Title: Amyloid-β Protein Protects Against Microbial Infection In Transgenic C. elegans and 5XFAD Mice

Authors: Deepak Kumar Vijaya Kumar†, Se Hoon Choi†, Kevin J. Washicosky†, William A. Eimer, Stephanie Tucker, Jessica Ghofrani, Aaron Lefkowitz, Gawan McColl, Lee E. Goldstein, Rudolph E. Tanzi*, and Robert D. Moir*

Affiliations:
1 Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, United States of America.
2 The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia.
3 Department of Psychiatry, Boston University, Boston, Massachusetts, United States of America.

*Correspondence to: Robert Moir, Ph.D. Genetics and Aging Research Unit, Massachusetts General Hospital, Building 114 16th Street Charlestown, MA 02129, USA Tel (617) 726 3746; Fax (617) 724 1823, Email moir@helix.mgh.harvard.edu
And
Rudolph E. Tanzi, Ph.D., Director, Genetics and Aging Research Unit, Massachusetts General Hospital, Building 114 16th Street Charlestown, MA 02129, USA Tel (617) 726 6845; Fax (617) 724 1823, Email Tanzi@helix.mgh.harvard.edu

†authors contributed equally to this study.

One Sentence Summary: β-amyloid protein oligomerization and fibrilization, while pathogenic for Alzheimer’s disease, may normally play a role in microbial entrapment and innate immunity.
**Abstract:** The amyloid-β peptide (Aβ) is a key protein in Alzheimer's disease (AD) pathology. We previously reported *in vitro* evidence suggesting Aβ is an antimicrobial peptide. Here we provide the first *in vivo* evidence showing high Aβ production protects against fungal and bacterial infections in mouse and nematode AD models. In Aβ-null mouse models low Aβ production is associated with attenuated resistance to infection. Regarding mechanism, we show Aβ oligomerization, a behavior traditionally viewed as intrinsically pathological, is necessary for the antimicrobial activities of the peptide. Soluble Aβ oligomers bind microbial cell walls, developing protofibrils inhibit pathogen host cell adhesion, and, finally, protease-resistant β-amyloid fibrils agglutinate and entrap the invading microbes. We also show that infection of 5XFAD mouse brain with *S. Typhimurium* bacteria rapidly seeds and dramatically accelerates β-amyloid deposition, which closely co-localizes with invading bacteria. Collectively, our findings raise the intriguing possibility that β-amyloid plays a protective role in innate immunity and infectious or sterile inflammatory stimuli may drive amyloidosis. These data suggest a dual protective/damaging role for Aβ, as has been described for other antimicrobial peptides.
**Introduction**

Neurodegeneration in Alzheimer's disease (AD) is mediated by soluble oligomeric intermediates generated during fibrilization of the amyloid-β protein (Aβ) (1). Overwhelming evidence supports Aβ's pivotal role in AD. However, despite remarkably high sequence conservation across diverse species (humans share Aβ42 sequences with coelacanths (2), a 400 million year old fish taxon) and extensive data showing broad activity spectra for Aβ, the peptide has traditionally been characterized as a functionless catabolic byproduct. Activities identified for Aβ in vivo are most often described as stochastic pathological behaviors. Oligomerization in particular is viewed as a pathogenic pathway and Aβ oligomers are assumed to be intrinsically abnormal. Scant consideration has been given to possible physiological roles for Aβ's activities.

Members of the evolutionarily ancient family of proteins, collectively known as antimicrobial peptides (AMPs), share many of Aβ’s purportedly abnormal activities, including oligomerization and fibrillization (3, 4). For AMPs these activities mediate key protective roles in innate immunity. AMPs are the first-line of defense against pathogens and act as potent broad-spectrum antibiotics and immunomodulators that target bacteria, mycobacteria, enveloped viruses, fungi, and protozoans, and in some cases, transformed or cancerous host cells (5). AMPs are widely expressed but are particularly abundant in brain (6) and other immunoprivileged tissues where actions of the adaptive immune system are constrained. While normally protective, AMP dysregulation can lead to host cell toxicity (7-11), chronic inflammation (8, 9, 11, 12), and degenerative pathologies (13). Particularly germane to Aβ's role in AD, AMPs are deposited as amyloid in at least three disorders (4, 14-18), including senile seminal vesicle amyloid and Isolated Atrial Amyloidosis, two of the most common human amyloidopathies (17, 18). Consistent with identity as an AMP, we recently reported that synthetic Aβ exhibits potent in vitro antimicrobial activity towards eight common and clinically relevant microbial pathogens (3). Furthermore, whole brain homogenates from AD patients show Aβ-mediated activity against *Candida albicans*. More recently, synthetic Aβ has been shown to protect cultured cells from influenza A (19) and herpes simplex (20). However, the biological relevance of protective in vitro Aβ activities requires validation in vivo. In this study we extend our original findings and show that Aβ expression inhibits infection in transgenic (Tg) mouse (5XFAD), *Caenorhabditis elegans* (*C. elegans*) and cultured mammalian cell models. Conversely, low Aβ expression in
APP-KO mice is associated with attenuated survival with infection. Most surprisingly, oligomerization and fibrilization appears to mediated Aβ's protective activity and cerebral infection with microbial cells seed and dramatically accelerates β-amyloid deposition in 5XFAD mice and Tg C. elegans. Our data suggest Aβ oligomerization and fibril formation may normally serve a protective role as a mechanism for microbial entrapment.
Results

Aβ-mediated protection was characterized in mice, *C. elegans*, and cell culture models of infection. *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium) was used as an infecting agent in mouse models. Nematode and cultured cell experiments used pathogenic (hyphal) *Candida albicans* (*Candida*) and *S.* Typhimurium.

*Aβ protects against meningitis in genetically modified mice.*

AMPs play a key role in fighting infections in the immunoprivileged brain. We first employed genetically modified mice to test for protective effects of elevated Aβ expression and attenuated resistance with low levels of the peptide. Four-week old 5XFAD Alzheimer’s transgenic mice constitutively express human Aβ in the brain at high levels but lack the β-amyloid deposits and features of neuroinflammation found in older animals (21). APP-KO mice lack the precursor protein required for murine Aβ generation (22). One-month old 5XFAD (n = 12), APP-KO (n = 15), and wild-type (WT) littermates (n = 11 and 15, respectively) received a single intracerebral injection of 65,000 colony forming units (CFU) of *S.* Typhimurium. Clinical progression to the moribund state was followed according to established grading criteria for mouse encephalomyelitis (Fig. S1A). Survival of Aβ-expressing 5XFAD mice was significantly (*P* = 0.009) increased compared to non-transgenic littermates (Fig. 1A). Consistent with increased resistance to infection, 5XFAD mice also showed significantly better clinical performance (*P* < 0.0001), reduced weight loss (*P* = 0.0008), and lower cerebral *S.* Typhimurium loads (*P* = 0.03) compared to WT controls (Figs. 1B-D). Consistent with immunodeficiency associated with low Aβ, APP-KO mice show a trend (*P* = 0.10) towards increased mortality with infection (Fig. 1E). Control injections using heat-killed bacteria did not lead to clinical decline or death in 5XFAD and WT mice (Fig. 1F), consistent with mouse mortality mediated by *S.* Typhimurium infection. Next, we confirmed high soluble Aβ and low insoluble β-amyloid levels in four-week old 5XFAD brain using formic acid extraction and anti-β-amyloid ELISAs (Fig. S1B). To confirm inflammation did not immunologically prime and protect 5XFAD mice against infection, we compared immune profiles of one-month old transgenic and WT brain. Consistent with previous reports showing an absence of immune activation (21), GFAP* astrocyte, Iba1* microglia, and level of ten cytokines in four-week old 5XFAD mice were not significantly elevated compared to WT littermates (Figs. S1C-E).
Increased survival of Tg C. elegans infected with Candida.

To further explore the ability of Aβ to afford protection against infection, we next tested transgenic C. elegans for resistance to Candida. Our nematode infection model uses two previously described C. elegans transgenic strains; GMC101 that express the 1-42 residue human Aβ isoform (Aβ42) (23) and CL2122, a control strain that express intestinal GFP (mtl-2:gfp) marker (as does GMC101) but does not express Aβ. Adult GMC101 nematodes ultimately develop age-progressive paralysis and β-amyloid deposition in the body wall muscle. For our experiments, developmentally synchronized L4 larvae were infected five days prior to the onset of paralysis. Aβ expression is driven by the unc-54 (which encodes a myosin heavy chain) promoter, active in body wall muscle (23) as well as in other tissues, including muscle cells of the gastrointestinal (GI) track (24). Amyloidogenic peptides under the unc-54 promoter have also been shown to translocate via vesicular transport to the gut of transgenic worms and Aβ has been proposed as a likely candidate for translocation via this mechanism (25). Immunohistological analysis of adult GMC101 using three different anti-Aβ antibodies confirmed Aβ localization in the body wall muscle and the gut lumen (Fig. S2A-B). Anti-Aβ antibodies did not label negative control CL2122 intestine or body wall cells. In addition, excreta from healthy GMC101, but not CL2122, worms were positive for anti-Aβ signal by immunoblot (Fig. S2C). While an origin for gut Aβ remains unclear, strong empirical evidence supports the peptides localization in the intestinal lumen of GMC101 nematodes. Thus, transgenic GMC101 nematodes appear to be suitable models for testing Aβ-mediated protective activities against intestinal pathogens.

Candida albicans (ATCC 90028) is an Aβ-sensitive microbial organism (3) and a well-characterized C. elegans intestinal pathogen that causes distention, penetrative filamentation, and death among WT nematodes two days following ingestion (26). Links between fungi brain infections and AD pathology have also recently emerged, including for C. albicans (27) and closely related Candida glabrata (28). We compared survival of control CL2122 (n = 56) and GMC101 (n = 59) nematodes following incubation (2 hrs, 20° C) on C. albicans lawns. Consistent with Aβ-mediated protection, GMC101 nematodes infected with Candida albicans showed significantly (P < 0.00001) reduced mortality as compared to control CL2122 worms that do not express Aβ (Fig. 2A). Consistent with mouse data, Aβ expressing nematodes were also protected from the C. elegans intestinal pathogen S. Typhimurium, with GMC101 worms
showing statistically significant ($P = 0.0005$) increased survival compared to CL2122 controls following infection with the bacteria (Fig. S3A).

**Protective antimicrobial activities of Aβ in cell culture**

To address the mechanism of protection, we next tested the ability of Aβ to protect host cell monolayers (HCMs) from infection using transformed cultured human brain neuroglioma (H4) and Chinese hamster ovary (CHO) HCMs. H4 lines include stably transformed H4-Aβ40 and H4-Aβ42 cells that selectively secrete the 1-40 residue Aβ isoform (Aβ40) or Aβ42, respectively (29). Processing of a BRI-Aβ fusion protein expressed by transformed H4 cells leads to constitute high-level expression and secretion of the encoded Aβ protein. For double transfected CHO-CAB cells, over-expression of β-amyloid protein precursor (APP) and the APP-processing protease β-secretase leads to APP cleavage and generation of multiple Aβ isoforms (30). Naïve H4 (H4-N) and CHO (CHO-N) are used as control cell lines. *Candida albicans* has been extensively characterized in HCM infection models and was used in our experiments as an infecting agent.

