Title: Alzheimer's Amyloid-β is an Antimicrobial Peptide: A review of the evidence

Authors: Maya L. Gosztyla[1], Holly M. Brothers[2], Stephen R. Robinson[3]

[1]Department of Neuroscience
The Ohio State University
Columbus, Ohio, USA

[2]Department of Psychology
The Ohio State University
Columbus, Ohio, USA

[3]Discipline of Psychology,
School of Health and Biomedical Sciences
RMIT University
Bundoora, Victoria, Australia.

Author for correspondence:
Stephen R. Robinson
Email: stephen.robinson@rmit.edu.au
Tel: +613 9925-7120

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Abstract

The amyloid-β (Aβ) peptide has long been considered to be the driving force behind Alzheimer's disease (AD). However, clinical trials that have successfully reduced Aβ burden in the brain have not slowed the cognitive decline, and in some instances have resulted in adverse outcomes. While these results can be interpreted in different ways, a more nuanced picture of Aβ is emerging that takes into account the facts that the peptide is evolutionarily conserved and is present throughout life in cognitively normal individuals. Recent evidence indicates a role for Aβ as an antimicrobial peptide (AMP), a class of innate immune defense molecule that utilizes fibrillation to protect the host from a wide range of infectious agents. In humans and in animal models, infection of the brain frequently leads to increased amyloidogenic processing of the Aβ precursor protein (AβPP) and resultant fibrillary aggregates of Aβ. Evidence from *in vitro* and *in vivo* studies demonstrates that Aβ oligomers have potent, broad-spectrum antimicrobial properties by forming fibrils that entrap pathogens and disrupt cell membranes. Importantly, overexpression of Aβ confers increased resistance to infection from both bacteria and viruses. The antimicrobial role of Aβ may explain why increased rates of infection have been observed in some of the AD clinical trials that depleted Aβ. Perhaps progress towards a cure for AD will accelerate once treatment strategies begin to take into account the likely physiological functions of this enigmatic peptide.

Keywords: Bacteria, virus, plaque, bioflocculant, innate immune system, infection

Amyloid-β is linked to Alzheimer's disease

In 1906, Dr. Alois Alzheimer presented a case study for the Meeting of Southwest German Psychiatrists. His patient, Auguste D., had suffered from profound and progressive dementia, personality changes and sleep disruption. After her death at age 55, Dr. Alzheimer examined her brain and noticed severe cortical atrophy, as well as intensely-stained protein deposits in the form of intraneuronal tangles and extraneuronal plaques [1]. The latter neuropathological feature has since become the primary focus of research on Alzheimer's disease (AD). These extracellular plaques are formed from the amyloid-β (Aβ) peptide, a 40- or 42-amino acid fragment of the amyloid precursor protein (AβPP). AβPP has two possible cleavage pathways. In the non-amyloidogenic pathway, AβPP is cleaved sequentially by α- and γ-secretases to form three soluble fragments. In the amyloidogenic pathway, AβPP is first cleaved by β-secretase, followed by γ-secretase, thereby releasing the AD-associated Aβ fragment [2].

The "Amyloid cascade hypothesis" has guided much of the research in AD for the past 25 years [3]. The hypothesis posits that the deposition of Aβ plaques initiates a series of pathological events which lead to the development of AD. Accordingly, the majority of AD drug candidates entering clinical trials have focused on depleting Aβ. Yet of more than 200 compounds advanced to at least stage 2 trials in the past 30 years, only four have received FDA approval for the treatment of AD; these four show limited therapeutic benefit and none target Aβ [4].

