ApoE attenuates unresolvable inflammation by complex formation with activated C1q

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Apolipoprotein-E (ApoE) has been implicated in Alzheimer’s disease, atherosclerosis, and other unresolvable inflammatory conditions but a common mechanism of action remains elusive. We found in ApoE-deficient mice that oxidized lipids activated the classical comple ment cascade (CCC), resulting in leukocyte infiltration of the choroid plexus (ChP). All human ApoE isoforms attenuated CCC activity via high-affinity binding to the activated CCC-initiating C1q protein (Kd~140–580 pM) in vitro, and C1q–ApoE complexes emerged as markers for ongoing complement activity of diseased ChPs, Aβ plaques, and atherosclerosis in vivo. C1q–ApoE complexes in human ChPs, Aβ plaques, and arteries correlated with cognitive decline and atherosclerosis, respectively. Treatment with small interfering RNA (siRNA) against C5, which is formed by all complement pathways, reduced C1q–ApoE complex formation with activated C1q and that the resulting C1q–ApoE complex emerges as a common player to impact brain inflammation and atherosclerosis. Thus, ApoE is a direct checkpoint inhibitor of unresolvable inflammation, and reducing C5 attenuates disease burden.

Human ApoE is a polymorphic multifunctional protein arising from three alleles at a single gene locus. However, a common mode of action of ApoE in physiology and disease has not been identified1–7. The human isoforms of ApoE, ApoE2, ApoE3, and ApoE4, differ by amino acid residues 112 and 158 located out of the N-terminal receptor-binding site, yielding proteins with distinct impacts on tissue homeostasis. Thus, ApoE may act through multiple pathways depending on its isoform, cellular source, the lipid moieties it binds, and multiple genetic and environmental risk factors. Notions regarding mechanisms of action of ApoE include isoform-specific domain–domain interactions8; involvement of lipoprotein receptors9; effects on cholesterol efflux10; maintenance of the blood–brain barrier (BBB)11; and binding extracellular molecules, including beta amyloid peptide and heparan sulfate proteoglycans12. This wide range of activities indicates that ApoE exercises its functions in complex territorialized tissue contexts. Here, we explored the roles of ApoE in the aging ChP, brain, and the aorta of mice and in two major prototypic human unresolvable diseases, that is, Alzheimer’s disease (AD) and atherosclerosis. We chose these diseases because both have been linked to ApoE: AD is the most common form of dementia, and the ApoE4 isoform predisposes to late onset AD (LOAD)13,14. Atherosclerosis is the leading cause of death worldwide15,16. Our data indicate that ApoE is a CCC checkpoint inhibitor by binding to activated C1q and that the resulting C1q–ApoE complex emerges as a common player to impact brain inflammation and atherosclerosis.

Results

ChP lipid deposits, inflammation, and interferon signatures. We focused on the ChP because the ChP is the major intracranial neuroimmunological interface that produces the cerebrospinal fluid (CSF), forms the blood–CSF barrier, exchanges signals between the brain and the circulation, and is the principal gateway for bloodborne leukocytes to infiltrate the central nervous system in inflammatory and degenerative brain diseases16–18. ApoE−/− mice develop spontaneous hyperlipidemia when maintained on normal chow (NC). To distinguish effects of human isoforms of ApoE and the separate impact of hyperlipidemia, humanized ApoE3-knockin

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of ApoE or in hyperlipidemic ApoE4-KI, but not in hyperlipidemic NC ApoE4-KI or NC or HFD ApoE3-KI ChPs, although HFD isoform-dependent IFN signature expression in ChPs. WT (5 mice); ApoE–/–, ApoE4–/–.

Fig. 1 | ChP lipid, inflammation, and interferon signatures. a, ChP sections were stained with oil red O (ORO) for lipid (red) and hematoxylin (HE) for nuclei (blue). Scale bar, 100 μm; representative images from b. ChPs and associated parenchyma per tissue area were quantified as described in Methods. b, WT (n = 3 mice); ApoE–/– (n = 9); ND ApoE3 (n = 6); HFD ApoE3 (n = 6); ND ApoE4 (n = 6); HFD ApoE4 (n = 9). c, Plasma cholesterol. WT (n = 6); ApoE–/– (n = 6); ApoE3 (n = 14); ApoE3 HFD (n = 17); ApoE4 (n = 8); HFD ApoE4 (n = 10). d, Epithelial cells were stained for cytokeratin (keratin, red), leukocytes (CD45, green), and nuclei with DAPI (blue). Phase contrast delineates the ChP. Dashed line indicates the border of ChP and the ventricle. Scale bar, 100 μm. e, CD68+ areas were quantified as described in Methods. 12 sections from 4 WT mice; 12 sections from 4 ApoE–/– mice. f, ChPs were stained for lipid with BODIPY (BO, green), endothelial cells (CD31, cyan), and macrophages (Iba-1, red) (left panel). TEM shows a single ChP macrophage-foam cell (middle panel) and lipid (BO, green) and immunoglobulin (Ig, red) (right panel). Scale bars, 10 μm. g, IFN-related genes in ChPs by ~18-fold and also localized at the luminal side of the epithelial cells (Extended Data Fig. 1a). Additionally, high-resolution and transmission electron microscopy (TEM) revealed leukocytes and macrophages in the CSF attached to the microvilli at the abluminal side of the ChP (Extended Data Fig. 1c, left and middle panels); some of the intraventricular macrophages accumulated lipid, yielding a foam cell-like appearance (Extended Data Fig. 1c, right panel). These data suggest that macrophages on both sides of the blood–CSF barrier engulfed lipid. Extracellular lipid provides a pathological surface for complement activation in other diseases, thus prompting us to consider complement activation in ChPs. Because extracellular ChP lipid appeared at the luminal side and the stromal space, we first considered the possibility that immunoglobulins (Igs) bind to the lipid droplets. In ApoE–/– ChPs, Igs colocalized with lipid inside the capillary lumen, the stromal space, and the lipid between the epithelial cells (Fig. 1f and Extended Data Fig. 1d). These data show that lipid and Igs accumulate outside of the blood–CSF barrier in the ChP. Bell et al. previously reported that ApoE deficiency and transgenic expression of ApoE4 in NC-fed ApoE4-KI mice were afflicted with BBB breakdown1. Igs, which we used as a marker of BBB breakdown,
accumulated in the perivascular space of the lipid-free brain parenchyma of ApoE−/− and NC or HFD ApoE4-KI mice, thus confirming the findings of Bell et al. However, there was no statistically discernable aggravation of BBB dysfunction as a function of hyperlipidemia (Extended Data Fig. 1e).

In view of the morphological ChP phenotypes in WT, ApoE−/− and ApoE-KI mice (Fig. 1 and Extended Data Fig. 1), we decided to examine differential ChP gene expression profiles in six groups of mice: WT, ApoE−/−, ND- or HFD-fed apoE-KI ApoE−/− and NC or HFD-fed ApoE4-KI mice. For this purpose, laser capture microdissection-based MIAME-compliant microarrays (https://www.ncbi.nlm.nih.gov/geo; accession nos.: GSE85774 and GSE85775) from ChPs of various mouse groups were examined. 241 differentially expressed ChP genes in the six transcripts were identified in gene ontology (GO) terms ‘immune system process,’ ‘transcription factor binding,’ ‘cell junction,’ and ‘ATP binding’ (Extended Data Fig. 1f and Supplementary Table 1). In ApoE−/− ChPs, most (81%) differentially expressed genes were downregulated compared with those in WT ChPs (Fig. 1g); however, 58% (7/12) of upregulated differentially expressed genes were downregulated compared with WT ChPs (Fig. 1g). Moreover, several genes that were downregulated in ApoE−/− ChPs were rescued in their ApoE-KI counterparts, indicating the presence of a C3b-initiated amplification loop that recruited factor H in both groups of mice. Interestingly, C1qa and C1qb transcripts were rescued in ApoE-KI versus ApoE−/− ChPs (Fig. 2i, and Supplementary Table 3).

