR agonist robustly increased Idol mRNA in primary mouse microglia (Fig. 1C). Genes encoding the canonical LXR targets Abca1, Srebp-1c, and Scl-1 were up-regulated in parallel, whereas ApoE mRNA expression was unchanged (Fig. 1C). Induction of Idol by an LXR agonist, in turn, resulted in a marked reduction of LDLR protein, consistent with its degradation (Fig. 1D). Abca1 protein was increased by LXR agonist in microglia as expected, but Lrp1 did not change.
We also found that overexpression of Idol decreased LDLR and triggered LDLR ubiquitination in Neuro2a mouse neuronal cells (fig. S1, B to D). Conversely, primary microglia from Idol−/− mice exhibited increased LDLR expression compared to wild-type microglia (Fig. 1E).

We next addressed the ability of Idol to regulate LDLR protein in the brain. Immunoblotting revealed striking increases in LDLR in the brains of Idol−/− mice (Fig. 1F). The amount of LDLR protein in wild-type and Idol−/− mice, consistent with a gene dosage effect (P < 0.05; Fig. 1F). These observations suggest that Idol plays a dominant role in determining LDLR expression in the brain under physiological conditions.

**Loss of Idol decreases Aβ and reduces amyloid plaque burden in a mouse model of Aβ amyloidosis**

On the basis of the above findings, we hypothesized that loss of Idol may attenuate the deposition of amyloid plaques. We bred Idol−/− mice with mice carrying APPswe and PSEN1dE9 transgenes (denoted APP/PS1 mice) (22). The extent of Aβ deposition in the brains of the progeny was analyzed at 7 months of age, a time when early amyloidosis is apparent. Because male and female APP/PS1 mice develop amyloid deposits at different rates (19), we analyzed both sexes independently. We first measured Aβ in the cortex by enzyme-linked immunosorbent assay (ELISA). Compared to APP/PS1;Idol+/− mice, insoluble and soluble Aβ40 and Aβ42 were prominently decreased in the cortex in both male and female APP/PS1;Idol−/− mice (Fig. 2A). The reduction in insoluble and soluble Aβ42 in the cortex of male APP/PS1;Idol−/− mice was also significant versus APP/PS1;Idol+/− mice (P < 0.01), suggesting that even partial inhibition of Idol activity was sufficient to suppress the accumulation of amyloid plaque.

We further analyzed protein expression in radioimmunoprecipitation (RIPA) buffer fractions from brains of APP/PS1;Idol+/− and APP/PS1;Idol−/− mice by immunoblotting (Fig. 2B). Idol genotype did not affect total APP protein, nor did it affect the expression of the cleavage enzyme BACE1 or its cleavage product β C-terminal fragment (β-CTF). We also assessed Aβ peptide in the RIPA fraction and total brain lysates and found markedly reduced Aβ peptide in APP/PS1;Idol+/− mice (Fig. 2, B and C).

We next characterized amyloid plaque deposition in a second cohort of mice by immunostaining with a biotinylated Aβ-specific monoclonal
Idol deficiency ameliorates neuroinflammation in a mouse model of Aβ amyloidosis

Activation of microglia is commonly observed in the brains of human AD and mouse models of Aβ amyloidosis (20). Fibrillar amyloid plaque is postulated to trigger detrimental neuroinflammatory responses, and its abundance correlates with the extent of microgliosis (23). To determine whether Idol affected microglia abundance in APP/PS1 mice, we stained brain sections with antibodies against CD45 (marker for activated microglia) and Iba1 (marker for resting and activated microglia) (Fig. 4). Consistent with the decreased Aβ plaque formation, neuroinflammation (as assessed by CD45 staining) was clearly decreased in APP/PS1;Idol−/− mice with Aβ-specific 82E1B antibody. Black stain indicates Aβ plaques. Scale bar, 250 μm. (E) Quantification of Aβ plaque load in the frontal cortex and hippocampus of APP/PS1;Idol+/+, APP/PS1;Idol−/−, and APP/PS1;Idol−/− mice by 82E1 antibody staining for Aβ. Error bars represent SEM. *P < 0.05, **P < 0.01 by Student's t test. n = 6.
Microglial abundance in APP/PS1 brains was directly proportional to the Idol gene dosage. Both heterozygous and homozygous knockout mice showed a decrease in the number of microglia in the cortex and hippocampus (Fig. 4, C and D; P < 0.01).