Interestingly, a problem shared by many of the anti-Aβ clinical trials is an increased incidence of infections among the study participants. For instance, meningoencephalitis developed in approximately 6% of trial participants who received the first active immunotherapy

that was targeted against Aß, AN-1792 [5-7]. An increase in infections, specifically orolabial herpes relapse, was reported in clinical trials of the β-site AβPP cleaving enzyme 1 (BACE1)-inhibitor E2609 [8], the γ-secretase inhibitor Semagacestat [9, 10], and the Aß-binding compound ELND005 [11, 12], while trials of the γ-secretase inhibitor Tarenfurbil reported increased rates of upper respiratory infections [13]. Skin and/or gastrointestinal reactions, often associated with infections, have been frequently observed in patients treated with active and passive immunizations against Aß, inhibitors of BACE1 or γ-secretase, or Aß-binding compounds [14-19]. In patients treated with the γ-secretase inhibitor Avagacestat, the rate of infections increased in a dose-dependent manner, with 42.5% of patients in the highest dose group developing infections, compared to 21.4% in the placebo group [20]. AβPP is expressed in many of the peripheral tissues where these infections were observed, including the skin, lungs, and intestines [21].

This relationship between Aβ inhibition and susceptibility to infection raises the possibility that Aβ plays a role in immune function. Indeed, several new lines of evidence indicate that Aβ may function within the innate immune system as an antimicrobial peptide (AMP), an ancient class of peptides with potent and broad-spectrum antimicrobial activity. The evidence in support of this intriguing possibility will be reviewed in the following sections.

Amyloidogenic peptides serve several physiological roles

The tendency of Aβ to aggregate into insoluble plaques has led many to consider it as an aberrant peptide with no physiological function. However, functional amyloidogenic peptides are common in biological systems, having been found in both prokaryotic and eukaryotic life forms [22]. For instance, amyloids fulfill multiple roles in humans: in red blood cells, amyloids help to facilitate cellular adhesion and movement [23], while amelogenin, the main component of the

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enamel protein matrix in teeth, self-assembles into amyloid-like structures *in vitro* and *in vivo* [24]. At least 31 human peptide hormones are stored in an amyloid-like conformation in secretory granules of the pituitary gland [25]. Thus the amyloidogenic nature of Aβ does not exclude the possibility that it may serve a physiological function.

Aβ's ubiquity hints at the likelihood of a functional role. The sequence of Aβ is highly conserved in the AβPP orthologs of more than 70% of vertebrate species, in addition to many invertebrates [26-28]. In cognitively normal humans, Aβ is present in the brain throughout life, as well as in the cerebrospinal fluid (CSF) and blood plasma [29-31]. Naked mole rats (*Heterocephalus glaber*) are the longest-lived rodent species, yet their brains naturally possess very high concentrations of soluble Aβ, comparable to those found in the 3xTg-AD mouse model of AD [32]. The widespread presence of Aβ in diverse evolutionary taxa suggests that it is maintained by positive selection.

Attempts to deplete Aβ often have negative consequences. In rat cortical neurons, depletion of Aβ by secretase inhibition or anti-Aβ antibodies results in loss of cell viability, while co-incubation with $Aβ_{40}$, the most common Aβ isotype, rescues cells in a concentration-dependent manner, with significant protective effects being observed at concentrations as low as 10 pm [33]. Bolkan and colleagues were the first to demonstrate a functional role for Aβ *in vivo* by showing that the *Drosophila* ortholog of human BACE1, dBACE1, is necessary for glial survival. RNAi knockdown of dBACE1 in photoreceptor neurons resulted in the progressive degeneration of glia within their target zone, the lamina cortex, indicating that the AβPP β-cleavage function of BACE1 within neurons is required for survival of their target glia [34]. In humans, clinical trials of anti-Aβ therapies frequently report adverse effects. In addition to the increased rate of infections, other negative outcomes include cancers, neurovascular

disturbances, and cortical atrophy [4, 19, 35], suggesting that this peptide may serve multiple physiological functions in humans.