Complement-driven ChP inflammation is attenuated by C5 siRNA. It is well recognized that oxidation-specific epitopes in extracellular lipid bind Igs and activate complement18,19, and complement activation results in surface opsonization by C3b; generation of locally acting anaphylatoxins, that is, C3a and C5a; and subsequent recruitment of leukocytes and tissue inflammation20. We hypothesized that the lipid deposits in ApoE−/− ChPs bind Igs with resultant activation of complement. IgG, C3a, and C5a were evident together with lipid in ChPs of ApoE−/− mice, but not in WT mice (Fig. 2a,b). The CCC-initiating C1q molecule and C4 colocalized with ChP lipid deposits (Extended Data Fig. 2a). Most complement constituents are produced by the liver and released into the circulation as inactive components, or they can be produced locally in tissues. Whereas several complement components were significantly expressed in the ChP, C5 transcripts were below the threshold level in ChP transcripts, indicating that ChP C5 was largely serum- and liver-derived (not shown). To examine whether ChP lipid-triggered CCC activation participates in leukocyte infiltration, we chose to specifically target liver-derived C5 using an siRNA that is exclusively taken up by the liver using an asialoglycoprotein receptor-specific tag, that is N-acetylgalactosamine (GalNAc) C5 siRNA (Fig. 2c). Liver C5 siRNA knockdown in ApoE−/− mice led to a large decrease of circulating C5 levels (Fig. 2c), without affecting blood lipoprotein concentrations, body weight, or circulating leukocytes (data not shown). Liver-targeted C5 silencing also resulted in substantial decrease of C5 protein deposits in the ChP (Extended Data Fig. 2b) and significantly attenuated CD45+ leukocyte, CD68+ macrophage and DC, and CD3+ T-cell infiltration in ApoE−/− ChPs (Fig. 2d–f). In contrast, IgG, C4, and C3 deposition were much less affected (Extended Data Fig. 2b,c). These data demonstrated that lipid-triggered complement cascade activation promoted ChP leukocyte infiltration. However, C3 and C4 were present at much lower levels in HFD ApoE4-KI ChPs versus ApoE−/− ChPs (Fig. 2g), despite similar amounts of ChP lipid (parallel sections from Fig. 1c) and respective serum C3 and C5 levels (Extended Data Fig. 2d), thus indicating that the presence of ApoE in the ChP prevented the marked complement cascade phenotype of ApoE−/− ChPs. ApoE colocalized with Igs and C1q (Fig. 2h and Extended Data Fig. 2e). Using an unbiased gene expression approach, that is, expression microarrays, we examined all complement-related gene signatures in ChPs. We identified six transcripts encoding CCC-specific constituents (c1qa, c1qb, c1qc, c2, c3ari, cira), which were selectively upregulated in ChPs of ApoE−/− mice compared with WT mice (Fig. 2j and Extended Data Fig. 2f). Subsequent to CCC activation, C3b (cleavage product of C3) initiates a constitutive amplification loop of the alternative complement cascade. Factor H (alternative complement pathway inhibitor) mRNA was detectable without differences between groups (Extended Data Fig. 2g); factor B and MASPI (components of the alternative and lectin pathways of complement activation) transcripts were below threshold levels. However, factor H protein accumulation was observed on lipid deposits of both ApoE−/− and HFD ApoE4 ChPs (Extended Data Fig. 2h), indicating the presence of a C3b-initiated amplification loop that recruited factor H in both groups of mice. Interestingly, C1qa and C1qb transcripts were rescued in ApoE-KI versus ApoE−/− ChPs (Fig. 2i, and Supplementary Table 3), and various complement regulators were expressed in ApoE−/− and ApoE-KI ChPs (Extended Data Fig. 2f). Taken together, these data revealed pronounced CCC activation in ApoE−/− but not in HFD ApoE3-KI, and less in HFD-fed ApoE4-KI mice. Additionally, we found that ApoE mRNA ranges in the top 50 of ~14,000 genes expressed in WT ChPs, indicating that ApoE is expressed at extraordinarily high levels in normal ChPs, ranging in expression in >99% of all expressed ChP genes (Supplementary Table 4).

ApoE inhibits CCC activity. The salient absence or low expression of key complement components in HFD ApoE4-KI ChPs led us to examine a role of ApoE in the classical, alternative, and lectin pathways14. ApoE was added to normal human serum (NHS), which was activated by pathway-specific buffers, and incubated with non-human erythrocytes, and lysis of erythrocytes was determined. All three variants, ApoE2, ApoE3, and ApoE4, reduced CCC activation, but not the alternative pathway (Fig. 3a). Furthermore, in a complement-mediated killing assay, Escherichia coli remained viable upon ApoE-supplemented NHS challenge, but were killed when complement was activated via the lectin or alternative pathways (Fig. 3b), indicating that ApoE inhibits CCC activity, but not the alternative or lectin pathways. All three ApoE isoforms inhibited deposition of C4b and the terminal complement complex (TCC) by ~80% (Fig. 3c), showing that ApoE acts early in CCC activation. Oxidized low-density lipoprotein (oxLDL) has been reported to activate the CCC12; we found that purified C1q indeed bound malondialdehyde-modified LDL (MDA-LDL) and oxLDL, but not native LDL (Extended Data Fig. 3a). Moreover, ApoE inhibited the CCC and reduced C4b deposition when the CCC was activated by MDA-LDL (Extended Data Fig. 3b). Notably, amyloid fibrils but not soluble amyloid triggered C3b deposition, thus reflecting complement activation (Extended Data Fig. 3c,d).
C1 complex, then C2 and C4 are cleaved to form the C3 convertase C4b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b. We incubated ApoE3 with C2 or C4 in the presence of the C1 complex, then C2 and C4 are cleaved to form the C3 convertase C3b2b3b.
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To determine the binding site in ApoE to C1q, ApoE peptides were generated and examined for their ability to reduce ApoE4-mediated CCC inhibition (Fig. 4a). ApoE peptide P139–152 but not P30–40, P74–85, or P210–232 abrogated CCC inhibition by ApoE4 (Fig. 4b), though P139–152 alone did not inhibit CCC activity (data not shown). Analyzing binding of the four ApoE peptides to C1q revealed binding of P139–152 to C1q, but not of peptides P30–40, P74–85, or P210–232 (Fig. 4c).

As monitored by electron microscopy, gold-labeled ApoE and gold-labeled ApoE139–152 peptide revealed binding to the C1q stalk but not to the globular heads, which mediate target binding (Fig. 4f).

ChP C1q–ApoE complexes correlate with cognitive decline. Though ChP lipid deposits have not been reported in AD, we searched for pathologies in human AD ChPs that may resemble the pathology of ApoE−/− and HFD ApoE4-KI ChPs. We studied 30 age- and gender-matched human brains with various stages of AD-associated pathologies, that is, Braak & Braak stages for neurofibrillary tangles (NFTs), Thal phase for Aβ plaque score, and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) for neuritic plaque (Aβ plaque with dystrophic neurites) burden (Supplementary Table 6). 13/30 patients had no signs of dementia (Braak & Braak 0–III, Thal phase 0–5, CERAD stage 0), whereas 17/30 patients exhibited dementia upon clinical neurological examination and showed marked AD pathologies (Braak & Braak IV–VI, Thal phase 1–5, CERAD stage B–C) (Supplementary Table 6).