To determine whether the differences in microgliosis reflected intrinsic differences in inflammatory properties between control and Idol-deficient microglia, we examined inflammatory gene expression. Loss of Idol did not change the expression of the signature microglial markers Cd45, Cd68, and Ym1 (fig. S2A). Furthermore, Idol deficiency did not affect the activation of microglia upon lipopolysaccharide (LPS) stimulation, as the induction of genes such as Il1b, Icam1, and Socs3 was not altered (fig. S2B). These data suggest that the difference in microglia abundance in APP/PS1 mice in the presence and absence of Idol is secondary to the difference in Aβ plaque load.

Loss of Idol facilitates ApoE and Aβ uptake and clearance

To investigate a mechanistic link between Idol and AD, we first tested the influence of Idol knockout on brain levels of the LDLR and its ligand ApoE. LDLR protein expression in APP/PS1 mice was higher in the absence of Idol, whereas LRP1 protein was unchanged (Fig. 5A). ApoE protein was correspondingly reduced in APP/PS1;Idol+/− mice compared to APP/PS1;Idol+/+ mice (Fig. 5A). We also observed lower levels of ApoE in phosphate-buffered saline (PBS)–soluble fractions and RIPA fractions of brain lysates from APP/PS1;Idol+/− mice (Fig. 5, B and C). The greater reduction observed in the PBS fraction is consistent with extracellular ApoE being the target of Idol and LDLR. ApoE has been shown to accumulate along with Aβ in amyloid plaques in both AD patients and mouse models of AD (6, 24). In line with our findings of reduced Aβ peptide in the insoluble fraction (Fig. 2A), there was a parallel reduction in ApoE protein in the insoluble fractions from APP/PS1;Idol+/− mouse brains (Fig. 5D; P < 0.05).

Extracellular Aβ can be cleared by neurons and other cell types in the brain through various receptors on the plasma membrane (25–27). Previous studies have suggested that LDLR and ApoE regulate extracellular Aβ by modulating its clearance by brain cells (28, 29). We therefore examined the effect of Idol on extracellular Aβ clearance. Small interfering RNA (siRNA)–mediated knockdown decreased Idol expression by ~60% and increased LDLR protein in mouse Neuro2a neuronal cells, hApoE3 knock-in astroglial cells, and BV2 microglial cells (Fig. 6A and fig. S3A). To assess the influence of Idol on Aβ uptake and clearance, we inhibited Idol with siRNA and then incubated the cells with synthetic Aβ peptide for 3 hours. We found that Idol knockdown strongly increased Aβ uptake in Neuro2a, hApoE3, and BV2 cells (Fig. 6B). Furthermore, Idol knockdown also decreased the amount of Aβ remaining in the media after 24 hours of incubation (Fig. 6C). We also examined the effects of loss of Idol on uptake of Aβ by primary mouse neurons. In contrast to the results obtained with the immortalized Neuro2a line, there was no difference in the ability of Idolfllox/fox control and Cre-infected Idol-deficient neurons to internalize fluorescently labeled aggregated Aβ42 peptide (Fig. 6D).

We extended these observations by analyzing the clearance capacity of primary microglia. Microglia lacking Idol showed increased ability to internalize fluorescently labeled aggregated Aβ42 peptide (Fig. 7, A and C). Similar results were obtained when Idol was acutely deleted from conditional knockout cells in vitro (fig. S3, B and C). Idolfllox/fox primary microglia treated with lentiviral Cre accumulated more Aβ42 peptide upon incubation compared to vector-treated cells. We observed a similar increased uptake activity of Idol-deficient cells toward ApoE-containing lipoproteins. Incubation of primary microglia with fluorescently labeled recombinant discoidal high-density lipoprotein (HDL) particles containing ApoE revealed accelerated uptake in cells lacking Idol (Fig. 7, B and D). Together, these data suggest that loss of Idol decreases Aβ deposition in the brain by enhancing the clearance of both Aβ- and ApoE-containing lipoproteins, primarily by microglia.

To assess directly the contribution of LDLR to increased uptake of Aβ by Idol-deficient microglia, we knocked down LDLR with siRNA in
Idol
deficiency largely abolished the effect of Idol deficiency on Aβ uptake. These data demonstrate that Idol regulates Aβ uptake in microglia, at least in part, by modulating LDLR levels, and strongly suggest that this mechanism contributes to the effects of Idol on Aβ deposition in APP/PS1 mice.