Infections can induce Aβ production

The identification of microbial DNA within Aβ plaques has given support to Robinson and Bishop's proposal [36, 37] that Aβ may aggregate in response to the presence of infectious agents in the brain. Wozniak and colleagues used an *in situ* polymerase chain reaction (PCR) to detect DNA of herpes simplex virus-1 (HSV-1) in brain tissue from six AD patients and five cognitively normal elderly individuals, who had all tested positive for HSV-1 infection. In the AD brains, 90% of Aβ plaques contained HSV-1 DNA and 72% of the DNA was associated with plaques. In contrast, 80% of plaques within the healthy brains contained HSV-1 DNA and 24% of the DNA was associated with plaques. The authors suggested that AD may result from higher than normal Aβ deposition in response to HSV-1 infection, either due to an overproduction of Aβ or a reduced rate of clearance [38]. Additionally, Miklossy examined three AD brains known to be infected with the spirochete *Borrelia burgdorferi* and found that the Aβ plaques colocalized with the bacterial antigen and DNA [39]. These results demonstrate that, at least in cases where infection is confirmed, a large proportion of Aβ plaques contain viral or bacterial DNA.

The association is further supported by direct causative evidence that infectious pathogens can stimulate the production of Aβ (Table 1). In human subjects, HSV-1 encephalitis is associated with reduced levels of $Aβ_{42}$ in the CSF compared to healthy controls [40]. A similar reduction is found in AD and serves as an indirect indication of enhanced $Aβ_{42}$ deposition in the brain [41]. HSV-1 infection of human and rat neuronal cultures activates the amyloidogenic pathway of AβPP processing while inhibiting Aβ degradation, leading to intracellular Aβ accumulation [42-46]. Wozniak and colleagues replicated these results *in vivo* by infecting

BALB/c mice with HSV-1 either intranasally or by ear scarification. $A\beta_{42}$ deposits were observed in temporal cortex sections five days after infection. Cell culture experiments revealed that intracellular concentrations of $A\beta$ were significantly increased as early as 24 hours post-infection and that infected cells increased their expression of both BACE-1 and nicastrin, a component of γ-secretase. An increase in these two enzymes is unusual, considering that HSV-1 infection activates double-stranded RNA-activated protein kinase (PKR), a defensive viral sensor that shuts down protein synthesis [46]. Interestingly, activation of PKR simultaneously triggers an increase in BACE1 activity, leading to an increased production of $A\beta$ [47]. Importantly, the same group later showed that antiviral treatments greatly reduce $A\beta$ accumulation in HSV-1 infected cells. Treatment with the antiviral agent Acyclovir, which inhibits replication of HSV-1, reduced the intensity of intracellular $A\beta$ staining to 28% of that in untreated infected cells, while also reducing the levels of BACE-1 and nicastrin [48]. A more potent antiviral, BAY 57-1293, further reduced $A\beta$ deposition in HSV-1 infected cells, while no intracellular $A\beta$ could be detected following treatment with a combination of the two antiviral drugs [49].

Kristen and colleagues showed that HSV-2 is also capable of altering $A\beta PP$ processing. Following exposure to HSV-2, neuroblastoma cells that overexpressed human $A\beta PP$ strongly increased their intracellular levels of $A\beta$. Infected cells displayed a decline in α-secretase activity and reduced levels of its proteolytic products, $sA\beta PP\alpha$ and α-carboxy terminal fragment (CTF). However, there were no changes in $A\beta PP$ expression or β-secretase activity, suggesting that the non-amyloidogenic pathway of $A\beta PP$ processing was selectively inhibited in response to HSV-2 infection, whereas amyloidogenic processing was unimpaired. Infected cells also showed a disruption of $A\beta$ autophagy, as evidenced by an accumulation of $A\beta$-containing autophagic compartments that failed to fuse with lysosomes [50]. A recent study suggested that

pseuodorabies virus (PRV), a member of the *herpesviridae* family related to HSV-3, may also increase Aβ deposition. In the brains of PS2-Tg2576 double transgenic mice that had been infected with PRV, the levels of insoluble $Aβ_{40}$ and $Aβ_{42}$ were 20-40 fold higher than in the brains of noninfected mice. Interestingly, the control mice (C57BL/6) also showed a significant (4-fold) increase in the brain levels of insoluble $Aβ_{42}$ in response to infection by PRV [51].

