Alternatives to amyloid for Alzheimer's disease therapies—a symposium report

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For decades, Alzheimer’s disease research has focused on amyloid as the primary pathogenic agent. This focus has driven the development of numerous amyloid-targeting therapies; however, with one possible exception, none of these therapies have been effective in preventing or delaying cognitive decline in patients, and there are no approved disease-modifying agents. It is becoming more apparent that alternative drug targets are needed to address this complex disease. An increased understanding of Alzheimer’s disease pathology has highlighted the need to target the appropriate disease pathology at the appropriate time in the disease course. Preclinical and early clinical studies have focused on targets, including inflammation, tau, vascular health, and the microbiome. This report summarizes the presentations from a New York Academy of Sciences’ one-day symposium entitled “Alzheimer’s Disease Therapeutics: Alternatives to Amyloid,” held on November 20, 2019.

Keywords: Alzheimer’s disease; amyloid; allopregnanolone; APOE; cognitive impairment; dementia; HDL; microbiome; microglia; neuroinflammation; tau; TREM2

Introduction

Alzheimer’s disease is the most common cause of dementia, afflicting approximately 5.5 million people in the United States and 24 million people worldwide. As the population ages, these numbers are expected to increase. The disease course is variable, but initial symptoms generally occur after the age of 65 with episodic memory trouble, followed by impairment in problem solving, attention, executive function, and language; and changes in behavior and personality that can include depression, delusions, and hallucinations. Death typically occurs 5–12 years from symptom onset. Biologically, Alzheimer’s disease is defined as the presence of extracellular amyloid beta (Aβ) peptide plaques, which typically develop decades before symptoms manifest, and intraneuronal accumulation of hyperphosphorylated tau (pTau) as neurofibrillary tangles (NFT) and neuropil threads. Synaptic and neuronal loss follows, leading to the symptoms described above.
Alternatives to amyloid for Alzheimer’s disease therapies

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For decades, the amyloid hypothesis has dominated Alzheimer’s disease research. The notion that extracellular accumulation and aggregation of Aβ peptide is the driving force behind Alzheimer’s disease and should therefore be the focus of drug development efforts has led to the failure of numerous anti-Aβ agents in clinical trials. With one notable possible exception, no amyloid-targeting therapy has been successful in delaying or preventing the progression of cognitive impairment in patients with symptomatic Alzheimer’s disease. The exception, aducanumab, is an experimental antiamyloid antibody in development by Biogen. In late 2019, Biogen announced that aducanumab demonstrated a positive effect on cognitive decline in one dosing group in one of two phase 3 trials after prematurely ending the trials because of futility. The company plans to submit its data to the U.S. Food and Drug Administration.

Even if aducanumab becomes the first approved antiamyloid therapy for Alzheimer’s disease, decades of failed trials indicate that Aβ may not be the ideal target for patients once they are symptomatic. While current evidence strongly supports a central role for Aβ aggregation in initiating the pathological cascade of Alzheimer’s disease, it seems that the mechanism may be less straightforward than initially anticipated. There is little correlation between amyloid deposition and degree of cognitive decline, and patterns of amyloid deposition do not correlate with patterns of hypometabolism via imaging. While amyloid accumulation is likely an upstream driver of Alzheimer’s disease pathology, downstream events like neuroinflammation and tau accumulation seem to correlate better with cognitive decline and may be the main drivers of neurodegeneration leading to dementia.

It is clear that a more comprehensive approach that considers multiple pathological processes in Alzheimer’s disease is needed. Central to identifying an appropriate target is determining the appropriate time in the disease course to modulate that target. An increased understanding of the disease pathology has the potential to pave the way for new targets, new therapies, and personalized treatment.

On November 20, 2019, experts in Alzheimer’s disease convened at the New York Academy of Sciences to discuss potential nonamyloid targets, including tau, neuroinflammation, innate immunity, and the gut microbiome.