Interestingly, 29 of the 30 brains showed various degrees of ChP lipid deposits (CERAD stage B-C) (Supplementary Table 6). 13/30 patients had no signs of dementia, whereas 17/30 patients exhibited dementia. The burden of ChP lipid deposits correlated with the pathology of ApoE−/− and HFD ApoE4-KI ChPs (Fig. 5a). Notably, cases with demented AD showed higher rates of lipid in ChPs versus those without dementia (Fig. 5a). Moreover, the burden of ChP lipid deposits correlated...
C1q–ApoE complexes indicate ongoing complement activity in Aβ plaques. Complement C1q and ApoE have been observed in human AD plaques. We examined whether C1q–ApoE complexes can be observed in human AD plaques. C1q, Aβ, phosphorylated Tau (pTau), and C3 colocalized with ApoE3 (Extended Data Fig. 6f–h) in human brains with AD. C1q–ApoE complexes were also observed in human AD plaques (Fig. 5i). Moreover, Aβ–ApoE complexes but not ApoE–pTau complexes were observed in AD plaques of those with dementia (Fig. 5i), extending and corroborating findings that ApoE binds to Aβ but not pTau in vitro26. These data add to earlier reports that ApoE, C1q, and C3 are detectable in human AD plaques by demonstrating the buildup of the C1q–ApoE and Aβ–ApoE complexes in brains from AD cases with dementia. We next searched for the C1q–ApoE complex in a mouse model of cerebral β-amyloidosis (the APPPS1-21 mouse carries mutations in both the APP and presenilin genes, leading to rapid onset amyloid lesions)27. High-resolution 3D confocal microscopy of C1q–ApoE complexes in brains from AD cases with dementia. We considered the possibility that liver C5 would have inflammatory effects inside the BBB, that is, the brain parenchyma, specifically in AD plaques. For this purpose, we treated APPPS1-21 mice with liver-specific C5 siRNA. C5 siRNA treatment significantly reduced the number and density of Aβ-plaque-associated microglia cells (Fig. 5m) and of Aβ-plaque-associated LAMP1 (Extended Data Fig. 7b). C5 siRNA also reduced the serum C5 (Fig. 5i) and the number and density of Aβ-plaque-associated microglia cells (Fig. 5m). The majority of Aβ–ApoE complexes were located inside X04+ Aβ plaque (Extended Data Fig. 7a and Supplementary Video 2). The presence of C1q–ApoE complexes is a marker for persistent CCC activity in vivo. We considered the possibility that liver C5 would have inflammatory effects inside the BBB, that is, the brain parenchyma, specifically in AD plaques. For this purpose, we treated APPPS1-21 mice with liver-specific C5 siRNA. C5 siRNA treatment significantly reduced the number and density of Aβ-plaque-associated microglia cells (Fig. 5m) and of Aβ-plaque-associated LAMP1 (Extended Data Fig. 7b). C5 siRNA also reduced the serum C5 (Fig. 5i) and the number and density of Aβ-plaque-associated microglia cells (Fig. 5m). The majority of Aβ–ApoE complexes were located inside X04+ Aβ plaque (Extended Data Fig. 7a and Supplementary Video 2). The presence of C1q–ApoE complexes is a marker for persistent CCC activity in vivo. We considered the possibility that liver C5 would have inflammatory effects inside the BBB, that is, the brain parenchyma, specifically in AD plaques. For this purpose, we treated APPPS1-21 mice with liver-specific C5 siRNA. C5 siRNA treatment significantly reduced the number and density of Aβ-plaque-associated microglia cells (Fig. 5m) and of Aβ-plaque-associated LAMP1 (Extended Data Fig. 7b). C5 siRNA also reduced the serum C5 (Fig. 5i) and the number and density of Aβ-plaque-associated microglia cells (Fig. 5m).
Fig. 5 | ChP C1q–ApoE complexes correlate with cognitive decline in Alzheimer's disease. a, Human ChP sections were stained with ORO and HE. Scale bar, 100 µm. ChPs lipid was quantified as described in Methods. Non-dementia (n = 13); dementia (n = 17). b, Pearson correlation of ChP lipid and neurofibrillary tangle stage (Braak & Braak). n = 30. c–e, ChP lipid correlated with Aβ score (Thal phase), neuritic plaque score (CERAD), and ApoE genotype. n = 30 biologically independent samples. f, ChP lipid correlates with dementia in ApoE3 and ApoE3 carriers. ApoE3 and ApoE3 non-dementia cases (n = 10) and dementia (n = 7). g, Human ChP sections were stained for C1q (green) and C5 (red). Scale bar, 100 µm. C5 percentage of lipid– ChP or lipid+ ChP from the same case was quantified as described in Methods. Lipid– (n = 7 biologically independent samples), lipid+ (n = 7). h, STED microscopy shows colocalization of C1q (green) and ApoE (red). Scale bar, 5 µm. i, Binding of C1q–ApoE in vivo by PLA. Anti-ApoE, anti-C1q, or no primary antibodies were used as controls. The number of C1q–ApoE complexes of lipid– ChP or lipid+ areas were quantified as described in Methods. Lipid– (n = 4 independent samples), lipid+ (n = 4). Scale bar, 5 µm. j, Human brain sections were stained for Aβ/ApoE, pTau/ApoE, C1q/ApoE, or C1q alone. Protein–protein binding in vivo was detected by PLA. Blue for nuclei. Scale bar, 5 µm. k, 16-week old AD (APPSS1–21) brain cortex sections were examined using the PLA assay for the presence of C1q–ApoE complexes, with methoxy-X04 to outline plaques. X04– (n = 5), X04+ (n = 5). Scale bars, 10 µm, l, Liver-targeted CS siRNA reduces serum CS in APPPS1–21 mice. Control (n = 4 mice), CS (n = 5). m, Brain sections were stained with iba1 for microglial cells (red), To-Pro-3 for nuclei (green), and X04 for Aβ plaque (blue). White dashed circle represents the area within a 30 µm radius. The number of iba1+/To-Pro-3+ cells per area were quantified (>30 µm radius represents non-Aβ plaque area). Plaques were further grouped into small plaques (X04% < 0% of 30 µm radius area), moderate plaques (X04% between 10 and 30% of 30 µm radius area), and large plaques (X04% > 30% of 30 µm radius area). Percentage of iba1+ positivity within a 30 µm radius of Aβ plaques and non-Aβ plaque areas were compared. 420 individual Aβ plaques and 40 fields of non-Aβ plaques from four control mice, 536 individual Aβ plaques and 51 fields of non-Aβ plaques from five CS-treated mice. Data in h,j are representative images from at least three independent samples. Data represent means ± s.e.m. Two-tailed Student’s t-test was applied to a, c–e, f, paired two-tailed Student’s t-test was applied to g, h, k; one-way ANOVA was applied to e; two-way ANOVA was applied to l, m.
plaque load was unchanged under these experimental conditions (Extended Data Fig. 7c,d). These data show that C5 cleavage contributes to Aβ pathology. Additionally, C1q–ApoE complexes were observed in 8-week old (adolescent) WT brain cortices (Extended Data Fig. 7e). As the complex only forms under conditions of activated C1q (described above), these data indicate that the C1q–ApoE complex may play a physiological role in normal brain homeostasis.

C1q–ApoE complexes in atherosclerosis. Our data raised the possibility that other unresolvable human diseases show similar pathological hallmarks that we identified in ApoE−/− ChPs in mice and human and mouse AD brains. We examined complement pathways during the early stages of atherosclerosis in ApoE−/− mice. When we mined gene expression signatures in WT versus ApoE−/− aortas5,6, we found nine complement-pathway-related transcripts (largely CCC related) to be >2-fold upregulated during development of aortic atherosclerosis (Fig. 6a, Extended Data Fig. 8a,b and Supplementary Table 7). The impact of CCC on atherosclerosis was examined by C5 siRNA treatment on ApoE−/− mice for 20 weeks (Fig. 6b). Treatment reduced both thoracic and abdominal atherosclerosis by ~65% in the total aorta (Fig. 6c). C5 siRNA reduced aortic root atherosclerosis and decreased macrophage density in plaques (Fig. 6d,e), without affecting blood lipid levels, body weight, or blood leukocyte counts (Extended Data Fig. 8c–g). We next evaluated C1q–ApoE complexes in human carotid atherosclerosis. Five healthy control arteries on autopsy (type 0–I; American Heart Association classification7), six early (type II–III) and nine advanced atherosclerotic plaques (type V–VII) (Supplementary Table 8) from carotid endarterectomy specimens were stained for CD68, C1q, ApoE, and C5 by DAB and hematoxylin. Representative images from human carotid atherosclerotic arteries on autopsy (type 0–I; American Heart Association classification7), six early (type II–III) and nine advanced atherosclerotic plaques (type V–VII) (Supplementary Table 8) from carotid endarterectomy specimens were stained for CD68, C1q, ApoE, and C5 by DAB and hematoxylin. 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Representative images from human carotid atherosclerotic arteries on autopsy (type 0–I; American Heart Association classification7), six early (type II–III) and nine advanced atherosclerotic plaques (type V–VII) (Supplementary Table 8) from carotid endarterectomy specimens were stained for CD68, C1q, ApoE, and C5 by DAB and hematoxylin. Representative images from human carotid atherosclerotic arteries on autopsy (type 0–I; American Heart Association classification7), six early (type II–III) and nine advanced atherosclerotic plaques (type V–VII) (Supplementary Table 8) from carotid endarterectomy specimens were stained for CD68, C1q, ApoE, and C5 by DAB and hematoxylin. Representative images from human carotid atherosclerosis plaque was determined by PLA. Intima (n = 3 independent samples), media (n = 3). Scale bar, 5 μm. i, High-resolution microscopy showed colocalization of lipid (green) and malondialdehyde epitopes (MDA2, red) in human atherosclerotic plaque. Scale bar, 10 μm. j, Representative images from at least three independent samples. k, Schematic representation of the C1q–ApoE complex. Locally produced and/or serum-recruited C1q is activated in situ by a variety of surface activators including oxidized lipid, oxDLDL, amyloid fibrils, and immunoglobulins. C1q activators have been implicated in diseases as varied as atherosclerosis and AD. After activation, C1q acquires an active conformation that allows initiation of the CCC, with resultant CCC-dependent physiological/beneficial or,
if the CCC becomes persistently activated, disease-associated inflammation, deserves future attention in both basic and clinical studies.

