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# Complement and microglia mediate early synapse loss in Alzheimer mouse models

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**Synapse loss in Alzheimer's disease (AD) correlates with cognitive decline. Involvement of microglia and complement in AD has been attributed to neuroinflammation, prominent late in disease. Here we show in mouse models that complement and microglia mediate synaptic loss early in AD. C1q, the initiating protein of the classical complement cascade, is increased and associated with synapses before overt plaque deposition. Inhibition of C1q, C3 or the microglial complement receptor CR3, reduces the number of phagocytic microglia as well as the extent of early synapse loss. C1q is necessary for the toxic effects of soluble  $\beta$ -amyloid ( $A\beta$ ) oligomers on synapses and hippocampal long-term potentiation (LTP). Finally, microglia in adult brains engulf synaptic material in a CR3-dependent process when exposed to soluble  $A\beta$  oligomers. Together, these findings suggest that the complement-dependent pathway and microglia that prune excess synapses in development are inappropriately activated and mediate synapse loss in AD.**

Genome-wide association studies (GWAS) implicate microglia and complement-related pathways in AD (1). Previous research has demonstrated both beneficial and detrimental roles of complement and microglia in plaque-related neuropathology (2, 3); however, their roles in synapse loss, a major pathological correlate of cognitive decline in AD (4), remain to be identified. Emerging research implicates microglia and immune-related mechanisms in brain wiring in the healthy brain (1). During development, C1q and C3 localize to synapses and mediate synapse elimination by phagocytic microglia (5–7). We hypothesized that this normal developmental synaptic pruning pathway is activated early in the AD brain and mediates synapse loss.

The degree of region-specific synapse loss is a stronger correlate of cognitive decline in AD than counts of plaques, tangles and neuronal loss (8, 9). To determine how early synapse loss occurs, we used super-resolution structured illumination microscopy (SIM) (10) to quantify synapse density in hippocampal CA1 stratum radiatum of familial AD-mutant hAPP (“J20”) transgenic mice (11). Quantification of colocalized pre- and postsynaptic puncta (synaptophysin and PSD95 (Fig. 1A); synaptotagmin and homer (figs. S1A–D)) revealed a significant loss of synapses in J20 hippocampus at 3–4 months old (mo), an age that precedes plaque deposition (11, 12). Synapse loss in pre-plaque J20 CA1 was

confirmed using electron microscopy (fig. S1G). Confocal imaging also showed synapse loss in CA1, CA3, and dentate gyrus of 3 mo J20 hippocampus but not in striatum (fig. S1E). Synapse levels were not altered in 1 mo J20 brains vs. wild-type (WT) littermates (fig. S1F), suggesting that the hippocampal synaptic loss at 3 mo is likely not a result of abnormal synaptic development.

We asked whether the classical complement cascade is upregulated in pre-plaque brains when synapses are already vulnerable. C1q immunoreactivity (13; antibody now available at Abcam) was elevated in J20 brains, as early as 1 mo and preceding synapse loss (Fig. 1B and fig. S1). C1q elevation was region-specific, particularly in hippocampus and frontal cortex, two regions vulnerable to synapse loss (14) (Fig. 1B and fig. S2A). C1q immunoreactivity was comparable between J20 and WT at P21 (fig. S2B), suggesting that elevated levels at 1 mo are likely not a developmental artifact. C1q was also similarly increased in hippocampus of another model of AD, the APP/PS1 mice (15) (fig. S2C). Importantly, SIM demonstrated colocalization of C1q with PSD95-positive puncta in 1 mo J20 hippocampus (Fig. 1C). A higher percentage of PSD95 colocalized with C1q in hippocampus of J20 mice than that of WT (Fig. 1D and fig. S3), suggesting that the C1q-associated synapses may be marked for elimination.

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Punctate A $\beta$  was found deposited in J20 hippocampus at 1 mo (fig. S4), long before A $\beta$  plaques deposit (11, 12), raising the question of whether C1q increase in these pre-plaque brains is dependent on soluble A $\beta$  levels. To test this, we injected the mice with Compound E, a  $\gamma$ -secretase inhibitor that rapidly decreases A $\beta$  production (12). Compound E markedly reduced soluble A $\beta$  levels in J20 mice; there was a corresponding reduction of C1q deposition (Fig. 1E), suggesting that A $\beta$  upregulates C1q. To further address whether the increase of C1q is dependent on soluble A $\beta$ , and if so, which species, we injected soluble A $\beta$  oligomers or monomers into lateral ventricles of WT mice. Hippocampus contralateral to injection site was examined to avoid any surgery-related effects. Oligomeric A $\beta$  (oA $\beta$ ), which is pre-fibrillar in nature and acts as a mediator of synapse loss and dysfunction in AD (4), but not the relatively innocuous monomeric A $\beta$  or vehicle, induced C1q deposition (Fig. 2A and fig. S5). A higher percentage of PSD95 colocalized with C1q in oA $\beta$ -injected mice vs. monomer-injected (Fig. 2B), in a manner similar to this colocalization in J20 mice. Together, these findings show an early and aberrant increase and synaptic localization of C1q in multiple AD model systems. Furthermore, fluorescent in situ hybridization demonstrated upregulated *C1qa* expression in microglia (fig. S6), implicating microglia as a major source of C1q in these pre-plaque brains.