An overview of nonamyloid targets for Alzheimer’s disease

David M. Holtzman from the Washington University in St. Louis started the meeting with a high-level overview of some of the potential targets for Alzheimer’s disease: Aβ, tau, microglia, apolipoprotein E (APOE), and sleep.

Holtzman began with a nod to Aβ. He argued that targeting Aβ early, before it begins to accumulate, or clearing Aβ deposits during the preclinical phase of disease, before symptoms manifest, might be effective strategies. Previous studies of Aβ-targeting therapies have tried to remove Aβ plaques later in the disease course, when other pathologies, such as tau accumulation and neuroinflammation, have developed and clinical manifestations have developed. Several ongoing trials are investigating antiamyloid treatments in nonsymptomatic patients who are either at high risk for developing amyloid plaques or are in the period of preclinical Alzheimer’s disease when they have amyloid plaques but they are cognitively normal. These studies include the Ante-Amyloid Prevention of Alzheimer’s Disease (A3) study, the Anti-Amyloid Treatment in Asymptomatic Alzheimer’s (A4) study, and the Dominantly Inherited Alzheimer Network Trial. The results of these trials will hopefully shed light on whether very early targeting of Aβ can delay or prevent disease progression.

Another target being actively investigated is tau. Tau, the primary component of intraneuronal NFTs, accumulates after Aβ plaques form. Imaging studies have shown that tau accumulation correlates better than amyloid plaques with both the timing and location of symptoms. Removing tau or preventing its ability to spread could be an effective strategy to delay or prevent symptoms in patients with preclinical to mild Alzheimer’s disease. An antisense oligonucleotide intended to decrease tau levels and active and passive immunization against tau are under investigation in clinical trials.

One area of research that has gained increasing attention for its role in disease progression is neuroinflammation. Many lines of evidence indicate that the innate immune response contributes to Alzheimer’s disease. Several genes related to Alzheimer’s disease, such as TREM2, APOE, and...
CD33, are highly expressed in the innate immune cells of the brain, the microglia. In a mouse model of tauopathy, eliminating microglia abrogated neurodegeneration, alluding to the impact that targeting microglia may have in affecting disease. Finding an appropriate strategy to target microglia in humans, however, will be more challenging. Some evidence suggests that activating microglial activity during the preclinical phase, during amyloid deposition, may be beneficial, while inhibiting activity may be beneficial after tau starts to accumulate. It may be possible to alter microglial activity by targeting its receptors triggering receptor expressed on myeloid cells 2 (TREM2) and CD33 or by altering lifestyle factors that can affect the innate immune system, such as sleep, exercise, and diet.

Holtzman also discussed the possibility of targeting APOE. APOE is the strongest genetic risk factor for Alzheimer’s disease. Holtzman suggested that decreasing APOE3 or 4 levels could be beneficial at multiple stages of disease. Targeting APOE3 or 4 early in disease could disrupt amyloid deposition, while targeting it later could attenuate the inflammatory response and mitigate neurodegeneration. By contrast, animal studies of amyloid deposition suggest that elevating APOE2 levels could be beneficial for that aspect of disease.

Holtzman also discussed the possibility of targeting sleep. There is a lot of evidence for a bidirectional relationship between Aβ, tau, and sleep/wake cycle. Fragmented sleep can exacerbate Aβ and tau release and increase both amyloid deposition and tau spreading, which can damage the sleep/wake regions of the brain and further disrupt sleep. Manipulating sleep not only influences acute memory and cognition but also disease pathology. In animal models, sleep deprivation increased amyloid plaques and tau spreading. How to target sleep is not yet clear; however, in mice, administration of an orexin receptor antagonist, which targets sleep-promoting neurons, also suppressed amyloid deposition. Orexin receptor antagonists are used to treat insomnia, though their effect on cognition in Alzheimer’s disease is not known.