We first compared naïve and transformed host cells for survival following infection with *C. albicans*. Viability was determined by pre-labeling host cells with BrdU and following anti-BrdU signal. Consistent with findings for 5XFAD mice and GMC101 nematodes, survival 28 hrs post-infection was significantly increased for Aβ over-expressing H4-Aβ40 ($P = 0.002$) and H4-Aβ42 ($P = 0.001$) transformed host lines compared to control H4-N cells with rank order H4-Aβ42 > H4-Aβ40 > H4-N (Fig. 2B). Survival of transformed CHO-CAB host cells was also significantly higher ($P = 0.004$) than control CHO-N lines. Additional independent assays of host cell viability (Figs. S4A-B) and HCM yeast loads (Fig. S4C) confirmed BrdU-viability data showing increased resistance of transformed H4-Aβ42 cells against *C. albicans*.

While media Aβ levels in our HCM models (Figs. S5A-B) fall within the physiological ranges reported for human CSF (2-20 ng/ml) (31), concentrations are two orders of magnitude (log$_{10}$) lower than the minimal inhibitory concentration (MIC) for fungicidal activities in microdilution MIC assays (3). We have previously reported that Aβ's antimicrobial activities show close parallels with those of LL-37 (3), an archetypal human AMP that remains protective at sub-fungicidal concentrations (32). Two linked, yet distinct activities mediate LL-37's protective anti-*Candida* actions at low peptide concentrations (32). The first is disruption of *C. albicans* host
adhesion. Host cell attachment is a prerequisite step for infection by many pathogens, including *C. albicans*. The second is agglutination of the resulting unattached yeast cells. Agglutination limits microbial access to host cells and also generates high local AMP concentrations within peptide/microbe aggregates. Accordingly, we next tested Aβ for anti-adhesion and agglutination activities using the HCM infection model. Hyphal *C. albicans* were incubated (2 hrs, 37 °C) in pre-conditioned media with transformed or naïve HCM prepared in slide chambers. Microscopic examination revealed fewer *C. albicans* attached to transformed Aβ-expressing HCMs compared to naïve monolayers (Figs. 2C and S6A). To confirm these data, HCMs and *C. albicans* incubation experiments were repeated in 96-well microtiter plates and *Candida* load in wells assayed immunochemically using anti-*Candida* antibodies. Data confirmed visual observations with statistically significant attenuation of *C. albicans* adhesion to transformed H4-Aβ42 (*P* = 0.001), H4-Aβ40 (*P* = 0.001), and CHO-CAB (*P* = 0.004) cells compared to naive control lines (Fig. 2D). Additionally, dramatic microbial agglutination was observed in transformed, but not naïve, wells following overnight incubation (Figs. 2E and S6B). Well images were analyzed for yeast aggregate signal. *Candida* aggregation was significantly elevated in transformed H4-Aβ42 (*P* = 0.00004), H4-Aβ40 (*P* = 0.0003), and CHO-CAB (*P* = 0.002) samples over naive control wells (Fig. 2F). For H4 cell lines, anti-adhesion and agglutination activities were consistent with host viability data with rank orders H4-Aβ42 > H4-Aβ40 > H4-N.

We next characterized cell-free conditioned culture media from HCMs for protective Aβ-mediated anti-adhesion and agglutinating activities. Yeast adhesion and agglutination were assayed in 96-well plates using the methods of Tsai *et al.*, 2011 (32). Briefly, for adhesion experiments hyphal *C. albicans* were incubated (2 hrs, 37 °C) with media samples and yeast adhering to host cell-free abiotic well surfaces stained with fluorescent Calcofluor White. Wells images were analyzed following overnight incubation for yeast aggregate signal. Consistent with Aβ-mediated activities, immunodepletion (ID) with anti-Aβ antibodies significantly attenuated H4-Aβ42, H4-Aβ40, and CHO-CAB media anti-adhesion (*P* = 0.009, *P* = 0.001, and *P* = 0.004, respectively) and agglutination (*P* = 0.001, *P* = 0.0005, and *P* = 0.004, respectively) activities (Figs. 3A-B). Analysis confirmed anti-Aβ-ID removed > 95 percent of the Aβ from samples used in experiments to confirm the anti-*Candida* activities of transformed media were specific for Aβ (Figs. S5A-B).
Consistent with yeast data, S. Typhimurium are agglutinated in H4-Aβ42 conditioned media. (Fig. S3B). H4-Aβ42 HCMs incubated with S. Typhimurium also have statistically significant ($P = 0.036$) lower intracellular infection compared to naïve H4-N cells (Fig. S3C-D).

Serial dilution experiments show anti-adhesion and agglutination activities are dose-dependent for both synthetic and cell-derived Aβ (Figs. 3C-D). However, synthetic Aβ peptide preparations had lower specific activities compared to cell-derived material. Co-factors secreted by host cells are unlikely to account for the increased potency of cell-derived Aβ since synthetic peptide incubations were performed in Aβ42-depleted conditioned media (H4-Aβ42-ID) from H4-Aβ42 HCMs. Anti-Aβ antibodies used to clear Aβ42 from H4-Aβ42 culture media prior to addition of synthetic peptides are specific for Aβ and not likely to deplete species acting as co-factors. Oligomerization has been shown to modulate a range of Aβ activities. Moreover, conditioned media from experimental cell lines are reported to contain oligomeric Aβ (33, 34) while our synthetic peptide preparations were pre-treated to remove oligomer species. Synthetic peptide pre-treatments included fractionation by preparative size exclusion chromatography (SEC) to remove species > 6 kDa. Characterization experiments using analytical SEC confirmed that immediately prior to experimental inoculation with yeast, cell-derived material contains a polydisperse population of soluble Aβ oligomers of between 8 and 50 kDa while synthetic peptides remain overwhelmingly monomeric (Fig. S5C).

To test whether oligomerization modulates Aβ's AMP activity, we generated synthetic Aβ oligomers and compared the antimicrobial activities of Aβ42 monomer, oligomer (ADDLs) (35), and high-order protofibril (> 600 kDa) preparations. Compared to monomeric peptide, ADDLs exhibited potentiated, and protofibrils attenuated, anti-adhesion (Fig. 3E) and agglutination (Fig. 3F) activities. Our data are consistent with a central role for soluble Aβ low-order (2-30 monomer units) oligomers in mediating the peptide’s AMP activities. Consistent with such a role, soluble Aβ is overwhelmingly oligomeric in vivo (36) and oligomers are key for the protective activities of a wide range of AMPs (37-40), including LL-37 (37, 41).

*Antimicrobial actions are mediated by heparin-binding activity of Aβ oligomers*

Binding of AMP peptides to microbial surfaces is a prerequisite step for anti-adhesion and agglutination activities. LL-37, contains a XBBXBX heparin-binding motif (where X is a hydrophobic or uncharged residue, and B is a basic residue) that mediates anti-adhesion and
agglutination activities by facilitating attachment of oligomeric species (37, 41) to microbial cell wall carbohydrates (32). Aβ also contains a XBBXBX heparin-binding motif between residues 12–17 (VHHQKL) (42, 43). Competitive inhibition by soluble microbial sugars is a hallmark for AMPs with activities mediated by lectin-like carbohydrate binding (32). Indeed, fungal and bacterial pathogens secrete specialized scavenging exopolysaccharides that target the heparin-binding domains of AMPs as a counter-measure to host mounted defenses (44). Soluble forms of mannan and glucan, the two most abundant carbohydrates in yeast cell wall, have been shown to inhibit XBBXBX-mediated binding of LL-37 to Candida (32, 45). We investigated if the anti-adhesion and agglutination activities of Aβ are similarly inhibited by soluble mannan and glucan. Live yeast cells were incubated in H4-Aβ40, H4-Aβ42, and CHO-CAB conditioned media in the presence or absence of mannan or glucan. Consistent with anti-Candida activity mediated by Aβ’s heparin-binding domain, mannan and glucan significantly attenuated anti-adhesion (P < 0.008) and agglutination (P < 0.003) activities of conditioned media from Aβ-expressing transformed cells (Figs. 3G-H).

We further characterized Aβ's binding to C. albicans and S. Typhimurium using a novel binding immunoassay. For this assay, samples were incubated in wells containing immobilized intact hyphal Candida or S. Typhimurium cells and bound Aβ detected immunochemically with an Aβ42-specific antibody. Aβ binding to Candida and S. Typhimurium was concentration dependent (Figs. 3I and S3E). Consistent with binding mediated by Aβ’s VHHQKL domain, for Candida signal from H4-Aβ42 media was significantly attenuated in the presence of glucan (P = 0.008) or mannan (P = 0.004) (Fig. 3J). Signal was also significantly reduce (P = 0.006) for negative control anti-Aβ-ID H4-Aβ42 media, consistent with assay specificity for Aβ42 binding. Consistent with findings for antimicrobial activities, heparin-binding of cell-generated Aβ oligomers was higher than synthetic monomeric peptide (Fig. 3I). Previous studies have shown Aβ oligomerization greatly increases carbohydrate-binding activity (42, 43). Heparin-binding AMP oligomers also show potentiated carbohydrate binding compared to monomeric species (46). Overall, our findings are consistent with soluble Aβ oligomers possessing an enhanced propensity to bind cell walls, engendering greater anti-adhesion and agglutination activities compared to monomeric synthetic peptide.
**Aβ fibrillization mediates Candida agglutination**

Binding of brain glycosaminoglycans induces Aβ fibrillization (47). Aβ's binding of cell wall and glycocalyx carbohydrates on microbial surfaces seem likely to also generate Aβ fibrils. While viewed solely as a part of Aβ's pathophysiology, fibrillization among AMPs is a normal protective behavior that mediates antimicrobial activities, including microbial cell and viral agglutination (48) and bacterial membrane perturbation (3, 4). Most recently, studies have shown the human AMP α-defensin-6 (HD6) forms fibrils that entangle and trap microbial cells (49). Thus, we next investigated a possible role for Aβ fibrillization in the peptides protective AMP actions. Analysis of early-stage (< 3 hrs post infection) Candida agglutination in H4-Aβ42 media using transmission electron microscopy (TEM) revealed clumped microbial cells entwined and linked by fibrils propagating from cell surfaces (Fig. 4). Candida albicans lack flagella and are not reported to produce extended fibrillar structures. Moreover, the fibrillar structures on yeast cell surfaces are labeled by anti-Aβ immunogold nanoparticles (anti-Aβ-Au). Anti-Aβ-Au binding to fibrils is ablated by co-incubation with synthetic Aβ peptide, consistent with Aβ-specific labeling (Fig. 4D). TEM analysis of early stage S. Typhimurium agglutinates in H4-Aβ42 conditioned media confirm bacterial cells are also bound and linked by fibrils (Fig. S3F).