To test whether C1q and oA $\beta$  act in a common pathway to eliminate synapses, we injected oA $\beta$  into lateral ventricles of *C1qa* knockout (KO) mice (16). Soluble oA $\beta$  induced a significant loss of colocalized synapsin- and PSD95-immunoreactive puncta in WT mice within 72 hours (Fig. 3A, left panel) (17). In contrast, oA $\beta$  failed to induce synapse loss in *C1qa* KO mice (Fig. 3A, right panel), suggesting that C1q is required for oA $\beta$ -induced synapse loss in vivo. To determine whether local, acute inhibition of C1 activation could similarly blunt the synaptotoxic effects of oA $\beta$ , we utilized an anti-C1q antibody, which blocks the classical complement cascade in vitro (ANX-M1 (Annexon Biosciences); see fig. S7 and Supplemental Methods). Co-administration of the ANX-M1 anti-C1q antibody, but not its IgG isotype control, prevented oA $\beta$  from inducing synapse loss in WT mice (Fig. 3B). Thus, blocking C1 activation by either genetic or antibody-mediated means lessened oA $\beta$ 's synaptotoxic effects.

To determine whether C1q is associated with synaptic dysfunction, we asked whether the established ability of oA $\beta$  to potently inhibit LTP (4) was dependent on C1q. We tested the functional effects of the ANX-M1 anti-C1q antibody in acute hippocampal slices treated with oA $\beta$ . IgG alone had negligible effects on LTP induction in WT mouse hippocampal slices and on the ability of oA $\beta$  to inhibit LTP; however, pre-treatment of hippocampal slices with the C1q

antibody significantly prevented the impairment of LTP by oA $\beta$  (Fig. 3C). Neither the ANX-M1 nor its IgG control altered basal synaptic neurotransmission (fig. S8). Collectively, these results in hippocampal slices and in mice support C1q as a key mediator of oA $\beta$ -induced synaptic loss and dysfunction.

In the healthy developing brain, C1q promotes activation of C3, which opsonizes subsets of synapses for elimination, a process that is significantly downregulated in the mature brain (5, 6). However, oA $\beta$  induced a significant C3 deposition in WT adult mice (fig. S7A, upper panel). This was significantly reduced in both the *C1qa* KO (fig. S7A, lower panel) and the ANX-M1 C1q antibody-treated WT mice (fig. S7B), suggesting that the C3 deposition in this model is downstream of the classical complement cascade. Consistent with these findings, a higher percentage of PSD95 colocalized with C3 in J20 and APP/PS1 brains (Fig. 3D and fig. S9). To determine whether C3 is necessary for early synapse loss in AD genetic models, we crossed APP/PS1 mice, which, similar to the J20 mice, had significant elevation and localization of C1q and C3 onto hippocampal synapses (figs. S2C and S9), to C3-deficient mice (18). Quantification of colocalized pre- and postsynaptic puncta demonstrated synapse loss in 4 mo APP/PS1 hippocampus as compared to WT; however, APP/PS1x*C3* KO mice did not display this synapse loss (Figs. 3E and F). Together, our data indicate that genetic deletion of *C3* ameliorates synapse loss in APP/PS1 mice, providing further evidence that the classical complement cascade mediates early synapse loss in AD mouse models.

Microglia express complement receptors and mediate synaptic pruning in the developing brain (1, 6), raising the question of whether this normal developmental pruning pathway could be activated to mediate synapse loss in the pre-plaque AD brain. Consistent with this hypothesis, microglia had elevated levels of the lysosomal protein CD68 in J20 hippocampus compared to WT, and less so in striatum, a less vulnerable region (figs. S1C and S10). Furthermore, in WT mice challenged with oA $\beta$ , microglia had significantly increased levels of CD68 immunoreactivity (Fig. 2C). However, in *C1qa* KO mice in which synapse loss was rescued, oA $\beta$  failed to induce such an increase (Fig. 2D), suggesting that microglia eliminate synapses through the complement pathway.

To directly test whether phagocytic microglia engulf synaptic elements, we adapted our in vivo synaptic engulfment assay (19) using intracerebroventricular injections of A $\beta$  in Homer-GFP mice (20) (Fig. 4A). oA $\beta$  induced a significantly higher volume of internalized Homer-GFP in microglia than monomeric A $\beta$  controls did at the contralateral hippocampus (Fig. 4B), indicating that microglia engulf synaptic elements when challenged with oA $\beta$ . Internalized Homer-GFP

often colocalized with CD68 (fig. S11A), suggesting that the engulfed synapses are internalized into lysosomal compartments in a manner similar to developmental synaptic pruning (6). Importantly, oA $\beta$  failed to increase synaptic engulfment in microglia lacking CR3 (21), a high affinity receptor for C3 expressed on macrophages (Homer-GFPxCR3 KO vs. Homer-GFP mice, which received tail vein injections of PBS or oA $\beta$  (Fig. 4C)). These data demonstrate that CR3 is necessary for oA $\beta$ -dependent engulfment of synapses by microglia.