AD and atherosclerosis share risk factors\textsuperscript{47}, whereas the second most common form of dementia, vascular dementia, has been closely related to LOAD. The incidence of AD is greatly enhanced in patients with both atherosclerosis and the ApoE4 allele, consistent with common mechanisms of disease progression\textsuperscript{48}. Our suggestion that the C1q–ApoE complex forms an active disease-relevant regulatory module is consistent with the frequent occurrence of autoimmune diseases or immune deficiencies in patients afflicted with genetic absence or loss-of-function mutations in C1q, C2, C4, and other components of the CCC and with the identification of both C1q and ApoE as major players in LOAD and atherosclerosis in genetic association studies\textsuperscript{49,50}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{A schematic diagram illustrating the proposed role of the C1q–ApoE complex in the development of atherosclerosis and AD.}
\end{figure}
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Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41591-018-0336-8.

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Author contributions
These authors contributed equally: C.W., P.F.Z., C.S., and A.J.R.H.; C.Y. and A.J.R.H. designed and performed experiments and wrote the manuscript; S.A., FEZ, C.S. designed...
and performed experiments and contributed to writing the manuscript; C.W. provided critical intellectual input for experimental design and wrote the manuscript; A.B. provided the C5 siRNA; Z.M., S.K.M., C.Z., Y.L., S.N., M.W., L.P., D.H., S.V.B., P.S., M.B., R.T.A.M., S.S., M.H., L.D.H., H.-H.E., J.P., J.H., S.R., T.A., I.H., and C.J.B. performed experiments or analyzed the data.

Competing interests
C.Y., A.J.R.H., A.B., S.K.M., S.A., P.F.Z., and C.S. declare competing financial interests. C.Y., S.K.M., and A.J.R.H. are owners of Easemedcontrol R & D GmbH & Co KG Munich, Germany; A.B. is employed by Alnylam Pharmaceuticals Cambridge, MA, USA; C.Y. and A.J.R.H. have been named inventors on a pending patent application related to treatment and diagnosis of unresolvable inflammatory diseases (EP18183584.4); A.B. has been named as an inventor on patent applications related to C5 including PCT publication WO2014160129, and applications and patents based thereon; S.A., P.F.Z., and C.S. have been named inventors on a pending patent application (DE 10 2018 100 377.3).

Additional information
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Methods

Mice. C57BL/6 WT and ApoE−/− mice were purchased from the Jackson Laboratories and housed in the animal facilities of Jena and Munich Universities. WT and ApoE−/− mice were fed a standard rodent chow under pathogen-free conditions. ApoE2-knock-in (ApoE2-KI) and ApoE4-KI mice on C57BL/6 background were purchased from Taconic, USA and maintained in the animal facility of Jena University under mouse chow or fed a high-fat cholate-containing diet (Altromin, Germany) containing 15.8% fat, 1.25% cholesterol, and 0.5% sodium cholate. The diet was started at the age of 62 weeks and continued for 16 weeks. APPPS1−21 mice were studied in collaboration with Mathias Jucker, Hertie Institute for Clinical Brain Research, University of Tübingen. Animal procedures were approved by Regierung Oberbayern according to guidelines of the local Animal Use and Care Committee and the National Animal Welfare Laws.

Human brain and choroid plexus tissues. All tissues were collected and provided by the Neurobiobank Munich, Ludwig-Maximilians-University (LMU) Munich according to the guidelines, and the protocols were approved by the ethics committee of Faculty of Medicine, LMU. The ApoE genotype was determined by means of PCR (Ezway PCR kit, Koma Biotech). AD-related pathologies (neurofibrillary tangles and beta amyloid) were determined according to the guidelines of the Brain Net Europe Consortium17, the density of neuritic plaques according to the plaque score modified from CERAD by the National Institute on Aging23. Patient characteristics are summarized in Supplementary Table 6.

Human carotid artery tissues. Atherosclerotic carotid plaques were obtained from carotid arteries of various human donors (10–70%) [55] after carotid endarterectomy. Healthy control carotid arteries were obtained from the Forensic Medicine Institute (type 0−1)11. Healthy control arteries comprised all three vessel layers: intima, media, and adventitia. Atherosclerotic plaques consisted mainly of the diseased intima resulting from the surgical intervention used for plaque excision24. The study was performed according to the Guidelines of the World Medical Association Declaration of Helsinki. The ethics committee of Faculty of Medicine, Technical University of Munich (TUM) approved the study, and written informed consent for permission to be included into the Munich Vascular Biobank was given by all patients. The baseline patient characteristics are summarized in Supplementary Table 8.

C5 siRNA injection. Mice were randomly separated into two groups. 5 mg/kg C5 siRNA targeting the liver (20 mg/ml in PBS) or control siRNA targeting luciferase (20 mg/ml in PBS) (provided by Alnylam Pharmaceuticals, Inc.) were administered subcutaneously every 2 weeks. Serum C5 protein levels were determined by means of ELISA. Complement C5-deficient DAB2 mouse serum (provided by Alnylam Pharmaceuticals, Inc.) was used as negative control for ELISA.

Atherosclerotic lesion analyses. Mouse aortas were prepared and stained by Sudan-IV for en face atherosclerosis analysis, as described previously24. The extent of atherosclerotic plaques was quantified in total aorta, thoracic aorta (1.5–2.5 cm), and abdominal aorta (70–150 mm)25. Atherosclerotic plaques were quantified in oil red O− and hematoxylin-stained serial sections of aortic root. Human carotid plaques were segmented in blocks of 3 to 4 mm, fixed in formalin overnight, decalcified in 0.5 M EDTA (pH 7.2) and embedded in paraffin (FFPE), as described previously26−28, or fresh frozen in Tissue-Tek (Sakura Finetek) for immunofluorescence staining of lipid. Hematoxylin- and Elastic Van Gieson (EvG) staining was performed to assess atherosclerosis. Specimens were divided into early (II−III) and advanced stages of atherosclerosis (−VII). In paraffin sections, atherosclerotic plaques were quantified using different dilutions to determine the best staining results with minimal background. After primary antibody incubation, visualization was performed using the LSAB ChemMate Detection Kit (Dako, Denmark) according to the manufacturer’s instructions. Slides were scanned by ScanScope microscope (Leica) to obtain digital images. All images were prepared as TIF files and quantified using image software.

Histology, immunofluorescence microscopy, and morphometry. For immunofluorescent labeling, tissues were dissected and embedded in Tissue-Tec (Sakura Finetek), frozen in isopentane, and stored at −80 °C. 20 μm whole mouse brain coronal sections or one hemisphere of AD mice were prepared according to the mouse brain atlas map29. Human and mouse brain sections were stained for oil red O and hematoxylin-stained serial sections of aortic root. Human carotid plaques were segmented in blocks of 3 to 4 mm, fixed in formalin overnight, decalcified in 0.5 M EDTA (pH 7.2) and embedded in paraffin (FFPE), as described previously26−28, or fresh frozen in Tissue-Tek (Sakura Finetek) for immunofluorescence staining of lipid. Hematoxylin- and Elastic Van Gieson (EvG) staining was performed to assess atherosclerosis. Specimens were divided into early (II−III) and advanced stages of atherosclerosis (−VII). In paraffin sections, atherosclerotic plaques were quantified using different dilutions to determine the best staining results with minimal background. After primary antibody incubation, visualization was performed using the LSAB ChemMate Detection Kit (Dako, Denmark) according to the manufacturer’s instructions. Slides were scanned by ScanScope microscope (Leica) to obtain digital images. All images were prepared as TIF files and quantified using image software.

Labeling of lipid with BODIPY. Stock solutions were prepared by dissolving 1 mg BODIPY 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene; Invitrogen) in 1 ml ethanol and stored at −20 °C. Tissue sections were fixed with 3% PFA, then stained with antibodies as controls. Apoptotic THP-1 cells (ATCC, TIB-202) were stained with Alexa Fluor 488 (green) and Alexa Fluor 546 (red) for lipid detection with BODIPY. Alexa Fluor 488 was determined with Duolink PLA kit according to manufacturer’s protocol. Leica confocal microscope (SP8, Leica, Germany) equipped with a 100x oil objective (NA 1.4) and LSM 710 equipped with ZEN 2011 software were used for image. Six fields per each sample were recorded; 3D reconstructions and the number of PLA signals per volume were performed using LAS-X software package (Leica, v1.2, Germany). Fluorescence was measured on cultured single cells.