Epifluorescent micrographs of ThS stained late-stage (> 12 hrs post infection) H4-Aβ42 yeast aggregates display the enhanced fluorescence and red shift that marks the presence of amyloid fibrils (Fig. 5A). Enhanced fluorescence was not observed for negative control yeast agglutinates. ThS fluorescence within H4-Aβ42 yeast aggregates co-localized with signal for anti-Aβ immunoreactivity (Fig. 5B). Congo red stained H4-Aβ42 yeast aggregates also show birefringence under polarized light, another marker for β-amyloid (Fig. S7). Scanning EM (SEM) micrographs of yeast aggregates from H4-Aβ42 media revealed an irregular material adhering to cell surfaces not present in Candida pellets prepared by centrifugation in Aβ-free media (Fig. 5C). Analysis of Candida cell surfaces by TEM revealed the adhering material to be filamentous and immunoreactive to anti-Aβ-Au (Fig. 5D). Co-incubation of soluble synthetic Aβ40 peptide abolished anti-Aβ-Au binding. Collectively, the data are consistent with microbial agglutination and entrapment mediated by Aβ fibrillization in our cell culture infection model.
**β-amyloid mediated pathogen entrapment in GMC101 nematode and 5XFAD mice**

We also investigated infection-associated Aβ fibrillization in our nematode and mouse infection models. Consistent with Aβ targeting and binding of yeast cells in our HCM model, *Candida* in the gut of recently infected (2 hrs post ingestion) GMC101 (Fig. 6A) nematodes were labeled by anti-Aβ-Au nanoparticles. Yeast in the gut of control CL2122 were not labeled by anti-Aβ-Au (Fig. S8A). Aβ fibrilization in GMC101 worms is normally confined to the body wall muscle. However, compared to infection-free nematodes, GMC101 worms with late-stage *Candida* infection shown enhanced ThS fluorescence in non-muscle tissue, including the GI track (Fig. 6B). High resolution micrographs of yeast cells in the GI track of GMC101 nematodes revealed clumped *Candida* embedded in material that has enhanced fluorescence following ThS staining (Fig. 6C) and is labeled by anti-Aβ antibodies (Fig. 6D). Consistent with Aβ-specific labeling, anti-Aβ signals (Figs. S2B) and enhanced ThS fluorescence (Fig. S8B) were absent from uninfected or *Candida* infected negative control CL2122 nematodes that do not express Aβ. Findings for *Candida albicans* infected GMC101 nematodes are consistent with the agglutinating and entrapment roles Aβ fibrils appear to play in our HCM model. Thus, gut Aβ fibrillization on *Candida* cell surfaces may mediate infection resistance in GMC101 nematodes.

Four-week old 5XFAD mouse brain is normally negative for β-amyloid deposits (21). However ThS and anti-Aβ staining of 5XFAD mouse brain revealed widespread β-amyloid deposition following 48 hrs infection with *S. Typhimurium* (Fig. 7). Moreover, anti-Salmonella and β-amyloid signals co-localize in 5XFAD brain suggesting bacterial cells induce Aβ fibrilization. TEM analysis also reveal bacterial cells embedded in fibrous material labeled by anti-Aβ-Au nanoparticles in 5XFAD, but not WT, brain sections (Fig. S8). Video of Z-section projections rotating through 360° show bacteria are not confined to the surface of Aβ accretions but are embedded within the β-amyloid deposits (Video S1). Consistent with fibrilization driven by proliferation of *S. Typhimurium* cells, β-amyloid deposits were absent from sham-infected one-month old 5XFAD controls injected with heat-killed bacteria. ThS and anti-β-amyloid antibodies did not label brain from negative control non-Tg littermates, confirming β-amyloid signals associated with *S. Typhimurium* in 5XFAD mice are specific for Aβ. Findings suggest invading microbial cells can seed and dramatically accelerate β-amyloid accumulation in 5XFAD mice. Data are also consistent with findings from HCM and *C. elegans* infection models.
that suggest this accelerated Aβ fibrilization is protective and leads to the entrapment of invading microbial pathogens.
Discussion

Our findings are consistent with a *bona fide* protective role for Aβ, *in vivo*, as an AMP. Expression of Aβ was associated with increased host survival in cell culture, nematode, and mouse infection models ([Figs. 1 and 2](#fig1-fig2)). Low Aβ expression was associated with higher mortality with infection in APP-KO mice. Our data are consistent with a protective role for Aβ in innate immunity that employs a classic AMP mechanism characterized by reduced microbial adhesion, agglutination, and entrapment mediated by peptide fibrillization. Moreover, activities mediating the peptides protective actions are well known Aβ behaviors. However, these same properties, e.g., oligomerization, fibrillation, and carbohydrate-binding, are also linked to Aβ's pathophysiology. While a protective/damaging duality is a novel proposition for Aβ's activities, this is not the case for classical AMPs. For example, LL-37 offers a germane model for the potential pathological consequences of normally protective AMP actions. LL-37 is essential for normal immune function and low expression leads to lethal infections (50). However, at elevated concentrations the peptides cytotoxic actions also damage host cells, particularly smooth muscle cells (51). The cytotoxic and proinflammatory activities of LL-37 are implicated in the pathogenesis of several major late-life diseases, including rheumatoid arthritis, lupus erythematosus, and atherosclerosis (52). Thus, a normally protective Aβ activity spectra that, when dysregulated also leads to AD pathology, is consistent with the actions of classical human AMPs.

Anti-adhesion and agglutination activities are distinct from AMP microbicidal activities, which typically require micromolar concentrations of peptide and involve different mechanisms (32). The anti-adhesion and agglutination activities we observed *in vitro* for cell-derived Aβ ([Fig. 3](#fig3)) fall within physiological concentration ranges reported for normal human CSF (1-5 ng/ml). Consistent with a normal *in vivo* protective role the highest cerebral concentrations of Aβ are in the leptomeninges (10-50 ng/ml) (53, 54), the brains first line of defense against infection and a tissue enriched for LL-37 and other innate immune proteins (55-57). The high specific activity observed for cell-derived material is consistent with our previous finding that Aβ in human brain extracts is a potent anti-*Candida* agent (3). Classical AMP expression can be either constitutive or inducible (58). Aβ expression is artificially constitutive in our transgenic mouse, nematode, and cultured cell models. As such, our models are not suitable for testing if infection normally
results in Aβ up regulation. However, data from other investigators suggest Aβ may be an inducible AMP. Host cell exposure to HSV-1 (59), HIV1 (59), spirochetes (60), or chlamydia (61, 62) increase Aβ expression.

In in vitro assays, cell-derived and synthetic Aβ oligomers were more potent against Candida than monomeric forms (Figs. 3C-F and S5C). The specific activities of synthetic ADDDLs, while higher than non-oligomerized peptide, remain lower than cell-derived Aβ species. Peptide posttranslational modifications may enhance the AMP activity of cell-derived Aβ oligomers. However, oligomer conformation is also likely to play a key role. Neurotoxicity has been shown to be highly dependent on the arrangement of Aβ peptides within oligomeric assemblies. Oligomer morphology may also modulate Aβ’s protective antimicrobial activities. Protocols for preparing ADDDLs and other synthetic Aβ assemblies are optimized for oligomer populations with neurotoxic, not antimicrobial, activities. Future protocols optimized for enhanced AMP activities may generate soluble synthetic Aβ oligomers with potencies that approach cell-derived material.

Aβ pathophysiology is thought to arise from an abnormal propensity to generate soluble oligomers. However, oligomerization is not a pathogenic behavior for AMPs and plays a key role in normal protective activities across this diverse group of proteins, including microbe agglutination and entrapment (48, 63), the targeting (37, 41, 64) and disruption of microbial cell membranes (38, 65-69), resistance to bacterial proteases (37, 38, 67, 70), and expanding the molecular diversity and protective functions of AMP families without commensurate genome expansion (39, 40). Our data and the widespread involvement of oligomerization in the protective actions of AMPs suggest that the brain's pool of soluble Aβ may normally include physiologically functional oligomeric species that mediate protective antimicrobial activities. The intrinsic polymorphic stoichiometry of Aβ oligomers may also play a protective physiological role in the peptides AMP actions. As has been shown with classical AMPs, diverse polymorphic oligomer pools target a broader spectrum of pathogens and are more resistant to AMP-targeting microbial proteases than homogenous peptide populations.

The lectin activity of Aβ oligomers is thought to promote brain amyloidosis (47). Studies to date have focused on accelerated Aβ fibrillization induced by binding of endogenous brain proteoglycans and glycosaminoglycans. However, our findings suggest Aβ oligomers also bind
microbial carbohydrates with high affinity (Figs. 3G-J). Carbohydrate-binding activity among AMPs is widespread and normally protective, playing a key role in helping peptides recognize and bind microbial pathogens (32). Heparin-binding AMPs have high affinities for the unique microbial carbohydrates found in cell walls but also bind host glycosaminoglycans (71).

Consistent with our findings for Aβ, binding of classical AMPs to microbial carbohydrate can lead to rapid peptide fibrilization and amyloid-mediated antimicrobial activities (72). Dysregulated carbohydrate-binding by Aβ may play a role in AD amyloidogenesis. However, a normal AMP role for Aβ would suggest that polymeric microbial cell surface carbohydrates may be the normal in vivo target for the heparin-binding activity of oligomeric species.

Long recognized as a key defensive strategy among lower organisms, AMP-mediated microbial agglutination is also emerging as an important part of human immunity (73). AMP fibrillization appears to play a central role in this important protective activity (48). Most recently, in vivo fibrillization of HD6 has been shown to mediate not only agglutination, but also microbial entrapment within an amyloid fibril network (49). Our findings suggest fibrillization is also involved in Aβ-mediated agglutination and leads to the entrapment of microbial cells by Aβ fibrils. Based on our findings, we propose a three-stage model for the protective activity of Aβ in vivo. Our model parallels the agglutination and entrapment actions of amyloidogenic HD6 (49).

First, the VHHQKL heparin-binding domain of Aβ mediates targeting and binding of soluble oligomeric species to cell wall carbohydrates (Fig. 8A). Bound oligomers then provide a nidus and anchor for Aβ fibril propagation. Second, growing protofibrils interfere with microbial adhesion to host cells (Fig. 8B). Third, Aβ fibrils link, agglutinate, and then entrap the unattached microbial cells in a protease-resistant network of β-amyloid (Fig. 8C). Consistent with our model for the antimicrobial activities of Aβ, classical human AMPs have also been shown to generate amyloid fibrils on microbial surfaces that agglutinate pathogens and inhibit infection (48).

Consistent with our AMP model for Aβ, APP-KO mice show a trend for reduced pathogen resistance (Fig. 1E). However the increase in infection-driven mortality among APP-KO mice was less dramatic than the increase in survival observed in the 5XFAD model (Fig. 1A). For AMP-null models immune impairment is often moderate because redundant activities among related members of antimicrobial peptide families can partially offset the loss of protection.
associated with low expression of individual AMP species (74). LL-37, the archetypal human AMP that serves as a model for Aβ's antimicrobial activity (3), is a member of the cathelicidin peptide family. In humans, serious immunodeficiency associated with low LL-37 expression typically lead to fatal infections in childhood if untreated (50). However, in mouse models, knockout (KO) CRAMP-KO mice lacking the murine LL-37 precursor protein (mCRAMP) show only a modest increase in mortality (≈ 10 %) with bacterial meningitis (75). Conversely, survival with infection among transgenic mice over-expressing human LL-37 is increased several-fold (76). LL-37 is the only cathelicidin expressed in humans. However, in addition to LL-37, mice express two other related cathelicidins. Overlapping activities from the remaining cathelicidin peptides is thought to partially protect from the loss of murine LL-37 in CRAMP-KO mice. APP-KO mice generate at least two Aβ homologues from amyloid precursor like proteins one (APLP1) and two (APLP2), which may likewise help mitigate loss of Aβ-mediated protection (77, 78). Consistent with this model, APP, APLP1, and APLP2 and their non-amyloidogenic processing products show extensive functional redundancy (79), likely because of the gene duplication origin for this protein family. APP-KO mice also have an important additional limitation as models for the loss of Aβ-mediated protection. APP itself may be involved in CNS immunity (80, 81). It remains unclear how loss of activities normally mediated by full-length APP can be excluded as the source of attenuated infection resistance in APP-KO mice.