To test whether inhibition in microglial engulfment leads to protection against oA $\beta$ -induced synapse loss, we performed tail vein injections of oA $\beta$  into WT and CR3 KO mice. oA $\beta$  induced synapse loss in hippocampus of WT mice, but not in CR3 KO mice (Fig. 4D). All CR3-positive microglia were P2RY12-positive (fig. S11), indicating they are resident cells (22). Altogether, these results suggest that resident microglia engulf synaptic material when challenged by oA $\beta$  through a complement-dependent mechanism.

Synaptic deficits occur in early AD and mild cognitively impaired patients before onset of plaques and are some of the first signs of the neuronal degenerative process (4, 23–25). Here we identify critical synaptotoxic roles of complement and microglia prior to plaque formation and neuroinflammation, in regions of hippocampus undergoing synapse loss. Using multiple experimental approaches, we demonstrate a region-specific increase of phagocytic microglia and accumulation of C1q and C3 on synapses in pre-plaque brains. Microglia in the adult brain, when challenged with synaptotoxic, soluble A $\beta$  oligomers, engulf synapses in the absence of plaque aggregates; deletion of CR3 blocks this process. Finally, inhibiting C1q, C3 or CR3 activity rescues synaptic loss and dysfunction.

Our data suggest a local activation of a developmental pruning pathway (5, 6) as a key mechanism underlying oA $\beta$ -induced synapse loss in pre-plaque AD brain. C1q is aberrantly increased by diffusible oA $\beta$  in a region-specific manner and deposits onto synapses, triggering the activation of downstream classical complement pathway and phagocytic microglia. Blocking A $\beta$  production in J20 mice significantly ameliorated C1q deposition in the hippocampus, and genetic or antibody-mediated inhibition of complement blocks oA $\beta$  from inducing microglial synaptic engulfment, synapse loss, and LTP inhibition. These complementary findings have direct therapeutic relevance.

We propose a model in which C1q and oA $\beta$  operate in a common pathway to activate the complement cascade and drive synapse elimination by microglia through CR3 (fig. S12). This could occur in multiple ways: soluble oA $\beta$  associates with synaptic membranes and other synaptic markers (4, 26); thus, oA $\beta$  bound to synapses may anchor C1q directly. Alternatively, oA $\beta$  binding to synapses may weaken the

synapse (4) and expose a C1q receptor. Although specific receptors for C1q at synapses are not yet known, C1q binds synapses in vulnerable regions undergoing synapse loss (our data here; (5, 27)). It is also plausible that oA $\beta$  and C1q may work indirectly to mediate synapse loss through cytokines such as TGF $\beta$  (7), through microglial or astrocytic activation, or through other mechanisms including MHCI/PirB, another immune pathway critical for synapse elimination in development and AD (28–30).

Finally, our studies show that resident microglia in the adult CNS phagocytose synapses when challenged by synaptotoxic oA $\beta$ , implicating microglia as potential cellular mediators of synapse loss. Although microglia and complement activation are prominently involved in plaque maintenance and related peri-plaque neuropathology, their roles have heretofore been largely regarded as a secondary event related to neuroinflammation (2). Our studies directly challenge this view and suggest that microglia and immune-related pathways can act as early mediators of synapse loss and dysfunction that occur in AD models before plaques form. Although the complement pathway may not be involved in all pathological routes to AD, including plaque-associated synapse loss, the work reported here provides new insights into how synapses are lost in AD. It will be important in future studies to examine whether this microglia or the complement-dependent pathway also plays a role in plaque-associated synapse loss or in other synaptopathies including tauopathies and Huntington's disease. If so, the work reported here may suggest complement and microglia as potential early therapeutic targets in AD and other neurodegenerative diseases involving synaptic dysfunction and memory decline.

## REFERENCES AND NOTES

1. S. Hong, L. Dissing-Olesen, B. Stevens, New insights on the role of microglia in synaptic pruning in health and disease. *Curr. Opin. Neurobiol.* **36**, 128–134 (2016). [Medline doi:10.1016/j.conb.2015.12.004](https://doi.org/10.1016/j.conb.2015.12.004)
2. T. Wyss-Coray, J. Rogers, Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. *Cold Spring Harb. Perspect. Med.* **2**, a006346 (2012). [Medline doi:10.1101/cshperspect.a006346](https://doi.org/10.1101/cshperspect.a006346)
3. M. E. Benoit, M. X. Hernandez, M. L. Dinh, F. Benavente, O. Vasquez, A. J. Tenner, C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid- $\beta$  neurotoxicity. *J. Biol. Chem.* **288**, 654–665 (2013). [Medline doi:10.1074/jbc.M112.400168](https://doi.org/10.1074/jbc.M112.400168)
4. L. Mucke, D. J. Selkoe, Neurotoxicity of amyloid  $\beta$ -protein: Synaptic and network dysfunction. *Cold Spring Harb. Perspect. Med.* **2**, a006338 (2012). [Medline doi:10.1101/cshperspect.a006338](https://doi.org/10.1101/cshperspect.a006338)
5. B. Stevens, N. J. Allen, L. E. Vazquez, G. R. Howell, K. S. Christopherson, N. Nouri, K. D. Micheva, A. K. Mehalow, A. D. Huberman, B. Stafford, A. Sher, A. M. Litke, J. D. Lambris, S. J. Smith, S. W. John, B. A. Barres, The classical complement cascade mediates CNS synapse elimination. *Cell* **131**, 1164–1178 (2007). [Medline doi:10.1016/j.cell.2007.08.012](https://doi.org/10.1016/j.cell.2007.08.012)
6. D. P. Schafer, E. K. Lehrman, A. G. Kautzman, R. Koyama, A. R. Mardinly, R. Yamasaki, R. M. Ransohoff, M. E. Greenberg, B. A. Barres, B. Stevens, Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* **74**, 691–705 (2012). [Medline doi:10.1016/j.neuron.2012.03.026](https://doi.org/10.1016/j.neuron.2012.03.026)
7. A. R. Bialas, B. Stevens, TGF- $\beta$  signaling regulates neuronal C1q expression and