Finally, Holtzman listed several more general strategies that do not directly target Alzheimer’s disease pathology but may address some of the consequences of neurodegeneration, such as neuroprotective agents, improving vascular health, maintaining a healthy lifestyle, and addressing risk factors, such as diabetes and obesity. He stressed that combination therapies will likely be necessary to address this complex disease.

Understanding variability in tau spreading

Bradley T. Hyman from Massachusetts General Hospital described his research in understanding why Alzheimer’s disease progresses rapidly in some patients and not in others. He focused on the spread of tau and NFTs through the brain as these features closely correlate with neuronal loss and symptoms. Tau is a microtubule-associated protein typically found along the neuronal axon. Several animal models have shown that tau can display prion-like spreading in Alzheimer’s disease in which tau escapes the axon into the extracellular space and is taken up by a neighboring neuron. Once there, it can affect endogenous tau in the receiving neuron, form NFTs, travel down the axon of the receiving neuron, and spread throughout the brain. Tau aggregation and spreading typically mirrors the progression of symptoms, beginning in the entorhinal cortex (EC), which is responsible for memory, and spreading to regions of the brain involved in executive function and language.

Hyman uses an experimental mouse model to investigate the effects of host characteristics on the rate of tau spreading in vivo. In his system, a cleavable green fluorescent protein (GFP)/tau fusion protein is transfected into the EC. Transfected neurons express both GFP and tau, whereas neurons that receive tau from pathological tau spreading express only tau.

Hyman showed that both the type of tau and host characteristics play a role in tau spreading. The tau mutant P301L, which is more likely to misfold and aggregate, propagated approximately 50–75% more quickly than wild-type tau. Age also played a key role in tau propagation. In older animals, tau propagation was faster, and tau was more likely to adopt an abnormal, unfolded conformation. Hyman stressed that while the characteristics of tau that enable it to propagate throughout the brain are important to understand, the vulnerability of certain regions of the brain to developing tau pathology is another important consideration. Hyman is currently investigating what human characteristics can affect tau spreading.
Panel discussion

The morning session concluded with a panel discussion moderated by Rudolph Tanzi with David Holtzman, Bradley Hyman, Roberta Brinton, and Marilyn Albert.

The panel emphasized the importance of targeting the appropriate pathology throughout the disease course. Targeting the initiating pathology, for example, Aβ accumulation, will likely only be effective early in the disease course, perhaps even before symptoms manifest. Brinton emphasized that the field will ultimately need a portfolio of therapies that includes combination treatments so that treatment can be tailored to a patient on the basis of their disease characteristics.

One challenge to achieving this personalized approach to treatment is the lack of biomarkers that adequately define the disease phenotype and assess risk of progression. Several longitudinal studies are underway to identify such biomarkers. Albert is involved in the BIOCARD study, which has been following over 250 cognitively normal people since 1995. She is hopeful that 25 years of biomarker data can help to identify a panel of biomarkers that predicts disease progression. Brinton described a study to identify risk factors for Alzheimer’s disease in 500 healthy women. The study showed that women who were at risk for metabolic syndrome and had the APOE gene were at an increased risk of cognitive decline. The results suggest that healthy lifestyle factors that reduce the risk of metabolic syndrome may reduce the risk for Alzheimer’s disease, even in patients with a genetic predisposition.

Additional evidence suggests that comorbidities, such as high cholesterol, may directly affect Alzheimer’s disease pathology. The panelists argued that more trials and data are not necessary to convince people to lead a healthy lifestyle to prevent Alzheimer’s disease—there are numerous reasons to promote general health. The bigger unmet need, Albert stated, is to figure out how to effectively convince people to change their behaviors. More information and data do not lead to behavior changes. Instead, more research is needed on effective strategies to affect habits and behavior.