**DISCUSSION**

Here, we investigated the impact of Idol, a physiological regulator of LDLR abundance, on the development of Aβ-dependent pathology in mice. Idol is robustly expressed in microglia, and its inactivation increases LDLR protein in the mouse brain. In a transgenic mouse model of amyloidosis, loss of Idol reduced amyloid plaque burden and microglial recruitment in a gene dosage-dependent manner. Mechanistic studies revealed that Idol does not directly modulate neuroinflammatory responses. Rather, Idol regulates the uptake and clearance of ApoE and Aβ by microglia. These results identify the LXR-Idol pathway as a previously unrecognized determinant of brain LDLR and ApoE and the development of AD-like pathology.

ApoE plays a central role in brain lipid homeostasis by mediating the endocytosis of HDL-like lipoprotein particles (30). ApoE is also a crucial...
factor in the development of AD (5, 6, 31, 32). Reducing ApoE in mouse models of amyloidosis greatly reduces plaque formation (7, 8, 33, 34). ApoE binds to lipoprotein receptors such as LDLR and LRP1. Inactivation of the Ldlr or Lrp1 gene increases, whereas overexpression of LDLR decreases, ApoE protein in the brain (19, 35–37). We have shown here that the LXR-Idol axis targets brain LDLR (but not LRP1) for degradation. Our data suggest that Idol affects amyloid plaque formation by modulating LDLR protein primarily in microglia and, consequently, ApoE protein in the brain.

Our results strengthen and expand previously established links between LDLR and ApoE expression and the amount of Aβ in the brain. However, the relationships between these factors remain controversial and an area of active investigation. For example, the connections between ApoE and Aβ uptake are complex. First, many ApoE receptors, including LDLR and LRP1, have been proposed to be involved in Aβ uptake (31). Second, the mechanisms by which ApoE protein affects Aβ uptake are not fully understood. One model proposes that ApoE binds to insoluble Aβ aggregates (38, 39) and thereby hinders their uptake by brain cells. Other studies suggest that ApoE may compete with soluble Aβ for the same endocytosis pathway (40). Others have suggested that lipidated ApoE facilitates removal of Aβ (29). Another recent study reported that LDLR mediates Aβ uptake through direct binding to Aβ, independent of ApoE (28). Although the exact mechanisms affecting Aβ clearance warrant further investigation, Idol’s ability to regulate LDLR levels and its ability to regulate ApoE clearance would both be predicted to affect Aβ uptake. The combined effect of Idol on both factors may explain its prominent effect on AD-like pathology.

Previous studies of the roles of the LDLR-regulating factors Idol and Pcsk9 were not predictive of the relative importance of these factors in the control of brain LDLR expression. Deletion of Pcsk9 in mice leads to increased LDLR protein in the liver but not in the brain (41, 42). By contrast, loss of Idol expression in mice has little effect on hepatic LDLR (13). Our discovery that Idol plays a major role in determining brain LDLR and ApoE levels under physiological and pathological conditions was thus unexpected.

The LXR signaling pathway also plays an important role in Aβ plaque biogenesis. LXR agonist treatment alleviates AD-like pathology in mouse models of amyloidosis, possibly by inducing Abca1 expression (29, 43–48). Abca1 affects the lipidation status of ApoE in the brain (49), which, in turn, may facilitate the proteolytic degradation of Aβ (29). We previously reported that loss of Lxra or Lxrb increases Aβ plaque burden in the APP/PS1 mouse (50). At first glance, the finding that knockout of the LXR target gene Idol reduces Aβ plaque burden may appear at odds with the consequences of loss of Lxr expression. However, it is important to keep in mind that loss of Lxra and Lxrb does not lead to complete loss of Idol expression (15). Furthermore, Lxra and Lxrb control the expression of an array of genes important for cholesterol metabolism, which may exert independent effects on the development of amyloidosis and neuroinflammation (51).