- developmental synaptic refinement. *Nat. Neurosci.* **16**, 1773–1782 (2013). [Medline doi:10.1038/nn.3560](#)
8. S. T. DeKosky, S. W. Scheff, Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann. Neurol.* **27**, 457–464 (1990). [Medline doi:10.1002/ana.410270502](#)
  9. R. D. Terry, E. Masliah, D. P. Salmon, N. Butters, R. DeTeresa, R. Hill, L. A. Hansen, R. Katzman, Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* **30**, 572–580 (1991). [Medline doi:10.1002/ana.410300410](#)
  10. S. Hong, D. Wilton, B. Stevens, D. S. Richardson, Structured Illumination Microscopy for the investigation of synaptic structure and function. *Methods in Molecular Biology: Synapse Development: Methods and Protocols*.
  11. L. Mucke, E. Masliah, G. Q. Yu, M. Mallory, E. M. Rockenstein, G. Tatsuno, K. Hu, D. Kholodenko, K. Johnson-Wood, L. McConlogue, High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. *J. Neurosci.* **20**, 4050–4058 (2000). [Medline](#)
  12. S. Hong, O. Quintero-Monzon, B. L. Ostaszewski, D. R. Podlisny, W. T. Cavanaugh, T. Yang, D. M. Holtzman, J. R. Cirrito, D. J. Selkoe, Dynamic analysis of amyloid  $\beta$ -protein in behaving mice reveals opposing changes in ISF versus parenchymal A $\beta$  during age-related plaque formation. *J. Neurosci.* **31**, 15861–15869 (2011). [Medline doi:10.1523/JNEUROSCI.3272-11.2011](#)
  13. A. H. Stephan, D. V. Madison, J. M. Mateos, D. A. Fraser, E. A. Lovelett, L. Coutellier, L. Kim, H. H. Tsai, E. J. Huang, D. H. Rowitch, D. S. Berns, A. J. Tenner, M. Shamloo, B. A. Barres, A dramatic increase of C1q protein in the CNS during normal aging. *J. Neurosci.* **33**, 13460–13474 (2013). [Medline doi:10.1523/JNEUROSCI.5341-13.2013](#)
  14. J. A. Harris, N. Devidze, B. Halabisky, I. Lo, M. T. Thwin, G. Q. Yu, D. E. Bredesen, E. Masliah, L. Mucke, Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein. *J. Neurosci.* **30**, 372–381 (2010). [Medline doi:10.1523/JNEUROSCI.5341-09.2010](#)
  15. J. L. Jankowsky, D. J. Fadale, J. Anderson, G. M. Xu, V. Gonzales, N. A. Jenkins, N. G. Copeland, M. K. Lee, L. H. Younkin, S. L. Wagner, S. G. Younkin, D. R. Borchelt, Mutant presenilins specifically elevate the levels of the 42 residue  $\beta$ -amyloid peptide in vivo: Evidence for augmentation of a 42-specific  $\gamma$  secretase. *Hum. Mol. Genet.* **13**, 159–170 (2004). [Medline doi:10.1093/hmg/ddh019](#)
  16. M. Botto, C. Dell'Agnola, A. E. Bygrave, E. M. Thompson, H. T. Cook, F. Petry, M. Loos, P. P. Pandolfi, M. J. Walport, Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* **19**, 56–59 (1998). [Medline doi:10.1038/ng0598-56](#)
  17. D. B. Freir, R. Fedriani, D. Scully, I. M. Smith, D. J. Selkoe, D. M. Walsh, C. M. Regan, A $\beta$  oligomers inhibit synapse remodeling necessary for memory consolidation. *Neurobiol. Aging* **32**, 2211–2218 (2011). [Medline doi:10.1016/j.neurobiolaging.2010.01.001](#)
  18. M. R. Wessels, P. Butko, M. Ma, H. B. Warren, A. L. Lage, M. C. Carroll, Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 11490–11494 (1995). [Medline doi:10.1073/pnas.92.25.11490](#)
  19. D. P. Schafer, E. K. Lehrman, C. T. Heller, B. Stevens, An engulfment assay: A protocol to assess interactions between CNS phagocytes and neurons. *J. Vis. Exp.* **88**, 51482 (2014). [Medline](#)
  20. T. Ebihara, I. Kawabata, S. Usui, K. Sobue, S. Okabe, Synchronized formation and remodeling of postsynaptic densities: Long-term visualization of hippocampal neurons expressing postsynaptic density proteins tagged with green fluorescent protein. *J. Neurosci.* **23**, 2170–2181 (2003). [Medline](#)
  21. A. Coxon, P. Rieu, F. J. Barkalow, S. Askari, A. H. Sharpe, U. H. von Andrian, M. A. Arnaout, T. N. Mayadas, A novel role for the  $\beta$  2 integrin CD11b/CD18 in neutrophil apoptosis: A homeostatic mechanism in inflammation. *Immunity* **5**, 653–666 (1996). [Medline](#)
  22. O. Butovsky, M. P. Jedrychowski, C. S. Moore, R. Cialic, A. J. Lanser, G. Gabriely, T. Koeglsparger, B. Dake, P. M. Wu, C. E. Doykan, Z. Fanek, L. Liu, Z. Chen, J. D. Rothstein, R. M. Ransohoff, S. P. Gygi, J. P. Antel, H. L. Weiner, Identification of a unique TGF- $\beta$ -dependent molecular and functional signature in microglia. *Nat. Neurosci.* **17**, 131–143 (2014). [Medline doi:10.1038/nn.3599](#)
  23. D. J. Selkoe, Alzheimer's disease is a synaptic failure. *Science* **298**, 789–791 (2002). [Medline doi:10.1126/science.1074069](#)
  24. S. W. Scheff, D. A. Price, F. A. Schmitt, E. J. Mufson, Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* **27**, 1372–1384 (2006). [Medline doi:10.1016/j.neurobiolaging.2005.09.012](#)
  25. S. W. Scheff, D. A. Price, F. A. Schmitt, S. T. DeKosky, E. J. Mufson, Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* **68**, 1501–1508 (2007). [Medline doi:10.1212/01.wnl.0000260698.46517.8f](#)
  26. S. Hong, B. L. Ostaszewski, T. Yang, T. T. O'Malley, M. Jin, K. Yanagisawa, S. Li, T. Bartels, D. J. Selkoe, Soluble A $\beta$  oligomers are rapidly sequestered from brain ISF in vivo and bind GM1 ganglioside on cellular membranes. *Neuron* **82**, 308–319 (2014). [10.1016/j.neuron.2014.02.027](#) [Medline](#)
  27. A. H. Stephan, B. A. Barres, B. Stevens, The complement system: An unexpected role in synaptic pruning during development and disease. *Annu. Rev. Neurosci.* **35**, 369–389 (2012). [Medline doi:10.1146/annurev-neuro-061010-113810](#)
  28. A. Datwani, M. J. McConnell, P. O. Kanold, K. D. Micheva, B. Busse, M. Shamloo, S. J. Smith, C. J. Shatz, Classical MHC I molecules regulate retinogeniculate refinement and limit ocular dominance plasticity. *Neuron* **64**, 463–470 (2009). [Medline doi:10.1016/j.neuron.2009.10.015](#)
  29. T. Kim, G. S. Vidal, M. Djuricic, C. M. William, M. E. Birnbaum, K. C. Garcia, B. T. Hyman, C. J. Shatz, Human LirB2 is a  $\beta$ -amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. *Science* **341**, 1399–1404 (2013). [Medline doi:10.1126/science.1242077](#)
  30. H. Lee, B. K. Brott, L. A. Kirkby, J. D. Adelson, S. Cheng, M. B. Feller, A. Datwani, C. J. Shatz, Synapse elimination and learning rules co-regulated by MHC class I H2-Db. *Nature* **509**, 195–200 (2014). [Medline doi:10.1038/nature13154](#)