Another key unmet need is to incorporate brain health into regular preventive care. As Tanzi pointed out, Alzheimer’s disease is not treated until patients already exhibit signs of dementia. In other chronic diseases, such as diabetes and cardiovascular disease, there is a larger focus on prevention and early treatment by monitoring biomarkers and risk factors. Holtzman noted that there are several methods to detect Alzheimer’s disease pathology before symptoms manifest, including imaging, cerebral spinal fluid (CSF), and blood tests. He is hopeful that tests will be clinically available soon to facilitate routine monitoring and assessment.

The panel also discussed how Alzheimer’s disease differs from normal aging-related memory loss. Albert explained that both human and animal studies have shown that normal aging-related memory loss is not due to neuronal loss, as in Alzheimer’s disease, but is associated with subtle changes in dendritic organization. While it is important to realize that Alzheimer’s disease is a distinct disease, it is also important to understand that there are likely different pathological mechanisms that lead to the biology and symptoms that we have termed Alzheimer’s disease. According to Hyman, people have started to talk about different strains of Alzheimer’s disease and, as we learn more about the disease mechanisms, we will likely begin to see that everyone’s disease is unique.

When asked what they are most excited about regarding the future of Alzheimer’s disease treatment, the panelists noted the upcoming results of prevention trials with anti-Aβ antibodies, the rich portfolio of investigational therapies, the potential for precision medicine, and the possibility for preclinical diagnosis and hence earlier treatment.

Targeting the immune system

Malú Gámez Tansey from the University of Florida College of Medicine discussed her work in targeting inflammation, specifically the proinflammatory cytokine tumor necrosis factor (TNF), in Alzheimer’s disease. Genetic analyses, neuroimaging studies, neurohistology, epidemiological, and most recently genome-wide association studies (GWAS) indicate that inflammation plays a central role in Alzheimer’s disease. The prevailing view of inflammation in Alzheimer’s disease is that accumulation of Aβ activates microglia, which attempt to clear the plaques. At some point, the plaques overwhelm the microglia.24 On the basis of the fact that over 60% of GWAS hits implicate immune-specific genes in risk for late-onset Alzheimer’s
Tansey proposed an alternate view wherein age-related immune dysfunction leads to dysfunctional microglia that are unable to clear plaques in Alzheimer’s disease. She suggested that similar immune dysfunction may be the precipitating mechanism for many other neurodegenerative diseases.

Somewhat paradoxically, immune system aging is associated with both immunodeficiency and increased inflammation. Impairments in the adaptive immune system coincide with an excess of proinflammatory cytokines, production of autoantibodies, and a corresponding loss of self-tolerance. Tansey speculated that age-related autoimmunity could lead the immune system to attack the misfolded and modified proteins associated with Alzheimer’s disease and perhaps other age-related neurodegenerative diseases like Parkinson’s disease.

If inflammation is a central player in Alzheimer’s disease, the question remains which components to target to prohibit pathological processes without affecting physiological processes. Recent clues have suggested that TNF may be such a target. TNF inhibitors are commonly used to treat rheumatoid arthritis (RA). Patients with RA taking TNF inhibitors have a lower incidence of Alzheimer’s disease compared with the general population, while those with RA not taking TNF inhibitors have a higher incidence.

Tansey and colleagues developed an inhibitor that selectively targets soluble TNF, and not the membrane-bound form, which is important for immunity and nerve myelination. XPro1595 is a TNF variant that does not bind TNF receptors but selectively heterotrimerizes with endogenous soluble TNF in the circulation and prevents it from engaging with its receptor and initiating signaling pathways. In mouse models, XPro1595 attenuated Alzheimer’s disease pathology by restoring synaptic function, slowing cognitive impairment, and ameliorating amyloid pathology. Tansey’s laboratory is now investigating how chronic peripheral inflammation due to lifestyle factors, such as high-calorie diets, can contribute to Alzheimer’s disease. Her laboratory recently reported that XPro1595 blocks multiple central and peripheral metabolic and inflammatory dysregulation induced by an obesogenic diet in mice. Studies are ongoing to investigate whether XPro1595 can ameliorate the harmful effects of obesogenic diets in preclinical models of Alzheimer’s disease–like pathology.