A limitation of the present study is that the relative importance of the three Idol targets for the clearance of ApoE and Aβ in vivo has not yet been tested directly. Although our cellular studies strongly suggest that LDLR is involved, we cannot exclude the possible contribution of the other two Idol targets, Apoer2 (ApoE receptor 2) and Vldlr (very low density lipoprotein receptor), to ApoE and Aβ levels in vivo. Another obvious limitation is that we used a mouse model to study AD-related pathology. We previously reported that the LXR-Idol-LDLR pathway is highly active in primates (13), but further studies will be needed to translate the current findings to humans.
Previous studies have suggested that Idol inhibition may have beneficial effects on plasma LDL in primates (13). Our present results provide a basis for investigating Idol inhibition as a therapeutic approach for AD. Although targeting E3 ligases with selective small-molecule inhibitors is challenging, there is precedence for this strategy (32). Our studies suggest that pharmacological inhibition of Idol merits further investigation as a means to modulate endogenous ApoE, LDLR, and Aβ in the AD brain. It is possible that modulating physiological LDLR protein expression in microglia by inhibiting Idol-dependent degradation could improve Aβ clearance and possibly delay the onset of AD pathology. The observation that loss of only one copy of the Idol gene affects Aβ-dependent pathology in mice suggests that even partial Idol inhibition could prove beneficial in the setting of AD.

MATERIALS AND METHODS

Study design

This study aimed to uncover the role of Idol in regulating brain LDLR receptor levels and its impact on ApoE clearance and Aβ amyloidosis. To accomplish this aim, Idol was genetically ablated in a mouse model of Aβ amyloidosis as well as in primary microglia, primary hippocampal neurons, and brain-derived cell lines. The effect of Idol deficiency on LDLR, ApoE, and Aβ amyloidosis was assessed by immunohistochemistry, protein/RNA quantitation, and immunocytochemistry. Data were collected from cell culture studies with the investigators aware of the sample identities. Data collection from mouse samples and all the data analysis in both tissue culture and mouse experiments were performed in a blinded fashion.

Animals

APPswe/PSEN1dE9 mice on a C57BL/6 background (APP/PS1 mice) were obtained from The Jackson Laboratory [005864, B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/J] (22). Idol+/− mice (13) were backcrossed to C57BL/6 mice for eight generations before mating to APP/PS1 mice. For histological analysis, mice were euthanized and brains were collected after transcardiac perfusion with 4% paraformaldehyde (PFA). Brains were then postfix with 4% PFA for 24 hours at 4°C, cryoprotected by 30% sucrose at 4°C, and embedded in optimum cutting temperature compound. Brain specimens for biochemical analysis were directly frozen in liquid nitrogen. All mouse specimens were collected at 7 months of age. All mouse experiments were approved and performed under the guidelines of the Animal Care and Research Advisory Committees at the University of California, Los Angeles (UCLA) and Mayo Clinic.

Primary culture

Primary microglia were isolated from the frontal cortices of 4- to 5-day-old mouse brains. Two brains per group were collected in ice-cold Hanks’ balanced salt solution, and meninges were removed. Frontal cortices were dissected out and transferred to Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS). Cortices were then gently triturated with a 1000-μl pipette tip and then centrifuged at 800 rpm for 5 min, and the pellets were resuspended in 30 ml of the above medium to prepare mixed glial cell cultures. Cells were plated in T-225 flasks and incubated at 37°C (5% CO2). After 24 hours, culture medium was changed to DMEM containing 10% FBS. Supernatants were centrifuged at 800 rpm for 3 min, and pellets were resuspended in DMEM containing 10% FBS and granulocyte-macrophage colony-stimulating factor (25 ng/ml). At 10 and 14 days after the isolation, microglia were detached from mixed glial feeder layers by shaking on an orbital shaker at 220 rpm for 20 min, supernatants were centrifuged at 800 rpm for 3 min, and pellets were resuspended in DMEM containing 10% FBS. Isolated primary microglia were plated in six-well plates at a density of 0.5 million cells per well and incubated at 37°C (5% CO2) until use. Primary neuron and astrocyte cultures were prepared as previously described (53, 54).
Reagents and primary antibodies

GW3965 was synthesized as previously described (55). LPS from Salmonella minnesota R595 (Re) was obtained from Enzo Life Sciences. Thioflavin S was purchased from Sigma (T1892). Ab(1–42)-HiLyte488 and Ab(1–42)-HiLyte555 were purchased from AnaSpec. siRNAs for mouse Idol (SasI_Mm02_00343514) and mouse Ldlr (SasI_Mm01_00084966) were purchased from Sigma (MISSION siRNA). Accell nontargeting siRNA pool and Accell mouse Ldlr (16835) SMARTpool were purchased from Dharmacon. The antibodies used were ABCA1 (Novus or HJ1, a gift from D. M. Holtzman), Ab(4G8, Covance or 82E1, IBL International), actin (Abcam), ApoE (K23100R, Meridian Life Science), BACE1 (D10E5, Cell Signaling Technology), β-Gal (Promega), CD45 (MCA1388, Bio-Rad), glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology), Iba1 (019-19741, Wako), LDLR (Cayman Chemical), LRP1 (Abcam), and mouse anti-tubulin (Abcam or EMB Millipore).