Additional studies have investigated the amyloid pathology that is associated with infection by human immunodeficiency virus (HIV). In an autopsy study of brain tissue from 162 individuals infected with HIV, who had died at an average age of 43 years, approximately half of the frontal cortices were found to contain Aβ plaques and intraneuronal inclusions of Aβ [52]. In cell culture aggregates from human AD brains, treatment with the HIV factor Tat led to increased concentrations of soluble Aβ and a reduced activity of the Aβ-degrading enzyme neprilysin [53, 54]. In a more recent study, exosomes containing mRNA and protein of the HIV Nef gene were isolated from HIV-infected humans. Neuroblastoma cells exposed to these exosomes showed increased rates of Aβ production and secretion [55]. These observations suggest that neurons respond to the presence of HIV by upregulating their expression of Aβ.

Bacteria are also capable of inducing Aβ deposition. When BALB/c mice were intranasally infected with *Chlamydia pneumoniae* that had been cultured from a human AD brain, the *C. pneumoniae* successfully infiltrated the olfactory bulb and persisted for at least three months post-infection [56]. Immunohistochemical analysis of the olfactory bulb and cerebrum revealed the presence of Aβ deposits, a subset of which were positive for thioflavin-s, an indicator of fibrillary Aβ. The number of deposits increased from an average of 7 per mouse at one month post-infection to 189 per mouse at three months post-infection [56]. However, a study attempting to replicate the above data reported key differences. Boelen and colleagues detected

Aβ deposits after infection with a respiratory isolate of *C. pneumoniae*, but failed to detect thioflavin-s-positive Aβ fibrils. They also noted that the number of deposits was substantially lower than had been observed in the previous study, with only one or two deposits per mouse [57]. A follow-up study by Little and colleagues, which used a laboratory strain of *C. pneumoniae*, reported that the number of Aβ deposits peaked at two months post-infection with an average of 60 plaques per mouse and then returned to control levels four months after infection [58]. Importantly, the strains of *C. pneumoniae* used by Boelen et al. and in the second study by Little et al. only persisted in the brain tissue for one week and one month post-infection, respectively. In contrast, the original study by Little et al. reported that the infection persisted for at least three months after the initial exposure. Thus the continuing presence of an infective agent may be necessary to maintain a high number of Aβ deposits [56].

Treponema palladium and *Borrelia burgdorferi* are spirochetal bacteria that cause neurosyphilis and Lyme neuroborreliosis, respectively. In humans, these diseases have a neuropathological course that resembles AD, including progressive dementia, cortical atrophy, neuroinflammation and Aβ deposition [59, 60]. Cultured rat neurons and glia exposed to *B. burgdorferi* or bacterial lipopolysaccharide (LPS) upregulate their AβPP expression and display morphological changes that resemble the amyloid deposits of AD after 2-8 weeks of exposure to the spirochetes [61]. The common oral bacterium *Porphyromonas gingivalis* has also been linked to Aβ deposition. AβPP-Tg mice infected by oral administration of *P. gingivalis* showed significantly increased levels of LPS in the brain, demonstrating the spread of the infection to the CNS. Infected mice had an increased load of Aβ plaques in the hippocampus, and higher concentrations of both $A\beta_{40}$ and $A\beta_{42}$ in the hippocampus and cortex. In mouse neuronal cell

cultures, exposure to LPS from *P. gingivalis* caused the cells to increase their secretion of $A\beta_{40}$ and $A\beta_{42}$ in a concentration-dependent manner [62].