A phase 1b trial of XPro1595 is currently enrolling in Australia. Eighteen patients with mild cognitive impairment (MCI) or early Alzheimer’s disease will receive XPro1595 once weekly for 3 months. The trial is investigating whether XPro1595 can mitigate biomarkers of inflammation and will also investigate the drug’s safety profile. The results could inform the design of longer, larger trials to investigate XPro1595’s effects on clinical manifestations of Alzheimer’s disease.

Targeting microglial activity via TREM2

Marco Colonna from the Washington University in St. Louis discussed the role of the macrophage receptor TREM2 in Alzheimer’s disease. TREM2 has been shown to bind to polyanions, including phospholipids, high-density lipoprotein (HDL), low-density lipoprotein, APOE, nucleic acids, and Aβ, though it is unknown whether any of these interactions are physiologically relevant. Early studies in Colonna’s laboratory showed that TREM2 is important for survival and proliferation of myeloid cells, including microglia.

Homozygous mutations in TREM2 or TREM2-related signaling molecules result in Nasu–Hakola disease, which manifests with early onset dementia, bone cysts, and osteoporosis. While the disease pathology is very different from that of Alzheimer’s disease, the symptoms highlight the importance of TREM2 expression in the microglia.

TREM2 variants have also been identified in patients with Alzheimer’s disease. Colonna hypothesized that changes in TREM2 activity may affect the ability of the microglia to contain Aβ plaques. In mice predisposed to Aβ accumulation, TREM2 deficiency reduced the microglia’s ability to circumscribe plaques. Microglia showed decreased colocalization with Aβ deposits and proliferated near plaques compared with when TREM2 is present. Plaques in TREM2-deficient mice were less compact, correlating with axonal swelling and neurite dystrophy.

To understand how TREM2 deficiency influences microglia, Colonna’s laboratory and collaborators used single cell RNA sequencing (scRNA-seq) to assess the microglial transcriptome in mice predisposed to Aβ accumulation. These analyses identified a population of disease-associated
microglia that is dependent on TREM2 expression. These microglia show upregulation of many activation markers, including APOE, MHC class 2, SPP1, CD11c, and CST7. In TREM2-deficient mice, many activation markers are also upregulated; however, this is accompanied by downregulation of several homeostatic markers, which is not observed when TREM2 is present. Colonna surmised that microglia undergo a two-stage activation—an early stage, which is independent of TREM2, and a late stage, which is dependent on TREM2.

Colonna’s laboratory is also using scRNA-seq and single nucleus RNA sequencing (snRNA-seq) to understand the transcription patterns of other types of cells in the brain and how the TREM2 variants observed in patients with Alzheimer’s disease affect microglial gene expression in humans. He suggested that pharmacological activation of microglial receptors may be a therapeutic strategy for Alzheimer’s disease.

**An epigenomic map of Alzheimer’s disease**

Manolis Kellis of the Massachusetts Institute of Technology presented his work on integrating genetic, epigenetic, transcriptomic, and other data to understand the genetic causes of Alzheimer’s disease. While genetics promises the ability to understand the mechanism of disease, identify new drug targets, and pave the way for personalized medicine, 90% of disease-associated single nucleotide polymorphisms are in noncoding regions, providing little insight into the mechanisms involved.

Kellis’s laboratory has worked with projects like ENCODE, Roadmap Epigenomics, the Blueprint projects, and the International Human Epigenome Consortium to develop an epigenomic roadmap that describes histone modifications, DNA accessibility, DNA methylation, and gene expression patterns across diverse cell types and tissues.