Transmission electron microscopy. Mice were put down by isoflurane and perfused transcardially for 2 min with PBS, then perfused for 8 mins with freshly prepared 4% PFA + 1% glutaraldehyde under 120 mm Hg pressure. Brain tissues were immersed in cacodylate buffer and cut at 4 μm using a cryostat (Leica, VT1000S). After three washes with cacodylate buffer, a post-fixation with 1% osmium tetroxide in cacodylate buffer was done, according to previous methods30. 0.5 μm sections of areas of interest were selected. 80 nm ultrathin sections were cut and stained with lead citrate and examined using an EM900 (Zeiss) TEM.

Blood–brain barrier assay. Mice were perfused with 200 ml 4% PFA under 120 mm Hg pressure. Ig was stained with donkey anti-mouse IgG conjugated Cy3 antibody. Collagen IV antisera was used to label basement membranes of blood vessels. Ig* signals located in the perivascular space were used to determine BBB integrity. For quantitative studies, cerebellum was used; three or four sections (120 μm interval) were stained with Ig; vessels with a diameter of more than 10 μm (representing arteries) were used.

Laser capture microscopy and generation of microarrays. LCM and microarray analyses were performed as previously reported31. Brain sections
were dissected using the PALM Micromanipulation system (Carl Zeiss Micromanaging). Trizol buffer was used to lyse tissues. RNA preparation and microarrays were done as reported previously. cDNA was synthesized, amplified, and purified, and then labeled with [γ-32P]ATP (0.5–1.2 Ci mmol⁻¹) and hybridized for 24 h in hybridization buffer according to Affymetrix protocols. Arrays were scanned immediately after staining, and raw data were scaled to 500 and exported for further analyses.

**Microarray data analysis.** Microarray data analyses were done as previously reported. Briefly, signal intensities were calculated from the raw data and scaled to an array trimmed mean of 500; R and Bioconductor were used in all further steps. Data were filtered prior to statistical analysis to remove genes with low expression or without significant changes between groups. Data were used from two groups and subjected to Student's t test. Use of data after applying filters with more stringent two-group tests were subjected to one-factor variance analysis (ANOVA). Tests with P values of P ≤ 0.01 were performed with Benjamini–Hochberg correction for multiple testing. Microarray data were deposited in the NCBI's gene expression omnibus (GEO; accession number GSE85774 and GSE85775 for ChPs, and GSE40156 for aortas).

**Proteins and antibodies used for in vitro assays.** Component complexes C2, C3, Cb, C4, C4b, C1q, C1s, Factor H, Factor I, and C4BP as well as all primary antibodies (anti C1q, A200/3b; anti C2, A212/18b; anti C3, A213/5a; anti C2, C3, C3b, C4, C4b, C1q, C1s, Factor H, Factor I, and C4BP as well as all Proteins and antibodies used for in vitro assays.

**Total volume of 100 µl containing 20 % NHS (pooled from healthy human blood donors), increasing amounts of ApoE, BSA, or Eβ (each 0.25–1.5 µM) and 2 x 10⁷ rabbit erythrocytes (Fiebig-Nährstofftechnik) in MgCl₂ (187 µM, pH 7.4). To analyze inhibition of hemolysis via the classical pathway, 1% NHS, increasing concentrations of ApoE (0.01–1.5 µM) or 0.44 µM ApoE4 plus 0.33 µM ApoE peptide together with 2 x 10⁷ sheep erythrocytes (Fiebig-Nährstofftechnik) were mixed in gelatin veronal buffer (GBV⁺⁺; Complement Technology). After preincubation of the proteins in NHS for 15 min at 37 °C, the NHS–protein mix was added to the erythrocytes and incubated for additional 30 min at 37 °C. Lysis of erythrocytes was determined by measuring the amount of hemoglobin in the supernatant at 414 nm. The results were calculated as hemolysis rate relative to the level of lysis in absence of ApoE; the latter was set as 100.

**E. coli killing assay.** Different amounts of ApoE (0.1–1 µM) together with 0.2 % NHS were preincubated for 10 min at 37 °C before adding E. coli pET200/D-TOPO (1,000 cells per sample). After an incubation of 30 min at 37 °C cells were plated to a LB-agar plate and cultivated overnight at 37 °C before counting colony forming units. To distinguish which complement pathway was effecive, the experiment was performed incubating ApoE3 with NHS in GBV⁺⁺ for all three pathways, with NHS in MgCl₂, EGTA buffer for the alternative, and with C1q-depleted serum in GBV⁺⁺ for the lectin and the alternative pathways. Bacterial survival without adding ApoE was set as 100.

**Complement activation assay.** The effect of ApoE on classical complement pathway C4b and C5b-9 deposition was analyzed by ELISA. The classical pathway was activated by coating either IgM (2 µg/ml) or MDA-LLD (1 µg/ml) to a microtiter plate. ApoE (0.25–2 µM), IgM (0.5 µM) were preincubated with 1 % NHS in GBV⁺⁺ for 15 min at 37 °C and added to coated plates for 1 h at 37 °C. Complement activation with NHS alone was set as 100%

**Cleavage and cofactor assays.** The influence of ApoE on C1s to cleave C2 and C4 was determined in fluid phase. ApoE3 (5 and 50 µg/ml) was preincubated with C1s (200 µg/ml) in GBV⁺⁺ buffer for 15 min at 37 °C, C2 (10 µg/ml) or C4 (10 µg/ml) for 30 mins at 37 °C. C4b cleavage activity of ApoE was determined by incubating C4b (20 µg/ml) with ApoE3 (5 and 50 µg/ml) or C4bBP (20 µg/ml) for 30 mins at 37 °C. C4b cleavage was followed by western blotting. Cofactor activity of ApoE was measured by incubating C4b (10 µg/ml) with factor I (FI) (5 µg/ml), C4bBP (20 µg/ml), ApoE3 (10 µg/ml) or combinations thereof (FI together with C4BP and FI together with increasing amounts of ApoE3 (0.1–100 µg/ml)) for 30 mins at 37 °C. C4b cleavage was analyzed by western blotting.

**Competition assay.** To verify binding of ApoE peptide 139–152 to C1q, different amounts of ApoE3, ApoE4, as a control (0.65–100 µM) were incubated with a constant concentration of C1q (0.6 nM) on immobilized ApoE3 (0.1 µM). Binding of ApoE3 to C1q or ApoE4 was studied using a sandwich ELISA. To verify whether ApoE competes with the C1sC1r tetramer for binding to C1q, C1q (0.6 nM) together with different amounts of the C1sC1r tetramer (1.53–100 nM) in PBS⁺⁺ were incubated with 0.1 µM immobilized ApoE3 for 1 h at 37 °C. After a subsequent step, C1q binding to ApoE was determined. 20 nM C1q was incubated with increasing concentrations of LDLR (0–20 nM) to test binding ApoE, binding of C1q and ApoE4 for 1 h at 37 °C. Coated by ELISA. Background binding of anti-C1q and anti-LDLR to immobilized ApoE was set at 0%.

**Binding assays, BLItz and surface plasmon resonance.** Biolayer interferometry (BLItz*, ForteBio) was used to examine binding of recombinant ApoE isoforms to C1, C2, C4, C4 complex components C1q, C1s, C1r, and to MBL and the binding of C1q to ApoE. Biotinylated proteins (ligands) were coupled to streptavidin-coated biosensors, and different analytes were added. Streptavidin-coated biosensors (VWR) were hydrated for 10 min in PBS with calcium and magnesium (PBS⁺⁺, Lenoir) before loading 20 µg/ml biotinylated ApoE3, 2, and 4 ng of ApoE for 120 sec. After a 30 sec baseline step 45 nM of complement protein analyte was associated for 240 s to the sensor followed by a 240 sec dissociation step. For biotinylation ApoE isoforms as well as ApoE were incubated with biotin (Thermo Fisher Scientific) for 30 min at RT. Samples were separated from unbound biotin using CentriPure Mini Spin columns (Biotec). Surface plasmon resonance was used to examine the binding of ApoE3 (1–100 nM) to C1q (immobilized to a 5.5 Biochip Chip) in real time. Binding of ApoE (1,000 nM) was determined upon addition of increasing amounts of C4⁺⁺ chelator EGTA (0.1–3 mM) in the binding buffer (75 mM NaCl, 5 mM Hepes, 1 mM CaCl₂).