RNA analysis

Total RNA was extracted from tissues and cells with TRIzol (Life Technologies). RNAs were reverse-transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems) or 2× SYBR Green Master Mix (GMO-SG2X-A300, Diagenode). Relative mRNA levels were calculated by the comparative Ct method using GenEx 5.3.2 (MultiD analyses) or QuantStudio 6 Flex (Life Technologies) and normalized to controls as indicated.
**Protein analysis**

Cultured cells were lysed in RIPA buffer [50 mM tris-HCl (pH 7.4), 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 0.1% SDS, 1 mM EDTA] and protease inhibitor cocktail (Roche). Proteins were sequentially extracted from brain tissues with PBS, RIPA, and 5 M guanidine buffer in the presence of protease inhibitors (19). For Western blots, equal amounts of proteins (10 to 40 µg) were separated on NuPAGE bis-tris gels (Invitrogen) or TGX gels (Bio-Rad), and membranes were probed with primary and secondary antibodies. Signals were visualized by chemiluminescence [ECL Plus (GE Healthcare) or TMA-6 ECL detection kit (Lumigen)]. Blots were quantified by densitometry with ImageJ software [National Institutes of Health (NIH)]. For quantifying Aβ, Aβ40 (KHB3481) and Aβ42 (KHB3441) human ELISA kits from Life Technologies were used.

**Histological analysis**

Brains were sectioned on a cryostat at 40-µm thickness. For immunofluorescence staining, free-floating sections were blocked with PBS containing 10% normal goat serum (NGS) at room temperature for 30 min, incubated with anti-Iba1 (1:1000) and anti-β-Gal (1:500) in blocking solution at 4°C overnight, and then incubated with Alexa Fluor 488–goat anti-mouse immunoglobulin G (IgG) (for β-Gal; Invitrogen) and Alexa Fluor 565–goat anti-rabbit IgG (for Iba1; Invitrogen) at room temperature for 1 hour. Sections were mounted on slides with ProLong Gold with DAPI (Life Technologies). For immunohistochemistry, free-floating sections were treated with 0.3% H₂O₂ and 1% SDS (for CD45 only) and blocked with tris-buffered saline containing 3% milk and 0.25% Triton-X (for Aβ) or 3% NGS (for CD45) or with PBS containing 1.5% NGS and 1% bovine serum albumin (for Iba1). Sections were then incubated with biotinylated 82E1 (1:500) or anti-CD45 (1:500) or anti-Iba1 (1:1000) in blocking solution at 4°C for 24 hours. Sections were incubated with biotinylated anti-rat or anti-rabbit IgG (for CD45 or Iba1, respectively; Vector Laboratories) in blocking solution at room temperature for 1 hour. Antibody binding was detected with Vectastain ABC Elite (Vector Laboratories) and DAB peroxidase (horseradish peroxidase) substrate kits (Fisher Scientific) supplemented with nickel solution. Sections were dehydrated and mounted on slides with Permount (Fisher Scientific). For thioflavin S staining, sections were mounted on Superfrost Plus slides (Fisher Scientific) and dried overnight, incubated in thioflavin S staining solution (50 ml of water + 50 ml of ethanol + 0.025 g of thioflavin S) for 5 min at room temperature, washed twice with 50% ethanol and water, and then mounted with Vectashield (Vector Laboratories). Images were captured on an AxioCam charge-coupled device camera (Zeiss) mounted on an Axioskop2 microscope (Zeiss). Staining was quantified using ImageJ software (19).

**Aβ uptake and clearance assay**

Neuro2a cells, hApoE3 knock-in astroglial cells, or BV2 cells were cultured at 37°C (5% CO₂) in DMEM containing 10% FBS, DMEM/F-12 containing 15% FBS and 1 mM sodium pyruvate, or DMEM containing 2% FBS and 1 mM sodium pyruvate, respectively. Cells were transfected with Idol siRNA or scrambled control using Lipofectamine RNAiMAX (Life Technologies). Forty-eight hours after transfection, cells were washed and cultured in serum-free medium containing 200 nM Aβ40. For Aβ uptake assays, cells were collected 3 hours after medium change, and intracellular Aβ levels were assessed by immunoblotting. For Aβ clearance assays, medium was collected 24 hours after medium change, and Aβ levels in the medium were assessed. All experiments were performed in duplicate or triplicate. Aβ40 monomers were prepared as previously described (56).