Taken together, these findings demonstrate that viral or bacterial infections shift AβPP processing toward the amyloidogenic pathway, resulting in increased rates of Aβ production and deposition. Thus perturbations in Aβ levels may indicate the presence of an infection. For instance, the reduced CSF titers of $A\beta_{42}$ in both HIV [40, 63] and bacterial meningitis [40, 64] may be a consequence of increased rates of Aβ deposition in brain tissues in response to the infection. Indeed, in eight patients with acute purulent bacterial meningitis who showed reduced levels of $A\beta_{42}$ in the CSF, successful treatment of the infection resulted in a rebound to control $A\beta_{42}$ levels [64], consistent with a curtailing of Aβ deposition in the brain.

Antimicrobial properties of Aβ

The idea that Aβ may serve as a part of the innate immune system was first proposed by Robinson and Bishop in what they termed the "Bioflocculant hypothesis." They posited that Aβ's aggregative properties could make it ideal for surrounding and sequestering pathogenic agents in the brain, and by so doing it would limit the spread of the pathogen and prepare it for phagocytosis. They noted that Aβ's positive charge would be attracted to the negatively-charged membranes of microbes [36, 37]. In a recent study, Pulze and colleagues identified a similar amyloid bioflocculant in humans. Neutrophil extracellular traps (NETs), a decondensed chromatin network produced by immune cells to entrap microbial pathogens, were shown to contain amyloid fibrils as a structural backbone. Amyloid structures that could be stained with Congo red and thioflavin-s were observed in activated neutrophils, and were found to colocalize with the DNA of the extracellular NET structures. The positive charge of the NET amyloid likely assists in targeting microbes, allowing it to distinguish between negatively-charged pathogen

membranes and zwitterionic host membranes [65]. In addition to neutrophils, activated eosinophils, monocytes/macrophages and mast cells can secrete NETs, suggesting that amyloid bioflocculants could be an integral part of human innate immunity [66-68].

Many AMPs have an amyloid structure. The β-sheet structure of these amyloids can spontaneously insert into membranes, forming channels that trigger death by ion dyshomeostasis [69]. This useful property led to conservation of amyloid AMPs across broad evolutionary taxa, such as longipin in the arachnid *Acutisoma longipes* [70] and microcin E492 in the bacterium *Klebsiella pneumoniae RYC492* [71]. A number of amyloid AMPs are known to function in humans. For instance, eosinophils, a type of white blood cell important for innate immunity, utilize the amyloidogenic major basic protein-1 (MBP-1) to combat pathogens [72]. In response to infection, MBP-1 released by eosinophils rapidly aggregates at the bacterial surface, leading to agglutination that limits the infection's spread and facilitating phagocytosis by immune cells [72, 73]. MBP-1 also displays bactericidal activity by triggering lysis or other disruptions of the bacterial cell membrane. This function is largely dependent on the aggregative nature of MBP-1, as prevention of amyloid fibrillation by using antibodies that bind to β-sheet-rich oligomers improves bacterial cell viability and reduces membrane damage [72]. Similar mechanisms are utilized by other human AMPs, including thrombin-derived C-terminal fragments, protegrin-1, and semen-derived enhancer of viral infection [74-76].

If Aβ is indeed an AMP, it ought to be released by activated immune cells, as has been demonstrated by several studies. Cultures of immortalized microglial cells constitutively express Aβ and upregulate its production following exposure to bacterial LPS [77]. Primary human monocyte cultures strongly increase Aβ secretion following lipoprotein phagocytosis or LPS stimulation [78], while monocytes activated with T-cell mitogens increase AβPP transcription,

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translation and secretion [79]. Like other amyloid AMPs, Aβ spontaneously forms cation channels in cell membranes, leading to cell death by ion dyshomeostasis [80-83]. Aβ also contains a U-shaped *β-strand-turn-β-strand* motif that is highly conserved in many amyloid AMPs [69].