Kellis now has developed epigenomic landscapes for over 900 traits and 800 cell types. Kellis uses these epigenomic maps to predict disease-relevant tissues and cell types. Using this approach, Kellis showed that genetic variants associated with Alzheimer’s disease are enriched in CD4+ monocytes, that is, microglia, but not in neurons. While the role of microglia in Alzheimer’s disease has been appreciated, Kellis’s work highlights the central role of microglia as the root of the genetic effect of Alzheimer’s disease. Kellis also showed that genetic changes occur early in the course of disease in immune cells and that immune activation precedes neuronal repression, further positioning the immune system and inflammation as a causal component of Alzheimer’s disease.

Kellis is also integrating genetic, epigenetic, and transcription data to understand disease variation across individuals. Kellis’s group has profiled genetic and epigenetic variation across approximately 750 postmortem samples from patients with Alzheimer’s disease from the ROS-MAP cohort. A good portion of the epigenome is malleable and changes with environment. These epigenetic sites may both affect and be affected by disease. Using his approach, Kellis has identified approximately 50,000 methylation sites that are determined by genetics, and therefore have a strictly unidirectional, causal effect on disease.

Finally, Kellis described his work on single-cell dissection of epigenomic and transcriptional variation using snRNA-seq in postmortem brain samples. He has evaluated 80,000 cells across 48 individuals who represent different stages. By clustering the cells based on epigenomic and transcriptional variation, Kellis identified distinct Alzheimer’s disease and non-Alzheimer’s disease subgroups within each cell type, as well as distinct cell type–specific signatures in different stages of disease.

His group is profiling other neuropsychiatric/neurodegenerative disorders and is developing multiplexed methods to scale up their approach to profile more patients and more cells.

**Promoting neuroregeneration in the Alzheimer’s brain with allopregnanolone**

Roberta D. Brinton from the University of Arizona presented her work on promoting neural regeneration using allopregnanolone to mitigate many of the dysfunctional processes seen in Alzheimer’s disease. Discovered in the early 1990s, allopregnanolone is a natural neurosteroid produced in high amounts during pregnancy that binds to the gamma-aminobutyric acid receptor and activates neural stem cells.

Preclinical studies in mice show that allopregnanolone not only increases neurogenesis but also promotes progression of cells into neurons and new neural circuits. Treatment with allopregnanolone promoted neural stem cell differentiation, generation of neuronal morphology, and activation...
of genes responsible for multiple aspects of pre- and postsynaptic membranes, as well as increases in axonal and dendritic length.

In a transgenic Alzheimer’s disease mouse model, a single administration of allopregnanolone improved learning and memory function to near normal levels within the time frame required for neural stem cells to regenerate, differentiate, and transition into the dentate gyrus.\textsuperscript{56,57}

An allopregnanolone therapy, Zulresso\textsuperscript{TM} (brenanolone), was approved by the U.S. Food and Drug Administration in 2019 for the treatment of postpartum depression. While the chemical compound is the same, Zulresso includes a dose of allopregnanolone that runs the risk of sedation and loss of consciousness.\textsuperscript{58} Brinton is investigating a lower dose that is more similar to the level naturally produced in the body during pregnancy. She has founded a company, NeuTherapeutics, to support the clinical development of allopregnanolone for patients with early stage Alzheimer’s disease and MCI.

Preclinical and early clinical studies show that allopregnanolone is safe and well tolerated, with no adverse events or amyloid-related imaging abnormalities observed to date. Brinton and colleagues have completed a 12-week phase 1b/2a study of intravenous administration of allopregnanolone in 24 subjects with MCI due to Alzheimer’s disease or early Alzheimer’s disease. The study investigated the safety profile of allopregnanolone as well as measures of cognition, brain volume, and biomarkers of response. Data from this trial are forthcoming.\textsuperscript{59}

Brinton is now conducting a bridging study to investigate the effects of intramuscular administration. In addition, a phase 2 trial of 200 patients is underway. Outcomes will include safety, cognition, magnetic resonance imaging (MRI) volumetrics, and activities of daily living. The study will also explore the predictive value of biomarkers on these endpoints.