Genetically modified mice that lack proteases (BACE1 and BACE2) for generating the Aβ family of peptides provide an alternative Aβ-null model. Consistent with our data, knockout BACE-KO mice that lack BACE have been reported to have dramatic immunodeficiency. While neonatal mortality is below two percent under sterile conditions, in less stringently antiseptic environments, up to half of pups born to BACE-KO mice die from infections within the first two-weeks of life (82). Benchmark tests for adaptive immunity have failed to identify defects in the response of BACE-KO mice to immune challenges. Findings for BACE-KO mice appear consistent with an innate immune deficiency and a possible normal protective role for Aβ. However, as with APP-KO mice, it is unclear how to demonstrate the immunodeficiency in BACE-KO mice is specific for a loss of members of the Aβ family of peptides. Additional data is required to conclusively link the etiology of BACE-KO mice immunodeficiency to low Aβ.
Our findings for Aβ and β-amyloid may have corollaries for amyloidopathies beyond AD. Protein fibrillization may be important not only for Aβ’s AMP activities, but also play a role in the normal actions of other amyloidosis-causing proteins. An association between amyloidosis and chronic bacterial infections has been recognized for almost a century (83) but the potential protective activities of host-generated amyloid have only recently emerged (4, 48, 84). At least six amyloidosis-associated peptides show antimicrobial activities, including amylin (85), atrial natriuretic factor (16, 86), prion protein (87), cystatin C (88, 89), lysozyme (5), and superoxide dismutase (90). Conversely, host AMPs have been identified that generate protective amyloids localized to infection sites (91). AA–type amyloidosis involves both systemic deposition of the acute-phase opsonin AMP serum amyloid A and has an infection-driven etiology (92, 93). It remains to be determined whether serum amyloid A or other amyloidosis-causing AMPs also engage in non-pathogenic fibrillation pathways that help protect against infection. However, should this prove to be the case, Aβ may be the first member of a new class of AMPs, in which amyloid generating activities protect against local infections, but can also lead to widespread pathological amyloidosis.

If confirmed, our model carries important implications for understanding the pathogenesis of amyloidosis in AD. Excessive β-amyloid deposition may arise not from an intrinsically abnormal propensity of Aβ to aggregate, but instead, be mediated by dysregulation of the brain’s innate immune system, e.g. the consequence of a response mounted to microbial or sterile inflammatory stimuli. Importantly, our new model is congruous with the amyloid hypothesis and the importance of Aβ and β-amyloid in the neurodegenerative cascade of AD. However, our model would shift the modality of Aβ’s pathophysiology from abnormal stochastic behavior toward dysregulated antimicrobial activities.

It is important to emphasize that while infection of 5XFAD mice with S. Typhimurium seeded and accelerated β-amyloid deposition, the presence of a CNS infection is not implicit in our proposed AD amyloidosis model. Our work has identified what we believe is the normal role of Aβ. What drives widespread β-amyloid deposition in AD remains unclear. Among sterile inflammatory diseases dysregulated innate immune responses rather than infections are emerging as drivers of pathology. Notably, two of the three confirmed AMP amyloidopathies are not linked to obvious infections (4, 15, 16). However a large body of data accrued over nearly a
century suggest genuine infection may also play a role in AD etiology (94, 95). Moreover, while a causal link to amyloidosis remains to be conclusively demonstrated recent epidemiological findings have given increased prominence to the "infection hypothesis", including studies linking brain fungi infection to AD (27, 28) and data showing risk for the diseases rises with infectious burden (96). Our findings do not constitute direct evidence of a role for infection in AD etiology. However, they do suggest a possible mechanism for pathogen-driven β-amyloid amyloidosis. Our data also suggest the possibility that a range of microbial organisms may be able to induce β-amyloid deposition, a possible reason for why a single pathogen species has not yet been identified that is overwhelmingly associated with AD. Future studies systematically characterizing microbial pathogens (viral, bacterial, fungal) in the brains of AD patients, e.g. by RNA Seq, will be necessary to further interrogate if specific clinical pathogens seed β-amyloid as part of the brain’s innate immune system. In any case, whether infectious or sterile inflammatory stimuli drive AD pathology the pathways that regulate innate immunity in the brain may offer significant new targets for therapeutic intervention.
Materials and Methods

Experimental Design. Protective activities associated with Aβ-expression were investigated in murine, nematode, and cell culture models of infection. Transgenic mice, nematode, and cell models were used that constitutively express human Aβ at high levels. Initial experiments tested for Aβ-mediated increases in survival with infection. Increased infection resistance is considered a hallmark for identity as an AMP. The mechanism of protection afforded by Aβ was then characterized in our cell culture monolayer model. We have previously shown remarkable parallels between Aβ activities and LL-37, a highly characterized human AMP. LL-37 was used as a model to elucidate the mechanisms for Aβ's targeting, anti-adhesion, and agglutination activities against microbial cells. Finally, nematode and mice models were tested to confirm the potential protective microbial entrapment role of Aβ fibrillization revealed by cell culture experiments.

Monomeric and oligomeric synthetic peptide preparation. Synthetic Aβ1-40 (Aβ40), Aβ1-42 (Aβ42), scrambled Aβ42 (scAβ42), and LL-37 peptides were prepared and purified by Dr. James I. Elliott at Yale University (New Haven, CT) using solid-phase peptide synthesis. To disrupt aggregates generated during synthesis (97) bulk powdered peptides were dissolved in 30% trifluoroethanol at 1 mg/ml, lyophilized (500 µl aliquots) in siliconized 1.7 ml sample tubes and incubated at room temperature (RT) for 18 hrs. Following incubation peptides were lyophilized and stored under nitrogen at -20 °C. Stock solutions were prepared the day of experimentation from dried peptide films by solubilization in 10 mM NaOH followed by dilution into phosphate-buffered saline (PBS). Preparations were then fractionated by SEC and peak monomer fractions (3-6 kDa) pooled and stored on ice while peptide concentrations were determined by bicinchoninic acid protein assay. Peptides in final stock monomer solutions were adjusted to 100 µM and used within 2 hrs. Analysis of chromatographs from re-fractionated Aβ42 monomer solutions confirmed that 6 hrs after initial fractionation, monomers remained the predominate (>92 %) species (data not shown) under our experimental conditions.

Synthetic Aβ42 oligomer preparations were generated using established protocols. Briefly, for protofibril generation synthetic Aβ42 peptide powder was directly solubilized (100 µM) in PBS and incubated overnight (98). Following centrifugation to remove insoluble material, the crude protofibril preparations were fractionated by SEC and high molecular weight fractions (> 600
kDa) pooled. ADDLs were prepared by direct solubilization (100 μM) and incubation (24 hrs) of Aβ42 peptide powder in phenol red-free F12 culture media (35). For experimentation, Aβ preparations were serially diluted into culture media. Final Aβ concentration in experimental samples was confirmed by densitometry analysis of anti-Aβ immunoblots using peptide standards of known concentration.

**Candida inoculants and lawns.** Freezer stocks of *Candida albicans* strain 90028 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). *C. albicans* stocks were maintained on yeast extract peptone dextrose (YPD) agar at 4 °C with subculture to fresh plates every two weeks.

*C. elegans* pathogenicity plates were prepared by streaking (10 μl) sterile 35m tissue culture plates (BD Falcon) with yeast grown overnight (30 °C) in YPD broth. Plates were incubated at 25 °C for 2 hrs to generate *C. albicans* lawn.

Synchronized hyphal yeast for cell culture experiments were prepared by single colony transfer of *C. albicans* stock to 5 mls of Minimal Sugar Medium (*Formedium*) and 48 hr static incubation at RT (99). Following pelleting (1,750 RCF for 2 mins) and washing with PBS (x2), starved yeast were resuspended in RPMI-1640 medium (Hyclone, Logan, UT) and concentration adjusted to 2.5 x 10⁶ cells/ml. Stock yeast in RPMI were diluted 10-fold into unconditioned culture media immediately prior to inoculation of host cell slide or culture plate wells. Yeast concentration in inoculates was determined using a BioRad TC20 automated cell counter and confirmed by counting CFU following serial dilution and streaking on agar.

**S. Typhimurium inoculants and lawns.** *Salmonella enterica* serotype Typhimurium SL1344 stocks were obtained from the ATCC. Colonies were maintained on agar and subcultured to fresh plates every three weeks. Inoculant stocks were prepared by transfer of a single *S. Typhimurium* colony to Luria-Bertani (LB) agar with 100 μg/ml streptomycin and incubation overnight in a shaker incubator (225 rpm at 37 °C). Pelleted (10,000 x 2 min) bacteria were washed twice with PBS, resuspended in inoculation media, and diluted to required concentration. Bacterial concentrations in stocks were determined by comparing inoculum turbidity to McFarland turbidity standards and confirmed by streaking on agar and counting CFU.

For mouse experiments, *S. Typhimurium* inoculants were pathologized prior to infection by incubation (90 min at 37 °C) in a CO₂ atmosphere. Pathogenicity plates for *C. elegans* were
prepared by streaking inoculate (10 µl) onto *Pseudomonas aeruginosa*- and *Salmonella enterica-* killing assay (SK) plates and overnight incubation at 37 °C. For HCMs, inoculant was added directly to the culture media of host cells.

**Counting CFUs.** Four-fold serial dilutions of samples were spread-plated (100 µl per plate) onto the surface of yeast extract peptone dextrose agar plates. Streaked plates were incubated overnight at 30° C and CFU counted manually. Plates with between 50 and 350 colonies were selected for use in calculations of yeast concentrations.

**Size exclusion chromatography (SEC).** Samples were loaded (400 µl) on a SEC-3 100Å column (Agilent Technologies, Santa Clara, CA), resolved at 1 ml/min using an Agilent Technologies 1200 series HPLC system, and 200 µl eluate fractions collected on ice. Synthetic peptide stock and protofibril preparations were resolved using PBS running buffer. Peak monomer (3-6 kDa) or high molecular weight (>600 kDa) fractions were identified from analysis of UV chromatographs and pooled. Culture media samples containing cell-derived or synthetic Aβ were resolved using unconditioned dye-free Dulbecco’s Modified Eagle’s Medium (DMEM) as running buffer. Eluate fractions were assayed for Aβ by β-amyloid ELISA. Molecular weight SEC standards (BioRad, Hercules, CA) were resolved under the same chromatographic condition as synthetic peptides.

**β-amyloid ELISA.** Samples were assayed for Aβ40 and Aβ42 using commercially available ELISA kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturer’s instructions. For mouse brain fractions, 5XFAD signal was blanked on WT signal.

**Immunodepletion.** Protein G Plus Agarose slurry (Pierce, IL) was pelleted, washed and incubated for 2 hrs at RT with 4G8 (epitope: Aβ17-24) monoclonal antibody (Covance, Princeton, NJ) or control mouse IgG in PBS. Following washing, beads were incubated with media samples for 2 hrs at RT under conditions equivalent to 10 µg of antibody per ml of media. Beads were pelleted and soluble fractions were removed, filtered (0.2 µm), and assayed to confirm Aβ depletion.

**Immunoblotting.** Samples were resolved by electrophoresis on SDS-PAGE (4-12 % Bis-Tris gels) and transferred to polyvinylidene fluoride membrane. Membranes were fixed with glutaraldehyde (0.1 %), blocked with bovine serum albumin (5 %), then incubated overnight at
4°C with mAb 6E10 (1:1000). Following washing, membranes were incubated with goat anti-mouse IgG-coupled to HRP. Blots were developed with chemiluminescence reagent (Pierce, Rockford IL) and signal captured using a VersDoc digital imaging system (BioRad, Hercules, CA). Blot incubations used Tris buffered saline, pH 7.4 containing 0.1 % Tween (TBST).