Several recent studies have shown that Aβ possesses antimicrobial activity against a variety of human pathogens (Table 2). Soscia and colleagues provided direct evidence that Aβ functions as an AMP. The 'minimum inhibitory concentration', defined as the "lowest concentration able to visibly inhibit growth overnight", for Aβ$_{40}$ and Aβ$_{42}$ was determined for twelve common pathogens, with the archetypal human AMP, LL-37, being used as a basis for comparison. Aβ displayed antimicrobial activity against eight of the pathogens, including gram-positive and gram-negative bacteria, and the yeast species, *Candida albicans*. For seven of these, Aβ had a potency equal to or exceeding that of LL-37. Additionally, homogenates from AD brains were shown to significantly inhibit the growth of *C. albicans* when compared to homogenates from non-demented brains. This difference was observed for the temporal lobe, where Aβ load is high in AD, but not for the cerebellum, where Aβ load is low. Within the temporal lobe homogenates, the level of *C. albicans* inhibition was significantly correlated with Aβ concentration, and the tissue's antimicrobial properties were attenuated by immunodepletion with anti-Aβ antibodies [84].

Spitzer and colleagues have confirmed the antimicrobial properties of Aβ [85]. Following incubation with Aβ$_{42}$, four bacterial species and the yeast *C. albicans* showed agglutination into large clusters, and Aβ was observed bound to the microbial surface. This effect was not observed with any other sizes of Aβ fragments. Scanning electron microscopy of

Enterococcus faecalis treated with $A\beta_{42}$ showed that the bacteria had acquired a dysmorphic shape and accumulated large amount of amorphic material between the cells. All five microorganisms exhibited reduced viability following $A\beta_{42}$ treatment, as demonstrated through flow cytometry, autofluorescence analysis, and culture plate seeding. By contrast, treatment of human THP-1 cells with identical concentrations of $A\beta_{42}$ did not result in agglutination or toxicity [85]. Spitzer and colleagues suggested that the capacity of $A\beta_{42}$ to selectively agglutinate pathogens could be due to the fact that the heparin-binding site of $A\beta$ has an affinity for the polysaccharides mannan and glucan, which are found in the cell walls of bacteria and fungi.

Other studies have expanded $A\beta$'s antimicrobial repertoire to include viruses [86]. In human primary neuronal and glial co-cultures, $A\beta$ was shown to be as effective as the antiviral Acyclovir at attenuating the pathological responses to HSV-1 infection [87]. Other groups confirmed the protective effects of $A\beta$ against HSV-1 infection in fibroblast, epithelial and neuronal cell lines [88], as well as in neuroglioma/glioblastoma co-cultures [42]. It appears that $A\beta$ interferes with the HSV-1 fusogenic protein gB, thereby preventing the virus from fusing with the plasma membrane and infecting the cell [86]. $A\beta$ is also protective against the H3N2 and H1N1 strains of influenza A virus, causing aggregation of viral particles and enhanced phagocytosis by neutrophils and macrophages [89]. Indeed, $A\beta$'s potent and broad-spectrum antimicrobial properties have prompted the suggestion that it could serve as a model for developing novel peptide antibiotics [90].

A recent study [91] confirmed $A\beta$'s *in vivo* function as an AMP. One-month-old 5XFAD mice and wild-type mice received injections of *Salmonella typhimurium* into the cerebral cortex. The 5XFAD mice, which constitutively express human $A\beta$, survived for a significantly longer period than the wild-type mice. Consistent with enhanced immunity, the 5XFAD mice also had

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lower clinical scores of encephalomyelitis progression, reduced weight loss and a lower *S.*

typhimurium load. These effects were unlikely to have been due to immunological priming, as no

significant differences were observed in the number of astrocytes or microglia in the brain, or in

the levels of ten different pro-inflammatory cytokines. In contrast, AβPP knockout mice showed

a non-significant trend toward accelerated mortality compared to the wild-type mice.