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**SUPPLEMENTARY MATERIALS**

[www.sciencemag.org/cgi/content/full/science.aad8373/DC1](http://www.sciencemag.org/cgi/content/full/science.aad8373/DC1)

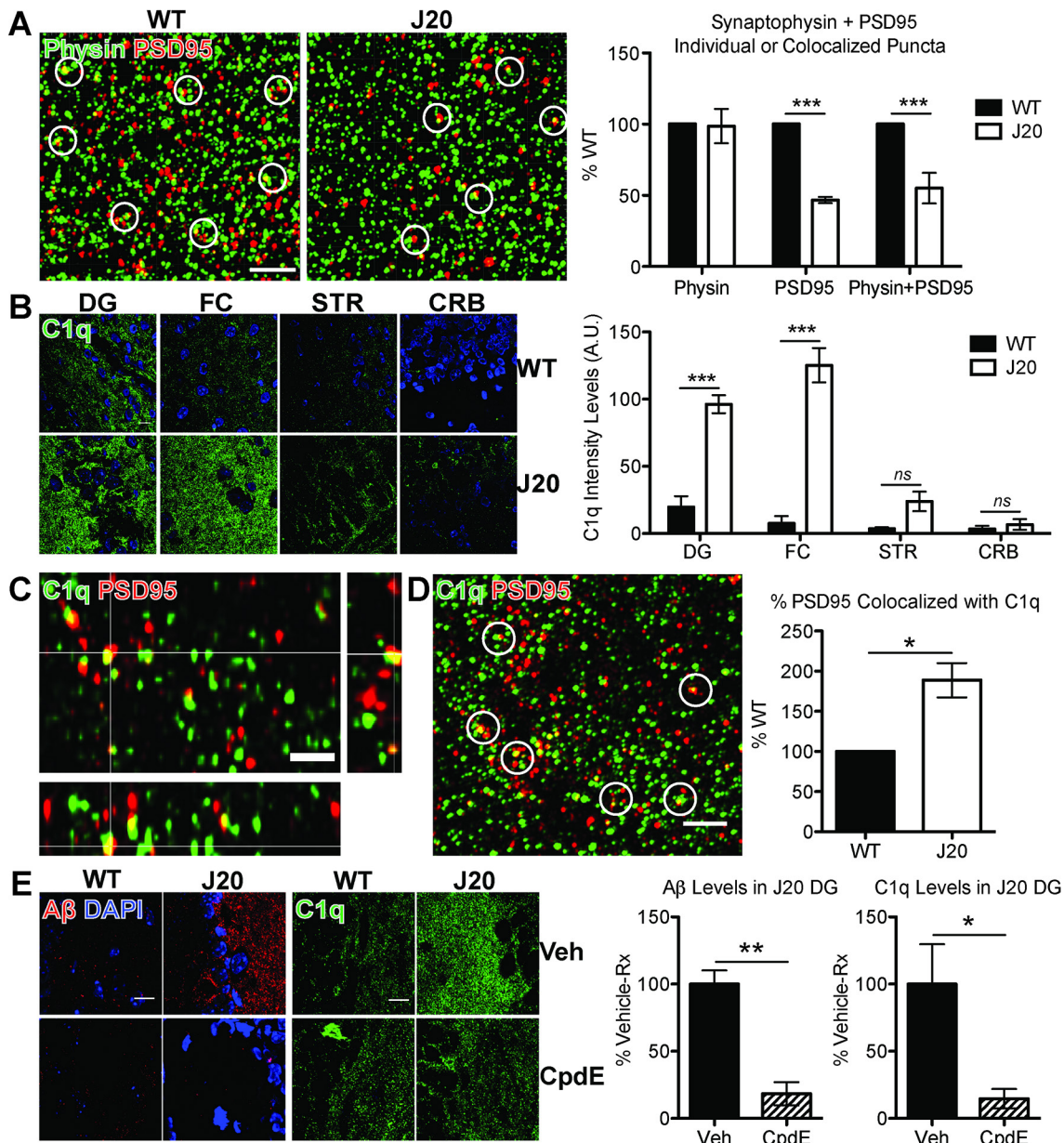
Materials and Methods

Figs. S1 to S12

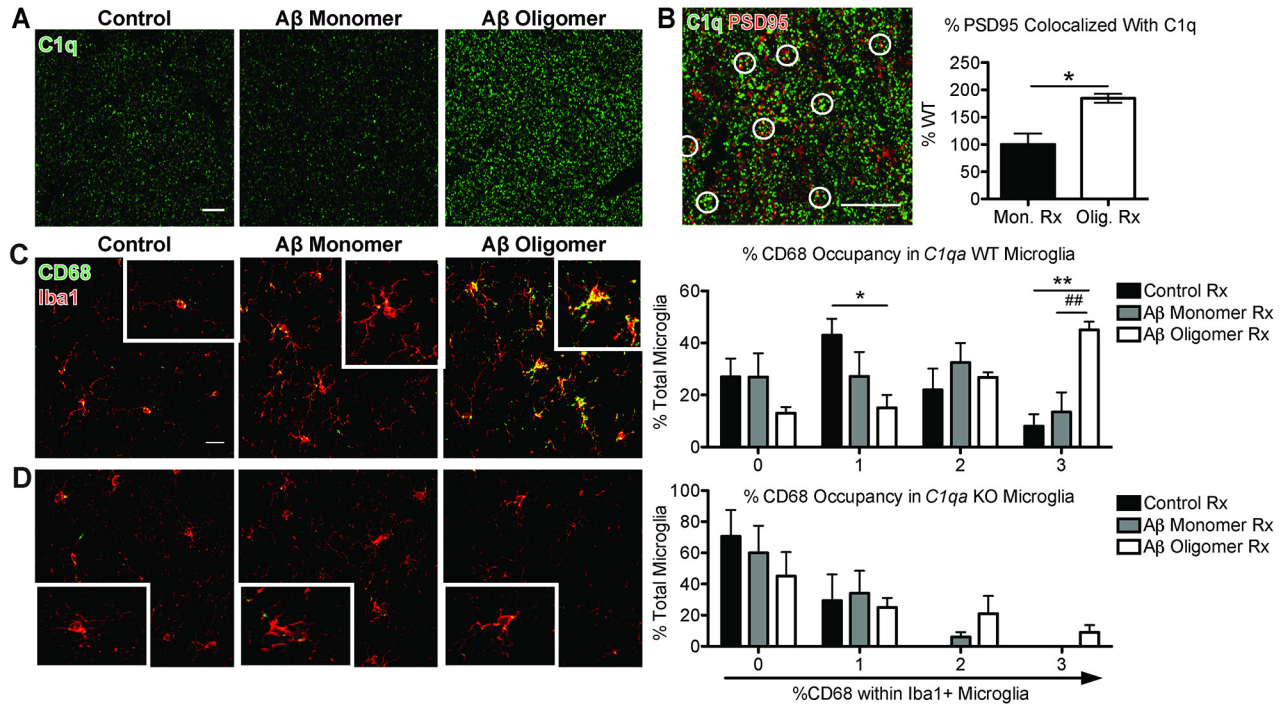
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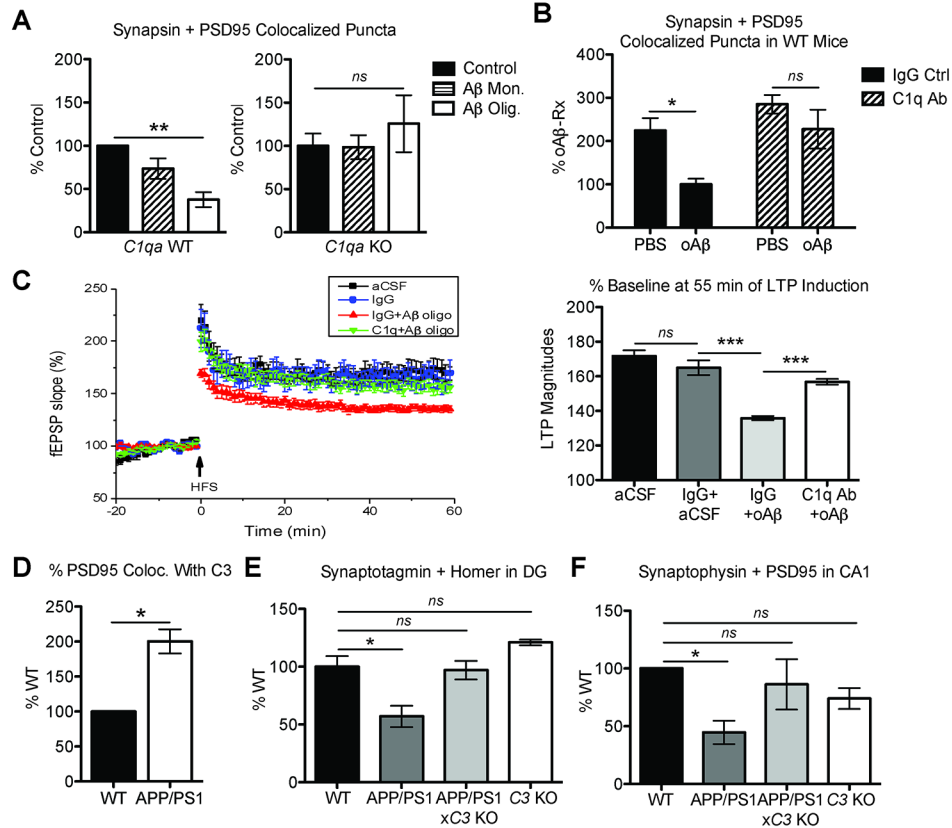
10.1126/science.aad8373



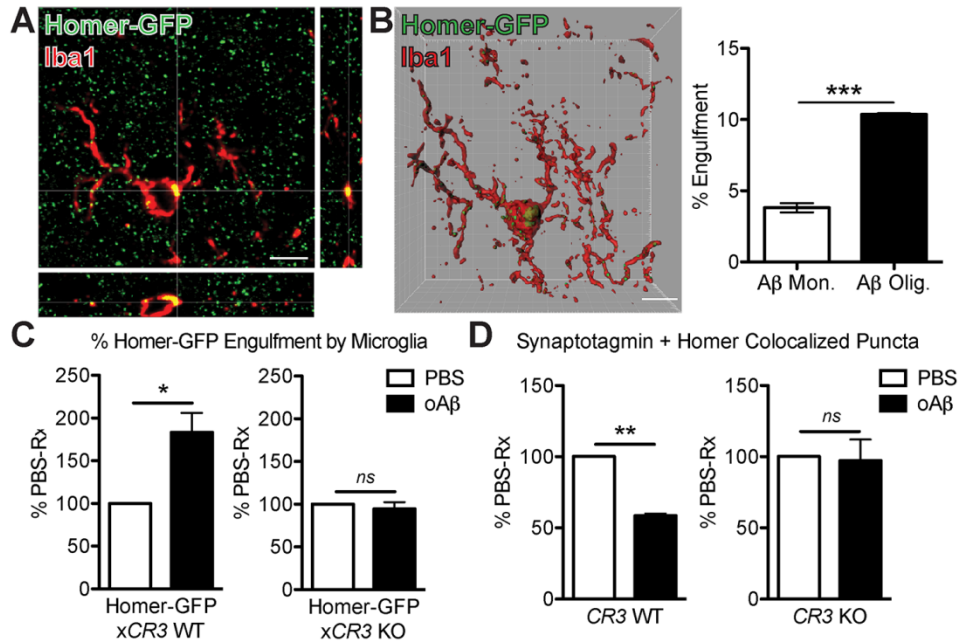