**Affinity measurement of ApoE binding to C1q.** Affinity constant (Kd) of association (ka) and dissociation (kd) constants of ApoE binding to C1q full-length proteins and C1s was determined by BLItz*. Streptavidin-coated biosensors were hydrated for 10 min in PBS⁺⁺. Recombinant ApoE3, ApoE4, plasma-purified ApoE3, or C1s (each 20 µg/ml) were loaded for 120 s via biotin onto the sensor. After a baseline step 45 nM of complement protein analyte was associated for 240 s followed by a 240 s dissociation step. Affinity values were generated by BLItz software analysis as an advanced kinetics experiment using MBL as reference value. Initial fluorescence analysis (NanoTemper) was used to determine the Kd of the binding between C1q and ApoE. Alexa 647-labeled ApoE3 (10 nM) or ApoE4 (30 nM) were incubated with different amounts of NHS (0.073–1196 nM) in PBS⁺⁺ for 30 min in the dark. After 10 min centrifugation, samples were transferred into standard capillaries, and initial fluorescence was measured using a Monolight NT.115Pico (LED power 60%, MSTR power 20%). To test whether the observed fluorescence changes are due to a binding event, an SD test was performed: the three samples with the highest and the lowest C1q concentration were centrifuged for 10 min at 15,000 g before removing the supernatant and adding SD mix (4% SDS, 40 mM DTT). After an incubation step of 5 min at 95 °C samples were transferred to capillaries and initial fluorescence was measured. The initial fluorescence analysis v2.0.2 was used to determine the Kd.

**Coimmunoprecipitation.** For coimmunoprecipitation of C1q (Invitrogen, clone 9A7) and ApoE (Invitrogen, clone 1H4), monoclonal antibodies were bound to protein G-coupled Dynabeads (Complement Technology). Beads were incubated with purified human ApoE (Merck) and/or purified C1q (Complement Technology) and/or NHS (2%) or C1q-deficient serum (dNHS, Complement Technology) (2%). NHS or anti-C1q antiserum with NHS were incubated with...
no precoated Dynabeads and were used as controls. Proteins were eluted from the beads with glycine buffer (pH 2.7), separated using SDS–PAGE and immunoblotted with goat anti-C1q antiserum (Complement Technology, A200) or goat anti-ApoE antiserum (Merck, 178479) and rabbit anti-goat antibody for detection. Purified C1q was added directly to the first lane of each gel as size marker for western blot.

**TEM for C1q and ApoE interaction.** To visualize single C1q protein particles by TEM, C1q (5 µg/ml) was diluted in PBS. In order to gold-label ApoE, biotinylated plasma-purified ApoE or ApoE139–152 (20 µg/ml) was incubated with streptavidin–gold complexes (5 nm gold, British BioCell International Ltd.) diluted 1:25 in PBS for 2 h at RT. C1q (10 µg/ml) was added to the ApoE–streptavidin–gold solution (1:1 mixture) and incubated under gentle shaking for 2 h at RT. To detect single C1q–ApoE complexes by structure, full-length ApoE3 (40 µg/ml) was directly labeled with EM-grade 6 nm gold particles (AURION-ImmunoGold Reagents & Accessories, The Netherlands). The probe (containing −2 × 10^14 gold particles/ml) was diluted 1:200 in PBS. Carbon-coated grids were hydrophilized by glow discharge at low pressure in air. Aliquots of C1q alone and C1q–ApoE–streptavidin–gold or C1q–ApoE–gold were adsorbed onto hydrophilic, carbon-coated grids for 1 min, washed twice with ddH2O, and stained on a drop of 2% uranyl acetate in ddH2O. Specimens were analyzed with a Zeiss EM902A electron microscope (Carl Zeiss) operated at 80 kV accelerating voltage, and images were recorded with a FastScan-CCD-camera 1,024 × 1,024 (TVIPS).

**Statistical analysis.** Significant differences between two groups were analyzed using GraphPad Prism 7 using the two-tailed unpaired Student’s t test or one-way ANOVA with multiple testing (Tukey). Values of *P* ≤ 0.05, **P* ≤ 0.01, ***P* ≤ 0.001 were considered statistically significant.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** All microarray data can be found under GEO database accession number GSE85774 and GSE85775 for ChPs. Microarray data for aorta had been published previously with the accession number GSE40156 (ref. 59). The remaining source data for figures in the manuscript will be made available upon reasonable request.

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Extended Data Fig. 1 | Lipid deposits, BBB, and ChP gene signatures. **a**, Vacuole (Va) represents lipid. Intercellular lipid (green) between two epithelial cells was quantified. 68 intercellular spaces from 3 ApoE−/− and 67 intercellular spaces from 3 WT mice were analyzed. Bar represents 1 µm. **b**, Lipid in ApoE−/− ChPs by TEM. Lymphocytes (left panel); macrophages/dendritic cells (DC) (middle panel); and ependymal cells contain lipid (right panel). Vacuole (Va) represents lipid. Bar 1 µm. **c**, ChPs were stained for cytokeratin (keratin, red) and leukocytes (CD45, green) (left panel); collagen IV (Co-IV, green) and CD68 (red) (middle panel). TEM shows single macrophage-foam cell/DCs adjacent to microvilli. Bar 10 µm. **d**, ChPs were stained with Ig (red) as described in Methods. Bars 10 µm. **e**, PFA-perfused brains were stained for Ig (Ig, red) and blood vessels (Col-IV, green) in the cerebellum. Perivascular Ig adjacent to blood vessels was quantified as described in Methods. WT (n = 3 mice); ApoE−/− (n = 3); ND ApoE3 (n = 3); HFD ApoE3 (n = 3); ND ApoE4 (n = 3); HFD ApoE4 (n = 3). Bar 10 µm. **f**, Laser capture microdissection (LCM)-based expression microarrays of ChPs. Heatmaps show transcript levels in GO terms immune system process, transcription factor binding, cell junction, and ATP binding. **g**, Genes that were down-regulated in ApoE−/− ChPs and rescued either in ApoE3-KI and in ND or HFD ApoE4-KI mice. WT (n = 5 mice); ApoE−/− (n = 4); ND ApoE3 (n = 6); HFD ApoE3 (n = 6); ND ApoE4 (n = 6); HFD ApoE4 (n = 6). Data in **c,d** are representative images from at least 3 biologically independent mouse samples. Data in **a,e,g** represent means ± s.e.m. Two-tailed Student’s t test was applied to **a,e,g**. Gene names and statistics in supplementary Tables 1, 3.
Extended Data Fig. 2 | Complement constituents in mouse ChPs. 