These results were mirrored for *C. albicans* infection in the nematode *Caenorhabditis*

elegans, which showed significantly reduced mortality in strains that expressed Aβ compared to

wild-type nematodes that do not express Aβ. Similarly, in human neuroblastoma cells and

Chinese hamster ovary cells exposed to *C. albicans*, the addition of picomolar concentrations of

Aβ to the culture medium enhanced cell survival and reduced fungal load, with $A\beta_{42}$ showing

greater anti-fungal potency than $A\beta_{40}$ [91]. These Aβ concentrations are comparable to those

found in the cerebrospinal fluid of healthy individuals and are below the minimum inhibitory

concentration by two orders of magnitude [84]. When cultured in medium containing Aβ, *C.*

albicans yeast cells were observed to be entrapped by Aβ fibrils. Similar entrapment also

occurred for *S. typhimurium* and *C. albicans* in mouse and nematode models, respectively [91].

The results described in this section provide compelling evidence that Aβ confers

immune resistance in living organisms, in line with the entrapment mechanism described by the

Bioflocculant hypothesis [36, 37], and they provide strong support for the idea that Aβ functions

as an AMP in the human innate immune system.

Implications for an infectious etiology of AD

The possibility that infection triggers the pathogenesis of AD has received attention for

several decades [92], and was recently the topic of an editorial coauthored by 29 prominent

neuroscientists and microbiologists [93]. An infectious etiology for AD is supported by results

from genome-wide association studies reporting that genes involved with immune regulation are associated with an increased risk of AD [94]. Notably, Apolipoprotein E (APOE), the major known genetic risk factor for sporadic AD, has been shown to influence susceptibility to viral and bacterial infections [95]. For instance, in HIV-infected individuals, APOE-ε4 homozygosity increases susceptibility to opportunistic infections and increases mortality [96]. A report by Dobson and colleagues found that the N-terminal region of APOE inhibited the infectivity of three viruses and two bacterial species [97]. This suggests that APOE may also function as an AMP, which is consistent with the protective effects of APOE against multiple types of pathogens [98]. As APOE is also present in Aβ plaques, these molecules may function together as components of the innate immune system.

A recent Next Generation Sequencing study reported that AD brains contain 5-10 fold higher bacterial reads than the brains of non-demented controls [99], and several studies from the Carrasco lab have observed a high prevalence of fungal infections in the brains and CSF of AD patients compared to none observed in non-AD patients [11, 100-104]. Meta-analyses have concluded that evidence of infection by periodontal bacteria, HSV-1, Epstein-Barr virus, spirochetes, or *C. pneumoniae* is strongly associated with increased AD risk [105-107]. Many of these pathogens have a high prevalence in the general population, and co-infection with multiple agents is common. Bu and colleagues demonstrated that increasing seropositivities of viral (HSV-1 and cytomegalovirus) and bacterial (*B. burgdorferi*, *C. pneumoniae*, and *H. pylori*) pathogens, as well as overall infectious burden, are independently associated with AD risk [108]. This was confirmed by a recent study that analyzed brain samples from 10 AD and 8 control brains. Immunohistochemical and PCR analyses revealed polymicrobial infections consisting of

bacteria and fungi in the samples. Notably, DNA of the gram-positive bacterium *Burkholderia* sp. was detected in 7/10 AD and 0/8 control samples [104].

The role of Aβ as an AMP can be interpreted in the context of a pathogen hypothesis for AD, in which a viral or bacterial infection of the brain may trigger the production, secretion and aggregation of Aβ. In some individuals a critical threshold may be surpassed, after which the rate of Aβ aggregation exceeds the capacity for clearance [109]. Thus by triggering an increase in the levels of extracellular Aβ, brain pathogens may contribute to the initiation of AD. The late age of onset of AD may reflect a declining resistance to infection and an increased permeability of the blood-brain barrier [110, 111], which would facilitate an increased rate of pathogen entry into the brain and/or a re-activation of latent infections.