**Aβ binding ELISA.** The wells of 96-well plates were coated with live yeast by overnight incubation (37°C) with synchronized *C. albicans* (50-250 CFU’s/well) in RPMI media (200 µl/well). Wells were washed to remove unattached yeast and adhering *C. albicans* cells then killed and covalently fixed in place by incubation (15 min at RT) with 4 % paraformaldehyde. Wells were blocked (2 hrs at RT) with 2 % BSA in PBS prior to incubation with experimental samples. Bound Aβ in wells was detected immunochemically by incubation (overnight at 4°C) with α-Aβ42-HRP (Covance) diluted 1:1,000 in blocking buffer and development with 100 µl of chemiluminescence reagent (Pierce, Rockford IL). Wells were washed (x5) with PBS between incubations.

**Mouse infection model.** Female 5XFAD(21) APP/PS1 doubly transgenic mice that co-overexpress and co-inherit FAD mutant forms of human APP (the Swedish mutation: K670N, M671L; the Florida mutation: I716V; the London mutation: V717I) and PS1 (M146L; L286V) transgenes under transcriptional control of the neuron-specific mouse Thy-1 promoter (Tg6799 line). 5XFAD lines (B6/SJL genetic background) were purchased from Jackson Laboratory and maintained by crossing heterozygous transgenic mice with B6/SJL F1 breeders. All 5XFAD transgenic mice were heterozygotes with respect to the transgene. Animal experiments were conducted in accordance with institutional and NIH guidelines.

One-month old mice received a single injection of 65,000 CFU (0.18-0.20 ml) of *S. Typhimurium* suspension at AP, -1.6; ML, +1.5; DV, -1.6/-1.1/-0.7 using a 5 µl Hamilton syringe with a 30-gauge needle attached to a digital stereotaxic apparatus and an infusion pump at a rate of 0.15 µl/min. After infusion was completed, the needle remained in place for 10 min before slow withdrawal. Mice were given food and water on the cage floor starting 24 hrs after the injection. Control sham infections used *S. Typhimurium* heat-killed prior to injection.

Clinical scores were recorded every 8 hrs according to a modified grading criteria for mouse encephalomyelitis (100, 101). Clinical criteria are summarized in Fig. S1a. Clinical progression
was followed to moribundity and then mice were sacrificed. Scores were recorded for each mouse and expressed as mean ± SEM.

**Assaying mouse brain bacterial load.** To determine bacterial loads, animals were sacrificed 24 hrs post-infection and tissues homogenized (1% Triton X in PBS) on ice under sterile conditions using a 0.4 µm pore-sized strainer. Homogenates were serially diluted and streaked on Luria Bertoni (LB) agar plates, incubated at 37 °C and CFU counted.

**Mouse tissue preparation and sectioning.** For immunofluorescence, mice were deeply anesthetized with a mixture of ketamine and xylazine, and perfused transcardially with 4% paraformaldehyde in cold PBS. Brains were postfixed overnight and then transferred into a 30% sucrose solution until sedimented. Coronal sections (40 µm) were cut from an ice-cooled block using a sliding microtome (Leica, Wetzlar, Germany). Sections were stored at -20°C in cryoprotective buffer containing 28% ethylene glycol, 23% glycol and 0.05 M phosphate until processing for analysis.

**Immunofluorescence labeling of mouse sections.** Immunofluorescence labeling was performed as previously described (21). Primary antibodies include, rabbit anti-GFAP (1:500, Dako, Fort Collins, CO) for astrocytes, rabbit anti-ionized calcium-binding adaptor molecule 1 (Iba1, 1:500, Wako, Osaka, Japan) for microglia, and anti-Salmonella polyclonal rabbit for S. Typhimurium. Bound primary antibodies were detected with anti-rabbit Alexafluor 594 (Invitrogen, 8889S). Cell nuclei in sections were stained with TO-PRO®-3 iodide (1:500, Life technologies, Eugene, OR). Fluorescent signals were detected and captured using a LSM Pascal 5 Carl Zeiss confocal laser-scanning microscope (Zeiss, Germany) and Zeiss LSM image browser or Nikon A1SiR confocal microscope.

**Cytokine assays.** Mouse brain homogenate prepared (1:5) in RIPA buffer (Sigma, St. Louis, MO) was centrifuged (45,000 g x 30 min) and supernatants removed for analysis. Supernatant cytokines levels were determined using the MesoScale Discovery (MSD, Rockville, MD) 96-well Mouse Pro-Inflammatory V-PLEX Assay according to the manufacturer’s instructions. Briefly, samples are incubated in wells containing an array of cytokine capture antibodies directed against Interleukins 1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p/70, Tumor necrosis factor α (TNFα), Interferon γ (IFNγ), and Keratinocyte-derived chemokine (KC/GRO).
Bound cytokines are detected immunochemically and signal read using an MSD Sector Imager 6000.

**Immuno and ThS co-stained mouse sections.** Immunofluorescence labeling was performed as described previously (102). Briefly, sections were incubated with primary anti-\textit{Salmonella} polyclonal rabbit IgG (1:1000) (PA1-7244, ThermoFisher Scientific) followed by secondary anti-rabbit Alexafluor 594 (1:500) (Invitrogen, 8889S) antibodies. For Aβ staining sections were incubated with mouse monoclonal antibodies 3D6 (Eli Lilly) (mouse brain sections) or 4G8 (nematode sections). Bound anti-Aβ antibodies were detected by incubation with anti-mouse Alexafluor 488 (1:500) (Life Technologies, A11001) antibodies. Following immunostaining, free-floating sections were incubated (8 min) with 0.002% Thioflavin S in TBS, rinsed twice for 1 min in 50% ethanol, washed for 5 min in TBS, and mounted with Prolong Gold antifade reagent (Life Technologies). Stained sections were analyzed by fluorescence confocal microscopy (Leica TCS SL, Leica Microsystems, Germany).

**\textit{Caenorhabditis elegans} model.** Two previously described transgenic \textit{C. elegans} strains were used in experiments. GMC101, \textit{dvIs100} [pCL354(unc-54:DA-Aβ1-42) + pCL26 (mtl-2: GFP)] nematodes express human Aβ42 in body wall muscle and green fluorescent protein (GFP) in intestinal cells (23). Control \textit{C. elegans} CL2122 \textit{dvIs15} (mtl-2: GFP) nematodes express GFP but not the Aβ42 peptide (23). Worm were synchronization prior to experimental treatments according to established protocols (103). Briefly, unhatched eggs were release by treating gravid worms with bleach. Following an overnight incubation, arrested L1 larvae were added to \textit{E. coli} OP50 lawns and incubated at 20 °C to generate synchronized L4 larval (48 hrs) or adult (60 hrs) nematodes.

For infection experiments, 100-150 synchronized L4 stage worms were incubated (2 hrs at 25 °C) on \textit{C. albicans} lawns, washed with M9 buffer to remove surface \textit{C. albicans}, and transferred to 6-well culture plates containing 1.5 ml/well of incubation media (79 % M9 buffer, 20% BHI, 10 µg/ml cholesterol in ethanol, and 90 µg/ml kanamycin). Nematodes were incubated at 25 °C and monitored daily for the distinctive distention and penetrative filamentation that characterizes \textit{Candida}-induced mortality.

**Nematode freeze-fracture and immunostaining.** Worms (L4) were transferred dropwise to poly-lysine coated slides and covered with a coverslip. Gentle pressure was applied to the
coverslip before the slide assembly was placed on a metal block and flash-frozen using liquid nitrogen. The coverslip was flicked off and fractured samples fixed by 5 min incubations with absolute alcohol followed by acetone. Dried samples were ringed with petroleum jelly and covered with a second coverslip. Slide staining was performed in a wet chamber. For immunostaining, slides were blocked for 15 min with blocking buffer (10% tween and 0.2 g/ml powdered milk in PBS) then incubated (1 hr at RT) with rabbit polyclonal anti-\textit{Candida} antibody (Abcam, ab20028) and/or anti-A\textbeta mAb 4G8. Following washing, slides were incubated with anti-rabbit and/or anti-mouse antibodies conjugated to AlexaFluor 568 and AlexaFluor 488 fluorescent dyes (Life Technologies), respectively. For ThS staining, slides were incubated 1 hr at RT with dye solution and PBS washed. Specimens were incubated with prolong Gold antifade reagent (Life Technologies) before viewing by CFM.

\textbf{Transmission Electron Microscopy (TEM) of nematode sections.} Worms were washed in M9 buffer, pelleted by centrifugation (500 g for 5 min), and incubated with fixative (4% para-formaldehyde, 0.2% glutaraldehyde, and 0.1 M sucrose in 0.1 M Sodium cacodylate). Fixed worms were then placed on a plastic surface and sliced with a clean razor blade several times at right angles. Sliced worms were incubated a further 60 min in fixative before rinsing with sodium cacodylate (Sigma-Aldrich, C4945) and embedding in 2% agarose (50-100 µl). Agarose preparations were sectioned into blocks, dehydrated by incubation (10 min) in progressively more concentrated solutions of ethyl alcohol, placed in straight LR white (Electron Microscopy Sciences, 14380) for 2 hrs, and finally embedded in gelatin capsules at 50° C overnight. Embedded blocks were diamond knife sectioned by ultramicrotome (Leica ultracut UCT) and sections absorbed to Formvar carbon coated copper grids (FCF100-Cu, Electron Microscopy Sciences, Hatfield, PA). Samples for immunostaining were blocked (1% BSA in PBS) before incubation (1:100 in blocking buffer for 30 min at RT) with primary antibody (mAb 4G8) followed by goat anti-mouse-IgG antibody covalently linked to nanogold particles. Washed specimens were fixed (1% glutaraldehyde for 10 min at RT), stained with uranyl acetate, and viewed using a JEM-1011 Transmission Electron Microscope (JEOL Institute, Peabody, MA).

\textbf{Immuno-histochemical nematode staining.} \textit{C. elegans} were washed in S-basal (23), fixed overnight in 10% (v/v) Neutral Buffered Formalin (NBF) at 4°C, embedded in agar (2% w/v in phosphate buffered saline) blocks and then fixed again (10% NBF overnight). Following processing of the agar blocks into paraffin, 5 µm sections were prepared, deparaffinised and
treated with 90% formic acid prior to Aβ immunohistochemistry with a 1:200 dilution of 1E8 mouse monoclonal (SmithKline Beecham) antibody (epitope: Aβ18–22). Antibody binding sites were detected with a peroxidase labelled streptavidin biotin system (Dako K0675) with a 3,3’-diaminobenzidine tetrahydrochloride (DAB) chromogen (Dako) resulting in a brown reaction product. Samples were counter stained with Harris Haematoxylin solution (Amber Scientific).

**Host cell monolayer model.** Host cell monolayers were prepared from naïve and transformed human neuroglioma (H4) or Chinese hamster ovary (CHO) cell lines. Stable transformed H4 cell lines that secrete Aβ40 (H4-Aβ40) or Aβ42 (H4-Aβ42) without over expression of the APP have been described previously (29). Stable transformed CHO-CAB cells co-expressing human ATCC Swedish mutation and β-site APP cleaving enzyme 1 (BACE1) were generated by transfecting a pcDNA3.1-BACE1-myc construct into CHO-APP751 cells that over-express mutant APP751 (K670N/M671L: Swedish mutation)(30).