**Fig. 1. C1q upregulation and deposition onto synapses precede pre-plaque synapse loss in J20 mice.** (A) Super-resolution SIM images of synaptophysin (green)- and PSD95 (red)-immunoreactive puncta in stratum radiatum of 3 mo J20 or WT hippocampus (CA1). Quantification of synaptic puncta or their apposition using Imaris indicates selective loss of PSD95 in J20 hippocampus as compared to their WT littermate controls. See fig. S1. (B) Region-specific upregulation of C1q (green) in 1 mo J20; DG=dentate gyrus, FC=frontal cortex, STR=striatum, CRB=cerebellum. See fig. S2. (C) Orthogonal view of SIM image showing colocalization of C1q (green) and PSD95 (red). (D) Higher % PSD95 colocalized with C1q in 1 mo J20 dentate gyrus vs. WT. (E) Compound E reduces deposited soluble A $\beta$  (red) and C1q (green) in 1 mo J20 dentate gyrus, with minimal effect on C1q levels in WT mice. Scale bar = 2 (A, C and D) or 10 (B and E)  $\mu$ m. Means  $\pm$  SEM;  $n$  = 3-4 mice per genotype or per treatment group per genotype. \* $P$  < 0.05 or \*\*\* $P$  < 0.001 using two-way ANOVA followed by Bonferroni posttest (A and B), two-tailed one-sample  $t$ -test (D) or two-tailed unpaired  $t$ -test (E).