Primary microglia Aβ uptake assays were performed as described (38). Aβ(1–42)-HiLyte488 or Aβ(1–42)-HiLyte555 was sequentially released in 10 mM NaOH and in PBS to 1 mg/ml and aggregated at 37°C for 19 hours. For acute Idol knockout in IdolΔ/Δ microglia, cells were infected with pUltra-hot (Addgene) or pUltra-hot-Cre lentivirus 2 days before the Aβ uptake assay. For LDLR knockdown and acute Idol knockout experiments, primary IdolΔ/Δ microglia were infected with adenovirus-associated virus [AAV5/TruFR–enhanced green fluorescent protein (EGFP) or AAV5/Cre-EGFP] 4 days before and treated with Accell siRNA (nontargeting siRNA pool or mouse Ldlr SMART-pool, Dharmacon) 3 days before the Aβ uptake assay. Primary microglia plated on PDL-coated coverslips were treated with 0.5 µM aggregated Aβ42-HiLyte488 or Aβ42-HiLyte555 diluted in DMEM. Cells were washed with PBS and warm 0.25% trypsin–EDTA and then fixed with 4% PFA. Coverslips were mounted on slides with ProLong Gold with DAPI. Images were captured and quantified as above.

Primary hippocampal neurons were infected with adenovirus-associated virus (AAV8/CaMKIIa-EGFP or AAV8/CaMKIIa-GFP/Cre) at 7 days in vitro (DIV), and Aβ uptake was assessed at 14 DIV. Analysis of Aβ uptake was performed as for microglia.

**HDL-ApoE uptake assay**

Reconstituted disoical POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine)–ApoE particles were prepared as reported previously (57, 58). The POPC–ApoE particles were stored at 4°C until use. Primary microglia were plated at 5 × 10⁴ cells per well in 24-well plates in complete DMEM. On day 2, the cells were washed once with DMEM without serum, and DMEM containing 10% lipoprotein-deficient serum and 4 µg ApoE/ml POPC–ApoE particles was added to cells. After incubating at 37°C for 30 or 60 min, cells were placed on ice and washed with PBS twice, followed by fixation in 4% PFA for 10 min. Cells were mounted, and images were captured and analyzed as described above.

**Statistical analysis**

For all analyses in this study, P values were calculated by Student’s t test. Detailed information regarding measurements, sample size, and error bars is presented in Materials and Methods and in figure legends. All data values from in vivo studies are presented in tables S1 to S6.

**SUPPLEMENTARY MATERIALS**

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Fig. S1. Idol decreases LDLR protein and promotes LDLR ubiquitination in mouse brain cells.

Fig. S2. Loss of Idol does not directly affect inflammatory responses.

Fig. S3. Idol inhibition promotes the clearance of Aβ by microglia.

Table S1. Data for Aβ40 shown in Fig. 1B.

Table S2. Data for Aβ40 shown in Fig. 1A.

Table S3. Quantification of Aβ40 levels in Fig. 2A.

Table S4. Quantification of thioflavin S plaque load in Fig. 2B.

Table S5. Quantification of CD45 load in Fig. 4B.

Table S6. Quantification of Iba-1 cells in Fig. 4D.

**REFERENCES AND NOTES**


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The E3 ubiquitin ligase Idol controls brain LDL receptor expression, ApoE clearance, and Aβ amyloidosis
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Idolizing a treatment for Alzheimer’s disease
A hallmark feature of Alzheimer’s disease is the accumulation of a protein called β-amyloid (Aβ) in the brain. In their study, Choi et al. demonstrate that Idol, a protein linked to cholesterol metabolism, also has a crucial role in the development of Alzheimer’s-like disease in mice. Mice engineered to lack Idol were protected from developing Aβ deposits. The authors further showed that this protection was associated with better clearance of Aβ and apolipoprotein E by macrophage-like brain cells called microglia. This discovery suggests that the Idol pathway may be a therapeutic target for preventing the formation of Alzheimer’s disease lesions.