**Targeting medial temporal lobe hyperactivity**

Marilyn Albert from Johns Hopkins University discussed her work on targeting medial temporal lobe hyperactivity to target memory dysfunction in patients with Alzheimer’s disease and MCI. The work spans decades of research, starting with functional MRI studies in the 1990s demonstrating that individuals with MCI show hyperactivity in the medial temporal lobe compared with cognitively normal individuals when performing a memory-related task. The degree of hyperactivity correlates with the rate of cognitive decline.\textsuperscript{60} By contrast, patients with Alzheimer’s disease dementia showed little to no activation in the medial temporal lobe.\textsuperscript{61,62} After several imaging studies, it has been concluded that medial temporal lobe hyperactivity changes during the course of MCI; it increases during early stages and decreases during later stages.\textsuperscript{63}

To better understand the mechanism behind this hyperactivity and whether it could be targeted to improve memory, Michela Gallagher from Johns Hopkins has studied age-related memory impairment in rodents. Decades of research has shown that while older animals show memory impairments on average compared with younger animals, there is a subgroup of older animals that do not experience age-related memory impairment.\textsuperscript{64} Albert showed that animals that experience age-related memory impairment have hyperactivity in the place cells found in the CA3 region of the hippocampus. Gallagher tested several antiepileptic medications to lower hippocampal hyperactivity. While several were successful in reducing hyperactivity, they did not improve memory. However, one agent, levetiracetam, was successful in reducing hyperactivity and improving memory, both in aged mice and in an Alzheimer’s disease transgenic mouse model.\textsuperscript{65,66} Levetiracetam is a common antiepileptic drug that is used in humans to treat seizures.\textsuperscript{67} The doses used in the animal studies and subsequent human studies were much lower than those used to treat epilepsy.

Next, Albert and colleagues wanted to see if the effects of low-dose levetiracetam in animals could be recapitulated in humans. Two small trials showed that low-dose levetiracetam improved task-related memory performance and reduced hyperactivity in the CA3 region of the hippocampus in subjects with MCI compared with placebo.\textsuperscript{62,68} A large, randomized, phase 3 clinical trial is currently enrolling to investigate the effects of an extended release formulation of low-dose levetiracetam, AGB101, in patients with MCI and amyloid positivity. The trial, which is sponsored by the National Institute on Aging and Agenebio, will follow patients for 78 weeks and investigate the treatment’s effect on dementia severity.\textsuperscript{69}
While it is still not clear why hyperactivity in the medial temporal lobe occurs in MCI or how it may contribute to Alzheimer’s disease, there are some clues. Hyperactivity correlates both with amyloid positivity and CSF pTau levels, suggesting that amyloid and/or tau may be increasing hippocampal activity. Albert also suggested that neuronal pentraxin 2 (NPTX2) may play a role. NPTX2 is a synaptic protein that helps to balance excitation and inhibition. Albert hypothesized that the lower levels of NPTX2 found in subjects with MCI can result in increased excitation, increased amyloid, and the spread of tau.

Cerebrovasculature health in Alzheimer’s disease

Cheryl L. Wellington from the University of British Columbia shared her research on understanding the role of the cerebrovasculature and lipoprotein metabolism in Alzheimer’s disease. In the highly vascularized brain, proper cerebrovasculature function is important for neuronal activity. In patients with Alzheimer’s disease, accumulation of Aβ in the cerebral arteries, known as cerebral amyloid angiopathy (CAA), can weaken cerebral blood vessels, making them more susceptible to damage, leakage, and stroke, and compromise blood flow throughout the brain. Up to 90% of patients experience some type of cerebrovascular dysfunction, including CAA, leaky blood–brain barrier (BBB), and changes in cerebral blood flow.