**Fig. 2. Oligomeric A $\beta$  increases C1q and microglial phagocytic activity.** (A and B) Soluble A $\beta$  oligomers in WT mice led to elevation of C1q (green) (A) and a higher % PSD95 (red) colocalization with C1q vs. monomers (B). (C and D) oA $\beta$  induced high levels of CD68 (green) immunoreactivity in Iba1-positive (red) microglia in WT mice (C), but not in those of C1qa KO mice (D). Both had negligible changes in morphology. See fig. S10. Scale bar = 10 (A), 5 (B) or 20 (C)  $\mu$ m. Means  $\pm$  SEM;  $n = 3$ -5 mice per treatment group per genotype. \* $P < 0.05$  using two-tailed  $t$ -test (B) or \* $P < 0.05$ , \*\* $P < 0.01$  vs. control-treated or ## $P < 0.01$  vs. A $\beta$  monomer-treated using two-way ANOVA followed by Bonferroni posttest (C).



**Fig. 3. Complement is necessary for synapse loss and dysfunction in AD models.** (A) A $\beta$  oligomers induced loss of colocalized synapsin- and PSD95-immunoreactive puncta in contralateral hippocampus of 3 mo WT mice (left panel); however, they failed to do so in *C1qa* KO mice (right panel). (B) Co-injection of A $\beta$  oligomers with the function-blocking antibody against C1q, ANX-M1, but not with its IgG isotype control, prevented synapse loss in WT mice. (C) Pre-treatment of hippocampal slices with the C1q antibody, ANX-M1, prevented A $\beta$ -mediated LTP inhibition (green) vs. IgG (red). IgG alone had a minimal effect (blue) vs. aCSF vehicle (black).  $n = 6-11$  slices per group. (D) % PSD95 colocalized with C3 is increased in APP/PS1 hippocampus vs. that of WT mice. (E and F) Genetic deletion of C3 prevents synapse loss in 4 mo APP/PS1 mice. Quantification of colocalized immunoreactive puncta for synaptotagmin and homer in dentate gyrus (E) or synaptophysin and PSD95 in CA1 stratum radiatum (F) of WT, APP/PS1, APP/PS1x C3 KO and C3 KO hippocampi. Means  $\pm$  SEM;  $n = 3-5$  mice per genotype or per treatment group per genotype. \* $P < 0.05$ , \*\* $P < 0.01$  or \*\*\* $P < 0.001$  using two-tailed one-sample  $t$ -test (D), one-way (A, C, E, F) or two-way (B) ANOVA followed by Bonferroni posttest.



**Fig. 4. Microglia engulf synapses via CR3 upon oligomeric Aβ challenge.**

(A) Orthogonal view of high-resolution confocal image shows colocalization of Homer-GFP and Iba1 (red). (B) 3D reconstruction and surface rendering using Imaris demonstrate bigger volumes of Homer-GFP puncta inside microglia of oAβ-injected contralateral hippocampus vs. those of monomer-injected. (C) Microglia of Homer-GFPxCR3 KO mice (right panel) show less engulfment of Homer-GFP when challenged with oAβ vs. those of Homer-GFP mice (left panel). (D) Aβ oligomers failed to induce synapse loss in contralateral hippocampus of CR3 KO mice (right panel) as they did in WT mice (left panel). Scale bar = 5 μm (A and B). Means ± SEM;  $n = 3$  mice per treatment group per genotype ( $n = 6-17$  microglia analyzed per mouse). \* $P < 0.05$  or \*\* $P < 0.01$  using two-tailed  $t$ -test (B) or two-tailed one-sample  $t$ -test (C and D).