Naïve H4-N and CHO-N cell lines were maintained in complete media containing DMEM, 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 100 U penicillin, and 100 µg/ml streptomycin. Complete media for transformed H4-Aβ40 and H4-Aβ42 cells included hygromycin (150 µg/ml) and media for CHO-CAB Zeocin (200 µg/ml) and G418 (200 µg/ml). Confluent host cell cultures were washed twice in PBS, trypsinized, pelleted by centrifugation, washed a second time to remove trypsin and residual antibiotics, and resuspended in antibiotic-free DMEM with 5 % FBS and 2 mM L-glutamine. Cell concentrations were determined by automated cell counter (TC20 BioRad, Hercules CA) and adjusted to 300,000 and 500,000 cells/ml for H4 and CHO lines, respectively. Host cell suspensions were transferred to the wells of Lab-Tek 8-chamber glass slides (Thermo Scientific, Waltham, MA) (200 µl/well) or the wells of 96-well culture plates (100 µl/well) and incubated for 24 hrs. Cell confluence in chamber slides and plate wells were confirmed by microscopic examination. Automated cell counter analysis of well trypsin extracts confirmed that in control uninfected HCMs naïve and transformed cell numbers did not diverge by more than 6 % prior to infection, or following the final experimental incubation (Fig. S4D).

Naïve and transformed culture media were conditioned for 36 hrs before inoculation with Candida. HCM in culture plates were infected by addition of Candida inoculant aliquots (10 µl) containing 2,000 or 250,000 CFU, respectively. For host cell survival experiments, Candida
were incubated with H4 and CHO cells for 28 and 36 hours, respectively. HCMs were then washed and assayed for host cell survival.

**Host cell BrdU labeling.** Subconfluent naïve and transformed H4 and CHO cells were incubated overnight (10 cm culture dishes) in complete culture media containing 10 mM BrdU. Confluent BrdU-labeled cell cultures were PBS washed (x3) to remove free BrdU then trypsinized and used for preparation of HCMs in 96-well culture plates. Following experimental treatments, plate wells were washed with PBS (x3) then fixed and permeabilized, and assayed according to manufacture’s instructions (Cell Proliferation BrdU ELISA, Roche).

**Host cell Calcein-AM viability assay.** Host cell viability was determined using a commercially available Calcein-AM cell viability assay kit (Life Technologies, Carlsbad CA). Assays were performed according to the manufacturer’s instructions for 96-well culture plates. While microbial esterases metabolize Calcein-AM, uptake of the dye is inhibited by yeast cell walls (104, 105) and assay interference from *C. albicans* mediated Calcein-AM hydrolysis was < 6 % of host signal under our experimental conditions (data not shown).

**Host cell DAPI staining.** The wells of 96-well culture plates were fixed by incubation (10 min at RT) with 4 % paraformaldehyde in PBS. Fixed wells were then stained under non-permeabilizing conditions by brief incubated (10 min) with detergent free TBS containing 1 µg/ml DAPI (Sigma-Aldrich, St. Louis, MO). Well fluorescence (Ex360 nm/Em460 nm) was captured following washing (x3). DAPI entry into yeast cells is retarded by the cell wall and assay interference from *C. albicans* staining was < 8 % of host signal under our experimental conditions (data not shown).

**Imaging *C. albicans* host cell adherence.** HCMs in 8-well chamber slides were infected with synchronized hyphal yeast (10,000 CFU/well) by addition of a 10 µl aliquot of freshly prepared *C. albicans* inoculate to culture media (200 µl/well) pre-conditioned for 36 hrs with host cells. Infected slides were incubated for 2 hrs, media removed by aspiration, wells washed with PBS (x3) and then fixed by 10 min incubation with 4 % paraformaldehyde. Fixative was removed and wells washed (x3) before incubation (30 min) with Calcofluor white M2R fungal surface stain (106) (Life Technologies). Wells were water washed and coverslipped before imaging by fluorescence microscopy (Ex360 nm/Em460 nm).
**Immunochemical detection of HCM adhering** *C. albicans*. Experiments were performed using HCMs prepared in white opaque 96-well culture plates. HCMs were infected with synchronized hyphal yeast (1,000 CFU/well) by addition of 10 µl of freshly prepared *C. albicans* inoculate to wells containing pre-conditioned (36 hrs) culture media (100 µl/well). Wells were incubated 18 hrs with yeast before aspiration of media, gentle washing with PBS (x3), and fixation by 10 min incubation with 4 % paraformaldehyde. Fixative was removed and wells washed (x3) before incubation (1 hr) with blocking buffer (2 % albumin in TBST). Wells were then incubated (2 hrs) with fresh blocking buffer containing a 1:5000 dilution of α-*Candida*-HRP antibody (Abcam, Boston MA). Wells were washed with TBST (x5) and fluorescent captured (Ex320 nm/Em420 nm) following development with QuantaBlu (Pierce, Rockford IL), a fluorescent HRP substrate.

**Viability assay for HCM adhering** *C. albicans*. Synchronized hyphal yeast (1,000 cells/well) were incubated (3 hrs) with HCMs in 96-well culture plates containing pre-conditioned (36 hrs) culture media. Wells were washed with PBS (x3) and then incubated with trypsin. Serial dilutions of trypsin extracts were streaked on agar and CFU counted.

**C. albicans adhesion assay for abiotic surfaces.** Experiments used a modified method of Tsai *et al*, 2011(32) to assay *C. albicans* adhesion to polystyrene in conditioned culture media. Synchronized hyphal yeast (1000 CFU/well) were incubated (37° C) in the wells of clear flat-bottom polystyrene 96-well microtiter plates containing host cell conditioned (36 hrs) culture media (200 µl/well). Incubation media was removed by aspiration and wells washed (x3) before incubation (30 min at RT) with PBS (200 µl/well) containing 10 µl of Calcofluor white fungal stain solution 6726 (ENG Scientific, Clifton, NJ). Following washing, attached hyphae were detected by measuring well fluorescence (Ex360 nm/Em460 nm).

**C. albicans aggregation assay.** Host cell conditioned (48 hrs) culture media (200 µl/well) was incubated (overnight at 37° C) with synchronized yeast (200 cells/well) in the wells of clear 96-well microtiter plates. Incubation media was removed and yeast pellets washed twice with PBS. During aspiration care was taken to minimize disturbance of settled yeast at the well bottom. Settled yeast pellets were resuspended in PBS and transferred to fresh wells. Low magnification (x4) bright field well images were captured at maximum condenser aperture. Images were then analyzed for yeast aggregates using ImageJ software (version 1.47) with the following procedure. Captured image files were first converted from 8-bit RGB to 8-bit greyscale then
further transformed to 1-bit black and white images using a conversion threshold of 86%. Well area covered by yeast aggregates was determined from pixel counts of transformed black and white images using the Analyze Particle tool with lower size threshold set to 50 pixels. Isolated black areas of less than 50 pixels (4-6 yeast cells) were not included in aggregate totals.

**Staining and antibody labeling of C. albicans aggregates.** Aggregated yeast were pelleted (2 min x 500 g), pellets washed with PBS (x2), transferred to glass slides in minimal volume, and excess buffer blotted off. Slides were air-dried to fix yeast and then carefully rinsed with water. For dye staining, slides were incubated in the dark at RT with 50 µl of Thioflavin S (5 min) or Congo red (60 min) staining solution then water rinsed. Specimens stained with ThS alone were viewed by fluorescence microscopy (Ex430nm/Em550nm). Congo red stained specimens were viewed by transmittance polarized light microscopy. ThS and Congo red staining solutions were prepared fresh according to the methods of Saeed and Fine, 1967 (107) and Putchler and Sweat, 1965 (108), respectively. For immunolabeling experiments specimens pre-stained with ThS were incubated (2 hrs at 4° C) with blocking buffer containing 1:1,000 dilution of mAb 4G8. Slides were TBST rinsed and then incubated (1 hr at RT) with donkey anti-mouse-IgG antibody covalently labeled with the red-fluorescent dye Alexa Fluor 594 (α-mouse-AF568) (Life Technologies). Thioflavin and anti-Aβ double labeled specimens were mounted with Prolong Gold antifade reagent (Life Technologies) before viewing with a fluorescence confocal microscope (Leica TCS SL, Leica Microsystems, Germany).

**TEM of microbial agglutinates.** Candida aggregates cells suspended in PBS (5 µl) were absorbed to Formvar carbon coated copper grids (FCF100-Cu, Electron Microscopy Sciences, Hatfield, PA). Grids were blocked with 1 % BSA in PBS (kept covered for 10 min at RT) then incubated (30 min at RT) with mAb 4G8 diluted 1:1,000 in blocking buffer. Grids were washed with PBS (x3) and incubated with goat anti-mouse-IgG antibody covalently linked to nanogold particles. Following three 5 min PBS washes and four 10 min water washes, specimens were fixed with 1 % glutaraldehyde (10 min at RT). Specimens were washed with water, stained with uranyl acetate, and then viewed using a JEM-1011 Transmission Electron Microscope (JEOL Institute, Peabody, MA).

**Statistical analysis.** Statistical analyses were performed with Prism software (version 6.0c) using two tailed t-tests to compare arithmetic means. Survival curves were compared using Log-
rank (Mantel-Cox) test and confirmed by Gehan-Breslow-Wilcoxon test. \( P \) values < 0.05 were considered statistically significant.
Supplementary Materials List:
Seven figures are included as supplemental materials

Table S1: Figure micrographs are representative of data from multiple repeat experiments and image fields.

Fig. S1: Aβ deposition and inflammation in 5XFAD mice prior to infection and criteria used for assessing clinical performance following infection.

Fig. S2: Aβ42 localizes to gut and muscle in GMC101 nematodes.

Fig. S3: Aβ expression protects GMC101 nematodes and H4-Aβ42 cells against S. Typhimurium.

Fig. S4: Confirmation of increased Candida resistance among transformed host cells using three independent assays.

Fig. S5: Transformed cell lines generate Aβ oligomers at levels found in the soluble fraction of human brain.

Fig. S6: Transformed H4-Aβ40 and CHO-CAB host cells resist Candida colonization and agglutinate yeast.

Fig. S7: Birefringence of Congo red stained yeast aggregates from H4-Aβ42 media.

Fig. S8: Anti-Aβ antibodies do not label CL2122 tissues or infecting yeast.

Fig. S9: β-amyloid co-localize with S. Typhimurium cells in 5XFAD brain.

Video S1; Z-section projection of 5XFAD mouse brain showing β-amyloid entrapment of S. Typhimurium cells.
References and Notes:


33. A. M. Weidner, M. Housley, M. P. Murphy, H. LeVine, Purified high molecular weight synthetic \( \text{A\beta}(1-42) \) and biological \( \text{A\beta} \) oligomers are equipotent in rapidly inducing MTT formazan exocytosis. *Neurosci Lett* 497, 1-5 (2011).