The utility of antibiotic, antiviral or antifungal drugs in the treatment of AD is an area that deserves further investigation. A small study on this topic had encouraging results, as eradication of *H. pylori* in infected AD patients was associated with reduced five-year mortality [112]. The antibiotics rifampicin and doxycycline have also shown promise for preventing memory decline in animal models of AD [113, 114], although human trials indicate they have little effect after the onset of symptoms [115]. These results suggest that resolution of the primary infection may be a necessary first step prior to anti-Aβ therapy, or even as a preventative measure.

The evidence reviewed here supports the view that one of the physiological roles of Aβ is to defend the brain and other tissues from microbial invasion. In the light of Aβ's likely function as an AMP, the high incidence of infections among clinical trials that have attempted to deplete Aβ is not surprising, as by removing this AMP, the pathogens that triggered the Aβ response will be left unhindered to multiply. Perhaps better progress will be made towards a cure for AD if

future treatment strategies take into account the likely physiological functions of this enigmatic peptide.

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Conflict of Interest Statement

The authors have no conflict of interest to report.

Pathogen	Pathogen type	Effects	References
Herpes simplex virus-1	Virus	↑Aβ deposition ↑AβPP phosphorylation ↑Amyloidogenic AβPP processing ↓ Non-amyloidogenic AβPP processing ↓ Aβ degradation ↓ CSF Aβ$_{42}$	[40, 42-46, 116, 117]
Herpes simplex virus-2	Virus	↑Aβ intracellular accumulation ↓ Non-amyloidogenic AβPP processing, ↓ Aβ degradation	[50]
Pseudorabies virus (suid herpesvirus-1)	Virus	↑ Insoluble Aβ	[51]
HIV-1	Virus	↑Aβ deposition ↑AβPP expression ↑Amyloidogenic AβPP processing ↓ Aβ degradation ↓ CSF Aβ$_{42}$	[40, 53-55, 63, 118-120]
Chlamydia pneumoniae	Bacterium	↑Aβ deposition	[56-58]
Treponema palladium	Bacterium	↑Aβ deposition	[60]
Borrelia burgdorferi	Bacterium	↑Aβ deposition ↑AβPP production	[59, 121, 122]
Porphyromonas gingivalis	Bacterium	↑Aβ deposition	[62]

Table 1. Summary of infections that can alter AβPP processing and Aβ deposition.

Pathogen	Pathogen type	Activity	References
Herpes simplex virus-1	Virus	↓ Infectivity, ↓ neuropathological responses in cell lines ↓ Viral replication	[42, 87, 88]
Influenza A	Virus	↓ Infectivity in cell lines, ↑ viral aggregation, ↑ immune phagocytosis	[89]
Enterococcus faecalis	Bacterium, gram-positive	↓ Growth in culture ↑ Aggregation ↑ Cellular dysmorphic shape	[84, 85]
Escherichia coli	Bacterium, gram-negative	↓ Growth in culture ↑ Aggregation	[84, 85]
Listeria monocytogenes	Bacterium, gram-positive	↓ Growth in culture ↑ Aggregation	[84, 85]
Salmonella typhimurium	Bacterium, gram-negative	↑ Survival, ↓ infection progression, ↓ Bacterial load in Aβ overexpressing mice	[91]
Staphylococcus aureus	Bacterium, gram-positive	↓ Growth in culture ↑ Aggregation	[84, 85]
Staphylococcus epidermidis	Bacterium, gram-positive	↓ Growth in culture	[84]
Streptococcus agalactiae	Bacterium, gram-positive	↓ Growth in culture	[84]
Streptococcus pneumoniae	Bacterium, gram-positive	↓ Growth in culture	[84]
Candida albicans	Fungus	↑ Survival in Aβ expressing nematodes ↑ Survival, ↓ fungal load in Aβ overexpressing cell lines ↓ Growth in culture ↑ Aggregation	[84, 85, 91]

Table 2. Antimicrobial properties of Aβ.

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