a, ChPs were stained for C1q (red) and C4 (green). Bar 100 μm. 
b, C5 siRNA treatment blocks C5 protein deposition in ApoE−/− ChPs. c, ChPs were stained for C3. Ig represents lipid. d, Serum C3 and C5. Serum C3 and C5 protein levels were measured by ELISA. ApoE−/− (n = 6 mice), HFD ApoE4 (n = 5). e, High resolution confocal microscopy shows colocalization of ApoE4 (ApoE, red) and Ig (green, represents lipid) in HFD ApoE4-KI ChPs. ApoE−/− ChPs serve as negative controls for ApoE staining. f, Complement regulators are expressed in ChPs. WT (n = 5 mice); ApoE−/− (n = 4); ND ApoE3 (n = 6); HFD ApoE3 (n = 6); ND ApoE4 (n = 6); HFD ApoE4 (n = 6). g, ChP Factor H expressed between WT and ApoE−/− mice. WT (n = 5); ApoE−/− (n = 4). h, ChP factor H protein in ChPs. White arrows indicate lipid positive areas. Data in a,b,c,e,h are representative images from at least 3 biologically independent mouse samples. Data in d,f,g represent means ± s.e.m. Two-tailed Student’s t test was applied to d,g; one-way ANOVA with Tukey posttest was applied to f; Gene names in supplementary Table 3.
Extended Data Fig. 3 | ApoE does not inhibit cleavage of C2 or C4 by C1s. a, C1q binds immobilized malondialdehyde-modified LDL (MDA-LDL) and oxLDL but not native LDL or gelatin. b, ApoE isoforms in normal human serum (NHS) were added to MDA-LDL-coated microtiter plates and C4b deposition was determined by specific antisera. c, d IgM, MDA-LDL, and Aβ fibrils but not soluble Aβ activate complement and cause C3b deposition. BSA, gelatin as negative controls; e, f ApoE3 was incubated with either (e) C2 or (f) C4 in the presence of C1s. C2 and C4 were cleaved to their active forms C2a (α’30) and C4b (α’83) via C1s as revealed by the cleavage products in western blot analyses. g, ApoE3 has no cofactor activity for factor I in the cleavage of C4b to inactive iC4b. ApoE3 was incubated together with factor I, C4BP and C4b, and cleavage products were detected by western blot analysis as indicated (α’25 and α’13). Full scanned blot images in e, f, g are available from source data figures. Data in a–d represent means ± s.e.m. of three independent experiments. Two-tailed Student’s t test. Data in e, f, g are representative from 3 independent experiments.
Extended Data Fig. 4 | ApoE binds to C1q but not to other complement components. a. ApoE isoforms bind to the C1 complex, but not to C4 or C2. Biotinylated ApoE was immobilized on streptavidin-coated sensors and incubated with C1 complex, C4, C2, or buffer. b. The C1 complex binds to immobilized ApoE isoforms. c. ApoE isoforms bind to C1 and factor H, but not to C3 or C3b. d. NHS-derived C1 binds to immobilized plasma-purified ApoE3 and to recombinant ApoE isoforms. e. C1q binds to immobilized plasma-purified ApoE3 and to all recombinant ApoE isoforms. f. Plasma-purified C1q was coated on a sensor chip (CM5) and plasma-derived ApoE (62-1000 nM) was injected into the fluid phase (75 mM NaCl, 5 mM HEPES, 1 mM CaCl2). g. Mannose-binding lectin (MBL) does not bind to C1q as determined by biolayer interferometry. h. Apolipoprotein A (ApoA) does not bind to C1q as determined by biolayer interferometry. i. C1q-ApoE complexes revealed by proximity ligation assay (PLA) on cultured human apoptotic cells (THP-1) were detectable when treated with NHS but not with C1q-depleted serum (dNHS). Data represent mean fluorescence intensity (MFI) ± s.e.m. of 16 cells for each group. Bar 10 μm. Data in b.c.d.e represent means ± s.e.m. of at least three independent experiments. Data a.f.g and h represent means of at least two independent experiments. Two-tailed Student’s t test.
Extended Data Fig. 5 | ApoE binds to the activated C1q; LDLR and C1sC1r tetramers do not compete with C1q-ApoE binding. **a**, ApoE-C1q interaction is dependent on Ca\(^{2+}\). Real-time binding of ApoE to C1q was followed using biosensor analyses. Binding of ApoE to C1q is reduced in a dose-dependent manner upon increasing amounts of EGTA (0.1–3 mM). **b,c**, co-immunoprecipitation of C1q-ApoE complexes; **(b)** anti C1q antiserum precipitate C1q-ApoE complexes composed of purified proteins with activated C1q, but not with inactive C1q from NHS; **(c)** Anti-ApoE antiserum precipitates C1q-ApoE complexes but no complexes from NHS. C1q-ApoE complexes were eluted with glycine buffer, then, C1q or ApoE proteins were separated by SDS-PAGE and immunoblotted using goat anti-C1q antiserum (left panel of **b**, and **c**) or goat anti ApoE antiserum (right panel of **b**) separately. Full scanned blot images in **b,c** are available from source data figures. **d**, ApoE peptide P\(_{139-152}\) but not ApoE peptide P\(_{30-40}\) competes with immobilized ApoE3 for binding to C1q in a dose-dependent manner. **e**, C1q antibody binding to C1q is not affected by SDS. **f**, C1q and LDLR bind simultaneously to ApoE. 20 nM C1q was incubated with increasing concentrations of LDLR to immobilized ApoE and binding of C1q and LDLR was followed by ELISA. Background binding of anti C1q and anti LDLR antisera to immobilized ApoE were set as 0%. **g**, ApoE does not compete with C1sC1r tetramers for binding to C1q. C1q in addition to increasing amounts of C1sC1r tetramers was added to immobilized ApoE3 and C1q binding was determined. Data in **d-g** represent means ± s.e.m. of at least three independent experiments. Two-tailed Student’s t test. Data in **a,b,c** are representatives of 3 independent experiments.
Extended Data Fig. 6 | Complement constituents in human ChPs and AD plaques. a–b, Human ChP sections were stained for C1q (green) / C3 (red) (a) and C1q (green)/ApoE (red) (b). c–d, ChP sections were stained for CD68+ macrophages/DCs (c) and collagen IV (Col-IV) to mark basement membranes. Phase contrast shows lipid deposits in ChPs. e, ChP sections were stained for ApoE (green) and factor H (red); no primary antibody as control (NA). f–g, human brain sections were stained for Aβ (green) / ApoE (red) (left panels), Tau phosphorylation (pTau, green) / ApoE (red) (middle panels), and C1q (green) / ApoE (red) (right panels) (f). Blue for nuclei. No primary antibody as control (g). h, AD brain parenchyma sections were stained for C3 (red) / ApoE (green). Bar 100 μm for a–h. Data in a–h are representative images from at least 3 biologically independent samples.
Extended Data Fig. 7 | Complement constituents in mouse brain. a, 16-week old APPPS1-21 mouse brain sections were stained with Aβ/ApoE complexes (red) by PLA, methoxy X04 for Aβ plaque (blue). High resolution confocal images show the spatial location of Aβ-ApoE complexes and Aβ plaque in 3D view (lower panel). Bars represent 10 μm. b, Brain sections were stained with methoxy-X04, ApoE, and LAMP1; the size of areas covered by methoxy-X04, ApoE, and LAMP1 was determined. ApoE/X04 and LAMP1/X04 (X04 > 150 μm²) were quantified. n = 123 plaques from 4 control mice, 147 plaques from 5 treated mice. Bars 100 μm. c, Aβ plaque was stained with methoxy X04 (X04). Number of plaques per section and number of plaque per area were quantified. control (n = 4 mice), C5 (n = 5). Bar 1000 μm. d, Total plaque volume was determined in 3D, plaques were further grouped according to the plaque volume. n = 71 random fields from 4 control mice, 88 fields from 5 C5 siRNA treated mice. Bar 100 μm. e, 8-week old C57BL6 brain cortex sections were examined for the presence of C1q-ApoE complexes with methoxy X04, ApoE, or C1q only antisera were used as negative controls. Bar represents 10 μm. Data in a, e are representative images from at least 3 biologically independent mouse samples. Data in b, c, d represent means ± s.e.m. Two-tailed Student’s t test was applied to b, c, d. Two-way ANOVA was applied to c, d.
Extended Data Fig. 8 | Complement and atherosclerosis. a, Expression microarray analyses of aortas. Heatmaps show GO terms *leukocyte migration, complement activation, phagocytosis, and cellular response to lipid*. 6 weeks WT (n=3 mice); 32 weeks WT (n=3); 6 weeks ApoE−/− (n=3); 32 weeks ApoE−/− mouse aortas. b, aorta alternative complement pathway genes (factor B, factor H, factor D) mRNA expression in 6 weeks and 32 weeks old WT and ApoE−/− mouse aortas. 6 weeks WT (n=3 mice); 32 weeks WT (n=3); 6 weeks ApoE−/− (n=3); 32 weeks ApoE−/− mouse aortas. c–d, plasma cholesterol and body weight. e–f, blood leukocytes and percentage. For c–f, control (n=11 mice); CS siRNA (n=12 mice). g, blood CD4+ T cells, CD8+ T cells, and B220+ B cells by flow cytometry. Control (n=6 mice); CS siRNA (n=6 mice). h, super-resolution microscopy shows colocalization of C1q (green) and ApoE (red) in human atherosclerotic plaque. Representative images from at least 3 biologically independent mouse samples. Bar 5 μm. Data in b,c,d,e,f,g represent means ± s.e.m. Two-tailed Student’s t test was applied to c,d,e,f,g. One-way ANOVA with Tukey posttest was applied to b. Abbreviations: WBC, white blood cells; RBC, red blood cells; PLT, platelets; LYM, lymphocytes; MO, monocytes; GRA, granulocytes. Gene names and statistics in supplementary table 7.
Extended Data Fig. 9 | Graphical presentation of the body of in vivo data. a, Pleiotropic impacts of single ApoE or single C1q molecules in brain as reported by others. Microglia cells are the major source of brain C1q. In response to Aβ plaques, resting microglia cells differentiate into plaque-associated microglia cells. Single actions of ApoE and C1q have been reported to be involved in multiple pathways as indicated in the Figure. Inactive C1q (yellow), activated C1q (red). b, Graphical presentation of the body of in vivo data. Three types of unresolvable inflammatory conditions were studied in 7 mouse models and in translational studies of human tissues, that is choroid plexus, aorta, and brain parenchyma.
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- Exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The exact test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection
- For histology analyses: ScanScope microscope (Leica) or normal microscope (Leica).
- For immunofluorescence analyses: confocal laser scanning microscope (CLSM) 510 META (Carl Zeiss, Germany) or Leica SP8 3X (Mannheim, Germany).
- For stimulated emission-depletion (STED) microscopy: Leica SP8 3X with STED.
- Transmission electron microscopy: EM900 (Carl Zeiss) and EM902A (Carl Zeiss)
- Laser capture microscopy (LCM): PALM MicroBeam system (Carl Zeiss MicroImaging).
- Microarray: Affymetrix GeneChip.
- Biolayer interferometry: BLItz™, ForteBio.
- Surface plasmon resonance: Biacore.
- FACs: BD Canto II.
- Image analysis: ImageJ, AxioVision Rel 4.6 software (Carl Zeiss) and Leica Application Suite (Leica).
- Data analysis: GraphPad Prism 7