Acknowledgments: His work was supported by grants from NIH (5R01AI081990-02), the Cure Alzheimer's Fund and The Helmsley Charitable Trust. The author would like to thank Prof Todd Golde for Aβ-expressing transfected cell line and Dr Ashley Bush for transgenic nematodes. First and second authors contributed equally to this study. D.K.V.K, S.H.C., K.J.W., R.D.M., and R.E.T were responsible for experimental design, data interpretation and manuscript writing. W.A.E., S.T., J.G., and A.L. conducted experiments. L.E.G. and G.M contributed to experimental design, data interpretation and developed host models.
Figures:

Figure 1: Aβ expression protects against S. Typhimurium meningitis in genetically modified AD mouse models. Transgenic 5XFAD mice expressing human Aβ and APP-KO mice lacking murine
APP were compared to genetically unmodified WT littermates for resistance to S. Typhimurium meningitis. One-month old mice were injected intracranially with S. Typhimurium and clinical progression followed to moribundity. Performance of 5XFAD (n = 12) mice compared to WT (n = 11) are shown following infection for A) survival ($P = 0.009$), B) clinical score ($P < 0.0001$), C) percent weight loss ($P = 0.0008$). D) S. Typhimurium load 24 hrs post-infection in right (ipsilateral) and left (contralateral) brain homogenates 5XFAD (n=4) and WT (n=4) mice shown as mean CFU ± SEM (*$P = 0.03$ and **$P = 0.04$). E) APP-KO (n = 15) show a trend ($P = 0.104$) towards reduced survival compared to WT (n = 15) littermates following infection. F) No significant change in survival was observed for control sham infected WT (n=6) and 5XFAD (n=6) mice injected with heat-killed S. Typhimurium. Statistical significance was calculated by Log-rank (Mantel-Cox) test for survival (A, E, and F), linear regression for clinical score and weight (B and C), and statistical means compared by t-test (D).
Figure 2: Aβ expression in nematodes and cultured cells increases host resistance to infection by Candida. Aβ-mediated protection against C. albicans (Candida) was characterized in C. elegans and host cell monolayer (HCM) mycosis models. Experimental nematodes included control non-Aβ expressing (CL2122) and transgenic human Aβ-expressing (GMC101) strains.
HCM lines included control naïve (H4-N and CHO-N) and transformed human Aβ-overexpressing (H4-Aβ40, H4-Aβ42, and CHO-CAB) cells. A) Survival curves for CL2122 (n = 61) and GMC101 (n = 57) nematodes following infection with *Candida* (*P* < 0.00001). B) Viability of naïve and transformed HCM following 36 hr incubation with *Candida*. Host cell viability was followed by pre-labeling HCMs with BrdU and then comparing wells for anti-BrdU signal. Signal of infected host cells shown as percentage of uninfected wells (*P* = 0.002, **P** = 0.001, and ***P** = 0.004). C) *Candida* adherence to host cells. Fluorescence micrograph of Calcofluor White stained *Candida* adhering to control H4-N or transformed H4-Aβ42 HCMs following 2 hr co-incubation in pre-conditioned culture media. D) Quantitate analysis of *Candida* host cell colonization. Adhering *Candida* were assayed immunochemically (*P* = 0.003, **P** = 0.001, and ***P** = 0.004). Anti-*Candida* luminescence signal was captured in arbitrary units (AU). E) Phase contrast micrographs of agglutinated *Candida* following overnight incubation with H4-N or H4-Aβ42 host cells. F) Quantitate analysis of *Candida* agglutination. Wells were compared for yeast aggregate surface area using image analysis software (*P* = 0.007, **P** = 0.002, and ***P** = 0.009). Bars in panel’s B, D, and F are means of six replicate wells ± SEM. Statistical significance was calculated by Log-rank (Mantel-Cox) test for nematode survival (A) and statistical mean comparisons by t-test (B, D, and F). Micrographs (C and E) are representative of data from three replicate experiments and multiple discrete image fields (Table S1A, supplemental materials).
Figure 3: Aβ’s protective actions in cell culture are mediated by anti-adhesion and agglutination activities against Candida. C. albicans (Candida) adhesion to abiotic surfaces and agglutination in the bulk phase were characterized in the presence of cell-derived or synthetic Aβ.
Following 36 hrs conditioning, host cell free culture media was collected from control naïve (H4-N or CHO-N) or transformed Aβ-overexpressing (H4-Aβ40, H4-Aβ42, or CHO-CAB) HCMs. Aβ-immunodepleted (ID α-Aβ) and control immunodepleted (ID IgG) media were prepared by incubation with immobilized anti-Aβ or non-specific antibodies. Experimental synthetic peptides included Aβ (Aβ40 and Aβ42), AMP positive control (LL-37), and negative control scrambled Aβ42 (scAβ42). A-B) Comparison of ID α-Aβ and ID IgG media's anti-adhesion (*P = 0.009, **P = 0.001, and ***P = 0.004) and agglutination (*P = 0.001, **P = 0.0005, and ***P = 0.004) activities. C-D) Anti-Candida activities of serially diluted conditioned media and synthetic peptides. E-F) Activities of synthetic Aβ42 monomer, ADDLs, and protofibril preparations. G-H) Conditioned culture media anti-adhesion (*P = 0.003 and **P < 0.0003) and agglutinating (*P < 0.02 and **P < 0.003) activities alone (Neat) or in the presence of soluble yeast wall carbohydrates (+Glucan or +Mannan). (I) Untreated, immunodepleted, or glucan (Glu) or mannan (Man) spiked H4-Aβ42 conditioned media were incubated with intact immobilized yeast cells in an Aβ/Candida binding ELISA (*P = 0.006, **P = 0.008, and ***P < 0.004). (J) Synthetic monomeric Aβ42 and cell-generated peptide from H4-Aβ42 cells were compared for Candida binding by Aβ/Candida binding ELISA. Synthetic peptide incubations (C and F) were performed in H4-Aβ42 conditioned culture media pre-treated to remove cell-derived Aβ by α-Aβ immunodepletion. Symbols and bars for A-J are statistical means of 6 replicate wells ± SEM. Statistical significance was by t-test.
Figure 4: β-amyloid fibrils propagate from yeast surfaces and capture and entwine *Candida* in H4-Aβ42 media. Early stage *C. albicans* aggregates harvested from H4-Aβ42 conditioned media were probed with α-Aβ-Au nanoparticles and analyzed by TEM. A) Yeast agglutination is mediated by fibrillar structures. Micrograph shows fibrils binding cells within yeast aggregates and linking *C. albicans* clusters. B) Fibrillar structures extending from yeast cell surfaces. C-D). α-Aβ-Au nanoparticle labeling of short fibrillar structures extending from *C. albicans* surfaces and long fibrils running between yeast clumps. E) Absorption experiment showing ablated α-Aβ-Au binding of fibrils extending from yeast in the presence of soluble synthetic Aβ peptide. Data is consistent with specific α-Aβ-Au labeling of β-amyloid fibrils. Micrographs are representative of data from three replicate experiments and multiple discrete image fields (*Table S1A*, supplemental materials).
Figure 5: *Candida* cells are entrapped by β-amyloid in H4-Aβ42 culture media. Late stage yeast (*C. albicans*) aggregates harvested from H4-Aβ42 media were probed for β-amyloid markers. A-B)
Yeast aggregates visible (VIS), green-fluorescence ThS (ThS FLU), red-fluorescence anti-Aβ (α-Aβ FLU), and superpositioned (VIS/FLU overlay) signals. Yeast aggregates generated with the control synthetic LL-37 peptide (A) are negative for ThS enhanced fluorescence. Yellow (B) denotes co-localization of anti-Aβ and ThS signals, the hallmark of β-amyloid. C) SEM analysis reveals fibrous material in H4-Aβ42 yeast aggregates that is absent from control C. albicans pellets prepared by centrifugation in H4-N media. D) H4-Aβ42 yeast aggregate incubated with anti-Aβ immunogold (α-Aβ-Au) and analyzed by TEM. First and second panels show labeling of fibrous material by α-Aβ-Au. Third panel shows inhibition of α-Aβ-Au nanoparticle binding by soluble synthetic Aβ peptide (α-Aβ-Au + Aβ peptide), consistent with specific labeling of β-amyloid. Micrographs are representative of data from two or more replicate experiments and multiple discrete image fields (Table S1A, supplemental materials).
Figure 6: Intestinal infection with *Candida* induces Aβ fibrillation in transgenic GMC101 nematode gut. Aβ42-expressing GMC101 *C. elegans* were infected with *C. albicans* (*Candida*) and...
probed for anti-Aβ immunoreactivity and β-amyloid markers using TEM and CFM. **A**) Micrograph shows positive labeling of yeast cell surface in GMC101 gut by anti-Aβ immunogold (α-Aβ-Au) two hours following *Candida* ingestion. **B-D**) Visible (VIS), red-fluorescence anti-*Candida* (α-*Candida*), green-fluorescence ThS (ThS) or green-fluorescence anti-Aβ (α-Aβ), and superpositioned (Overlay) signals from freeze fracture nematode sections with advanced *Candida* infections. Yellow denotes signal co-localization. Figure **B** compares uninfected and infected worms. Figures **C** and **D** show ThS and anti-Aβ signals for gut yeast aggregates. Uninfected and infected CL2122 nematode controls were negative for anti-Aβ and enhanced ThS fluorescence signals (**Figs. 2S and S8**, supplemental materials). Micrographs are representative of data from three or more replicate experiments and multiple discrete image fields (**Table S1B**, supplemental materials).
Figure 7: Infection-induced β-amyloid deposits co-localize with invading S. Typhimurium cells in 5XFAD brain. Four-week-old WT mice or transgenic 5XFAD animals expressing high levels of human Aβ were injected with viable S. Typhimurium bacteria. Mice were also injected with heat-treated S. Typhimurium cell debris as a negative control for the injection procedure. A-B) Visible
(VIS), red-fluorescence anti-Salmonella (α-Salmonella), green-fluorescence ThS (ThS) or green-fluorescence anti-Aβ (α-Aβ), and superpositioned (Overlay) signals from brain sections prepared 24 (A) or 48 (B) hrs post-infection. Panels are representative signals from multiple image fields captured as z-sections by CFM from three repeat experiments Yellow denotes signal co-localization. Z-series projection showing β-amyloid surrounding and entrapping bacteria colonies in a rotating 3-dimension section of 5XFAD brain is also included in Video S1, supplemental materials. Micrographs are representative of data from three replicate experiments and multiple discrete image fields (Table S1C, supplemental materials).
Figure 8: Model for anti-microbial activities of soluble Aβ oligomers. Proposed mechanism mediating microbial anti-adhesion, agglutination, and entrapment activities of Aβ oligomers. A) Oligomer binding of microbial cell surface carbohydrates mediated by Aβ’s heparin-binding domain. B) Bound oligomers and propagating fibrils inhibit host cell attachment. C) Elongating fibrils agglutinate and finally entrap unattached microbial cells within a protease-resistant amyloid matrix.
Supplementary Material:

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Supplemental Table S1: Figure micrographs are representative of data from multiple repeat experiments and image fields. Tables show the number of discrete image fields analyzed and replicate experiments performed to generated figure micrographs.
Supplemental Figure S1: Aβ deposition and inflammation in 5xFAD mice prior to infection and criteria used for assessing clinical performance following infection. Supplemental data for
5XFAD mouse infection model A) Grading system used for clinical assessment following cerebral injection of 65,000 CFU of S. Typhimurium. B) Confirmation of low Aβ deposition in 4-week old mice prior to injection with S. Typhimurium. Brain homogenates were centrifuged (105,000 g for 2 hrs), soluble fraction removed and pellet extracted with formic acid. Fractions were then assayed for Aβ isoforms by human-specific β-amyloid ELISA. C) Sample photomicrographs of mouse cortex and hippocampus sections stained for cell nuclei with Hoechst stain (blue) then immunoprobed (red) for GFAP+ astrocytes (left) or Iba1+ microglial (right) cells. D) Quantification of GFAP+ astrocyte and Iba1+ microglia signals from cortex and hippocampal micrographs. E) Multi-array profile (V-PLEX) of brain homogenate cytokines, including interleukin 1β (IL-1β), IL-2, IL-4, IL-5, IL-10, IL-12p/70, Tumor necrosis factor α (TNFα), Interferon γ (IFNγ), and Keratinocyte-derived chemokine (KC/GRO). Signals (B, D and E) shown as mean (eight mice per group) ± SEM. Data confirm high soluble Aβ and low insoluble β-amyloid in young 5XFAD brain. Findings are also consistent with the absence of potentially protective inflammatory conditions in 1-month old 5XFAD brain.
Supplemental Figure S2: Aβ42 localizes to gut and muscle in GMC101 nematodes. Infection-free control (CL2122) and Aβ-expressing (GMC101) nematode sections were characterized for tissue
localization of anti-Aβ immunosignal. A) Immunohistochemically stained transverse microtome nematode sections showing anti-Aβ signal (brown) in body wall muscle and gut lumen. Sections were probed with an Aβ-specific antibody (mAb 1E8). B) Longitudinal freeze-fracture C. elegans sections showing anti-Aβ fluorescence signals for two antibodies in body wall muscle and gut. Sections were probed with anti-Aβ monoclonal antibodies 6E10 (mAb 6E10) for top two panels and 4G8 (mAb 4G8) for bottom micrographs. Left panels are visible (VIS), middle show red (top two panels) or green (bottom two panels) -fluorescence anti-Aβ (α-Aβ), and right superpositioned (Overlay) signals. Data confirm Aβ is found in body wall muscle and gut of GMC101, but not control CL2122 nematodes. C) Aβ-expressing (GMC101), but not control (CL2122), worms excrete Aβ. Healthy synchronized L4 stage GMC101 (n = 191) or CL2122 (n = 192) worms were individually selected and transferred to fresh agar plates (10 worms per plate) and cultivated for 48 hrs. Plates were washed with water and effluents pooled collected. Samples were briefly centrifuged at low speed to pellet worms, supernatants removed, lyophilized, and reconstituted in SDS sample buffer. Control Aβ42 peptide (Lane 1), CL2122 (Lane 2), and GMC101 (Lane 3) samples were resolved on SDS-PAGE, immunoblotted, and probed with an anti-Aβ antibody (mAb 6E10). Consistent with Aβ in the gut lumen, GMC101 samples were positive for anti-Aβ signal that resolved with control synthetic Aβ42 peptide (Syn. Aβ42). Data is representative of findings from three repeat experiments.
Supplemental Figure S3: Aβ expression protects GMC101 nematodes and CHO-CAB cells against *S. Typhimurium*. Aβ-mediated antimicrobial activity against *S. Typhimurium* was tested in *C. elegans*
and HCM infection models. A) Transgenic (GMC101) (n = 57) and control (CL2122) (n = 61) C. elegans were incubated (1 hr) on S. Typhimurium lawns and nematode survival followed. Consistent with data from S. Typhimurium/mouse infection models, GMC101 worms expressing Aβ42 show statistically significant higher survival with infection (P = 0.0005) compared to control C. elegans by Log-rank (Mantel-Cox) test. B) S. Typhimurium were incubated (3 hrs) in culture broth with (Broth + Aβ42) or without (Broth Alone) synthetic Aβ42 (1 µM). Consistent with C. albicans data, bacteria were agglutinated in the presence of Aβ. C-D) Transformed (CHO-CAB) or naïve (CHO-N) CHO host cell monolayers (HCMs) were incubated (1 hr) with S. Typhimurium. Following repeated washing, wells were treated with the cell-impermeable antibiotic gentamycin to kill extracellular bacteria. Wells were then fixed or trypsinized. Fixed wells were probed immunochemically for red fluorescent anti-Salmonella (α-Salmonella) signal and CFM micrographs captured. Trypsin extracts were streaked onto agar and well microbial load determined by counting CFU. Micrograph (C) and CFU assay data (D) confirm attenuated intracellular S. Typhimurium infection for Aβ-expressing CHO-CAB cells compared to control naïve CHO-N HCMs. The attenuated infection with Aβ expression shown by CFU assay data (D) is statistically significant by t-test (*P = 0.036). E) Aβ binding of S. Typhimurium was tested in a Aβ/bacteria binding ELISA. Serial dilutions of synthetic Aβ42 peptide were incubated in wells containing intact heat-fixed S. Typhimurium cells. Wells were probed with anti-Aβ42 antibody and luminescence signal captured in arbitrary units (AU). Data are consistent with concentration dependent binding of S. Typhimurium cell surfaces by Aβ. F) Aβ-induced S. Typhimurium agglutinates were analyzed by transmission electron microscopy (TEM) for fibril-mediated bacterial aggregation. Micrograph shows early stage bacterial agglutinates (1 hrs) from CHO-CAB conditioned media (CHO-CAB Media). Consistent with C. albicans agglutination data, analysis reveals fibrillar structures binding and linking S. Typhimurium cells in CHO-CAB bacterial aggregates. Data points for figures D-E are presented as statistical means of 6 replicate wells ± SEM.
Supplemental Figure S4: Confirmation of increased Candida resistance among transformed host cells using three independent assays. Monolayers of naïve (H4-N) and Aβ-overexpressing transformed (H4-Aβ42) H4 host cells were prepared in the wells of 96-well culture plates. *C. albicans* were added to H4-N or H4-Aβ42 monolayers or to wells lacking host cells (Yeast Alone). Wells were incubated for 28 hrs before washing to remove unattached cells. 

**A)** Host cell viability in unfixed HCM was determined by incubation with Calcein-AM, a synthetic esterase substrate that is trapped within the cytoplasm of viable cells following intracellular hydrolysis to an impermeable fluorogenic product (*P = 0.000001*). 

**B)** To assay host cell DNA wells were fixed with paraformaldehyde, incubated with DAPI under non-permeabilizing conditions, and relative well fluorescence measured (*P = 0.00001*). 

**C)** For Candida load, wells were given a brief incubation with *Candida* (2 hrs) then trypsinized. Yeast were assayed by streaking trypsin extracts onto agar and counting CFU (*P = 0.013*). Data (panels A-C) are consistent with findings from BrdU and anti-Candida assays, with H4-Aβ42 host cells showing increased resistance to yeast infection compared to naïve controls. 

**D)** Host cell monolayer stability under our experimental conditions was characterized. Naïve (H4-N and CHO-N) and transformed (H4-Aβ40, H4-Aβ42, and CHO-CAB) host cell monolayers were prepared for infection then given sham inoculations (sterile media) before incubation (28 and 36 hours for H4 and CHO cell lines, respectively). Wells were trypsinized at the beginning (Start) and end (Finish) of incubations and host cells in trypsin extracts counted using an automated cell counter. Data confirmed plating densities of naïve and transformed host cell monolayers are closely matched and remain stable over experimental incubations in the absence of infecting *C. albicans*. Bars (panels A-D) show mean signal of six replicate wells ± SEM. Statistical significance was by t-test.
Supplemental Figure S5: Transformed cell lines generate Aβ oligomers at levels found in the soluble fraction of human brain. Levels and oligomerization of synthetic and cell-derived Aβ were characterized by ELISA, immunoblot, and size exclusion chromatography (SEC). Culture media was conditioned 36 hrs with naïve (H4-N and CHO-N) or transformed (H4-Aβ40, H4-Aβ42, and CHO-CAB) cell monolayers in 96-well culture plates. Immunodepleted (ID) transformed samples (ID-H4-Aβ40, ID-H4-Aβ42, and ID-CHO-CAB) were also prepared by incubation of conditioned media with immobilized anti-Aβ antibodies. Samples were then analyzed for A) Aβ40 and Aβ42 concentrations by amyloid-β sandwich ELISA and B) total anti-
Aβ signal by SDS-PAGE and immunoblot. ELISA data is expressed as mean (triplicate wells) ± SEM. Western blot analysis included synthetic Aβ42 control peptide (10 ng/ml) in Lane 1. ELISA and immunoblot data confirm Aβ levels in transformed media fall within the range reported for CSF and the soluble fraction of human brain. C) Synthetic Aβ monomer stocks were fractionated on preparative SEC to remove oligomeric species. Pooled monomer synthetic Aβ fractions (3-6 kDa) were then diluted into conditioned H4-N culture media. Freshly prepared synthetic Aβ40 (Aβ40 in H4-N media) and Aβ42 (Aβ42 in H4-N media) monomers in H4-N media were then resolved a second time along with conditioned media from transformed H4 (H4-Aβ40 media, H4-Aβ42 media) and CHO (CHO-CAB media) cells by analytical SEC under non-denaturing conditions. Eluate fractions were assayed for Aβ by amyloid-β sandwich ELISA. Bars show mean signal of duplicated ELISA wells ± SEM. Analytical SEC data confirm synthetic Aβ monomers freshly diluted into H4-N media retain a predominantly monomeric confirmation However, anti-Aβ signal from SEC fractions and SDS-PAGE immunoblots are consistent with previous reports of predominately non-covalent oligomeric assemblies for cell-derived Aβ. Overall, data confirm that Aβ in our cell culture model is expressed at physiological CNS levels and, like soluble Aβ in brain, is overwhelmingly oligomeric. Data also confirm monomeric confirmation for synthetic Aβ40 and Aβ42 preparations immediately prior to experimentation.
Supplemental Figure S6: Transformed H4-Aβ40 and CHO-CAB host cells resist *Candida colonization and agglutinate yeast*. Extended data for Figs. 2C and 2E comparing naïve control (H4-N and CHO-N) and transformed (H4-Aβ40 and CHO-CAB) Aβ-overexpressing HCMs for *Candida* adhesion and yeast agglutination. A) HCMs were incubated (2 hrs) with *C. albicans* in slide chambers, washed to remove unattached *Candida*, adhering yeast stained with Calcofluor White, and fluorescence signal captured. B) Micrographs of agglutinated *Candida* following overnight incubation with HCMs. Data confirm that, like H4-Aβ42 cells, Aβ-overexpressing H4-Aβ40 and CHO-CAB host cell lines show anti-adhesion and agglutinating activities against *Candida*. 
Supplemental Figure S7: Birefringence of Congo red stained yeast aggregates from H4-Aβ42 media. Bright field images of Congo red stained H4-Aβ42 Candida aggregates under normal (Unpolarized light) or polarized (polarized light) light. Aβ-induced aggregates display the hallmark apple-green birefringence under polarized light of β-amyloid.
Supplemental Figure S8: Anti-Aβ antibodies do not label CL2122 tissues or infecting yeast.

Extended data for Figs. 6A and 6D showing negative anti-Aβ immunoreactivity in control non-Aβ expressing worms (CL2122). A) TEM analysis confirms anti-Aβ immunogold nanoparticles (α-Aβ-Au) do not bind to the surface C. albicans in the intestine of control nematodes. B) By CFM, uninfected and Candida-infected CL2122 sections are negative for anti-Aβ signal. Data are consistent with Aβ-specific labeling in GMC101 nematodes.
Supplemental Figure S9: β-amyloid co-localize with S. Typhimurium cells in 5XFAD brain.

Four-week-old WT mice or transgenic 5XFAD animals expressing high levels of human Aβ were injected with viable S. Typhimurium bacteria. Brain sections were prepared 48 hrs post-infection, probed with α-Aβ-Au nanoparticles, and imaged by TEM. Inserts in lower left of micrographs are high resolution images showing α-Aβ-Au binding (black arrows). Consistent with infection-induced Aβ fibrillization and β-amyloid mediated microbial entrapment, bacterial cells in 5XFAD brain are associated with insoluble deposits labeled by α-Aβ-Au nanoparticles. Micrographs are representative of data from multiple discrete image fields.
Supplemental Video S1: Z-section projection of 5XFAD mouse brain showing β-amyloid entrapment of S. Typhimurium cells. Four-week-old 5XFAD mice were given cerebral injections of S. Typhimurium bacteria. Brains of mice with late stage infection (60 hrs post-injection) were perfused, sectioned, and probed with mAb 3D6 and anti-Salmonella antibodies. Z-series anti-Aβ (red) and anti-Salmonella (green) fluorescence signals were captured by CFM and rotating z-projections generated using image analysis software (NIS Nikon). Yellow denotes signal co-localization. Data from three replicate experiments and multiple discrete image fields (Table S1C, supplemental materials).