Data analysis
- All images were prepared as TIF files by imageJ or Leica LAS-X (V1.2) software and exported into Adobe Illustrator CD6 for figure arrangements.
- 3D reconstructions were performed using LAS-X software package.
- For microarray, signal intensities were calculated from the raw data and scaled to an array trimmed mean of 500, R and Bioconductor were used in all further steps. Data were filtered prior to statistical analysis to remove genes with low expression or without significant changes between groups. Data were used from two groups and subjected to Student’s t-test. Use of data after applying filters with more
than two groups were subjected to one-factor variance analysis (ANOVA). Tests with p values P \leq 0.01 are performed with Benjamini and Hochberg correction for multiple testing;
Lysis of erythrocytes was determined by measuring the amount of hemoglobin in the supernatants at 414 nm. The results were calculated as haemolysis rate relative to the level of lysis in absence of ApoE, the latter was set as 100%;
Significant differences between two groups were analyzed by GraphPad Prism 7 using the two-tailed unpaired Student’s t-test or one-way ANOVA with multiple testing (Tukey). Values of *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001 were considered statistically significant.

Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Microarray data were deposited in the NCBI’s gene expression omnibus (GEO; accession number GSE85774 and GSE85775 for ChPs, and GSE40156 for aortas).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Sample size
Preliminary experiments were performed when possible to determine the sample size of animal required, taking into account of local ethical and reductionist animal use. The sample size for each experiment have been listed in the figure legends and methods.

Data exclusions
For microarray, data were filtered prior to statistical analysis to remove genes with low expression or without significant changes between groups.

Randomization
Mice were randomly separated into two groups. 5 mg/kg C5-siRNA targeting the liver (20 mg/ml in PBS) or control siRNA targeting luciferase (20 mg/ml in PBS).

Blinding
Mice and human samples were examined in a blinding and randomization way.

Reporting for specific materials, systems and methods

Materials & experimental systems
n/a Involved in the study
- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

Methods
n/a Involved in the study
- ChIP-seq
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Unique biological materials

Policy information about availability of materials

Obtaining unique materials

C5 siRNA and control siRNA were provided by Alnylam Pharmaceuticals, Inc. The material transfer agreement was signed between Klinik university of munich and Alnylam.

Antibodies

Antibodies used

All commercial and in house generated antibodies were listed in Methods section. Immunofluorescence:

- anti-mouse collagen IV (2150-1470; AbDserotec), immunoglobulins (715-166-151; Dianova), immunoglobulin isotype that do not react with mouse Ig (017-160-006; Dianova), anti-human/mouse C3 (A213, ComplementTech), anti-human/mouse C3a (A218, ComplementTech), anti-human CS (A220, ComplementTech), anti-mouse C5 (ab11898, Abcam), isotype for C5 (ab27478, Abcam), anti-mouse C1q (HM1096BT, Hycult Biotech), anti-human C1q (ab71089, Abcam), anti-mouse C4 (HM1046, Hycult Biotech), anti-human Factor H (A312, Quidel), anti-mouse Factor H (HM1119F, Hycult Biotech), anti-human ApoE (ab52607, Abcam), anti-human ApoE (178479; Calbiochem), anti-mouse ApoE (ab183597, Abcam), anti-mouse CD68 (EMB11, DAKO), anti-mouse CD31 (553370; BD PharMingen), anti-mouse iba-1 (019-19741; WAKO), anti-mouse C1q (HM1046, Hycult Biotech), anti-human Factor H (A312, Quidel), anti-human/mouse cytokeratin (Z0622; DAKO), anti-human collagen IV (CIV22, DAKO), anti-beta-amyloid (4G8, Biolegend), anti-phospho-Tau (AT8, Thermofisher), anti-iba1 (polyclonal, WAKO), anti-LAMP1 (1D4B, Abcam), TO-PRO™-3 Iodide (642/661) (T3605, Thermo Fisher Scientific) or DAPI for DNA. Anti-malondialdehyde (MDA)-lysine epitope antibody was in-house produced as described previously.

Proximity ligation assay (PLA):

- rabbit anti-human ApoE (ab52607, Abcam), and mouse anti-C1q (ab71089, Abcam), mouse anti-beta-amyloid (4G8, Biolegend), mouse anti-phospho-tau (AT8, Thermo Fisher), rabbit anti-mouse ApoE (ab183597, Abcam), mouse anti-C1q (HM1096BT, Hycult) with no or/and one primary antibody as controls; rabbit anti-ApoE antibody (Acris Antibodies, Herford, Germany), mouse anti-C1q antibody (Thermo Scientific, Massachusetts, USA).

Validation

The specificity of ApoE antibody is verified by the absence of signal in Immunofluorescence in samples from ApoE knock out mice.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

THP-1 cell line, ATCC® TIB-202™

Authentication

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

negative for mycoplasma contamination

Commonly misidentified lines

(See ICLAC register)

no

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

All mouse details are provided in methods section. C57BL/6j WT and ApoE-/- mice were purchased from the Jackson Laboratories and housed in the animal facility of Jena and Munich Universities. ApoE3 knock-in (ApoE3-KI) and ApoE4-KI mice on C57BL/6 background were purchased from Taconic, USA. APPPS1-21 mice were studied in collaboration with Mathias Jucker, Hertie Institute for Clinical Brain Research, University of Tübingen.

Mice were housed in the animal facilities of Jena and Munich Universities. Mice were maintained under mouse chow or fed a high fat cholate-containing diet (Altromin, Germany) containing 15.8 % fat, 1.25 % cholesterol, and 0.5 % sodium cholate. The diet was started at the age of 62 weeks and continued for 16 weeks. Animal procedures were conducted according to guidelines of the local Animal Use and Care Committee and the National Animal Welfare Laws.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.
Human research participants

Policy information about studies involving human research participants

Population characteristics

Human brain and choroid plexus tissues from patients with and without dementia. All cases were collected and provided by the Neurobiobank Munich, Ludwig-Maximilians-University (LMU) Munich according to the guidelines of the local ethics committee. Patient characteristics are summarized in extended Tabl.2. All Human artery tissues were provided by Munich Vascular Biobank. Human atherosclerotic plaques were obtained from patients with high-grade carotid artery stenosis (>70%) after carotid endarterectomy. Healthy control carotid arteries were obtained from the Forensic Medicine (type 0 – I). The baseline patients characteristics is summarised in extended Tabl.3.

Recruitment

Only human tissues were examined.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
☒ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Blood was collected into 1-2 ml of 5 mM EDTA, mixed well, and made up to 10 ml by adding ice cold FACS buffer and centrifuged at 400 G for 6 min at 4° C, followed with incubation with erythrocyte lysis buffer (ACK buffer) at RT for 5-6 mins. Lysis reaction was stopped by adding 5-10 ml of FACS buffer and centrifuged at 300 G for 5 min at 4° C. The pellet was resuspended in required amount (~ 300 μl) of FACS buffer. Cells were strained through 70 μm cell strainer again before analysis. Single cell suspensions were stained with fixable blue dead cell stain kit (L23105, invitrogen), CD45 (47-0454-81, ebioscience), CD4 (25-0041-82, ebioscience), CD8 (11-0081-82, ebioscience), and B220 (25-0452-82, ebioscience).

Instrument

Data were acquired on BD Canto II.

Software

Data were analyzed by using FlowJo software.

Cell population abundance

No FACS sorting used.

Gating strategy

Total blood cells were gated on FSC/SSC, live, leukocytes positive (CD45+), CD4+ or CD8+ or B220+ (see Extended Fig.8g)

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.