Wellington focused on the effects of lipoproteins in the cerebrovasculature. ApoE is one of the most important lipoprotein genes in Alzheimer’s disease. ApoE is particularly interesting lipoprotein as it is made both in the brain by astrocytes, microglia, and pericytes, and outside of the brain by the liver and tissue macrophages. Importantly, as ApoE does not cross the BBB, the “brain” and “peripheral” pools of ApoE are distinct. The role of peripheral apoE in Alzheimer’s disease has received little attention. HDL circulates in the bloodstream and is a heterogeneous lipoprotein that contains over 90 proteins, and ~6–9% of HDL particles contain liver-derived ApoE (HDL-ApoE). HDL is best known for its vasoprotective properties; however, little is known about its effect on cerebral blood vessels. Epidemiological data suggest that HDL may protect against dementia, and animal models show that low HDL levels lead to increased vascular amyloid and inflammation, while addition of that HDL can reduce CAA and soluble amyloid and brain Aβ levels.

While these studies suggest that HDL has a protective effect on cerebral vessels, key differences between mouse and human lipoprotein metabolism mean that these results need to be interpreted with caution. To understand how lipoproteins affect Aβ clearance across the arterial wall, Wellington’s group has developed a bioengineered cerebrovascular model that incorporates many of the features of cerebral vessels, including an anatomically correct vascular anatomy, functional BBB, and dynamic perfusion.

The model has revealed interactions between ApoE and HDL with respect to CAA and Aβ clearance. Introducing ApoE2 into the brain side and HDL into the peripheral side of the vessel preferentially facilitated Aβ42 transport across the vessel over Aβ40. This is consistent with the observation that Aβ40 is the predominant species in CAA. HDL also attenuated Aβ-induced monocyte binding. Wellington showed that HDL uses distinct mechanisms to attenuate Aβ accumulation and Aβ-induced monocyte binding. The former is independent of the HDL receptor scavenger receptor class B type I, while the latter is not.

Wellington stressed that, with over 90 proteins and 300 lipids, there are many forms of HDL and that the specific composition of HDL is likely important for its function. In the cardiovascular field, HDL-ApoE, which is 6–9% of total HDL, is emerging as a strong biomarker for protection against coronary heart disease. Clinically, total HDL is measured with little regard to HDL composition. New assays are being developed to measure the HDL-ApoE subfraction in clinical settings that may provide additional insight on the types of HDL that are most potent at maintaining cerebrovascular health. In addition, Wellington is hopeful that synthetic HDL therapies being developed for coronary heart disease may be leveraged in patients with Alzheimer’s disease.

Effects of the microbiome on amyloid deposition

Sangram S. Sisodia from the University of Chicago presented his research in understanding how the microbiome can influence amyloid deposition. Several lines of evidence indicate that the
gut microbiome can regulate brain function by affecting the host immune system and point to a relationship between the gut and brain in some neurological diseases. Single-cell RNA-seq analyses of microglia demonstrate that the microbiome can control the maturation, function, and morphology of microglia, that the microglia are involved in clearing amyloid deposits and, as several speakers throughout the day showed, that dysfunction in microglia can contribute to amyloid accumulation and pathology, it stands to reason that the composition of the microbiome may be involved in microglial function and ultimately amyloid deposition.

To test this, Sisodia and colleagues treated transgenic mice susceptible to Alzheimer’s disease with a combination of antibiotics that targets various types of bacteria in the microbiome. Administration of either low-dose antibiotics throughout the animals’ lifespan or short-term administration of high-dose antibiotics shortly after birth did not affect the overall amount of bacteria but had similar effects on the microbiome composition. Male mice treated with antibiotics showed a 40–60% decrease in Aβ plaque burden; however, antibiotics had no effect on plaque burden in female mice. Consistent with this, male mice treated with antibiotics showed a change in the chemokine and cytokine profile compared with vehicle-treated mice, demonstrating the impact of the microbiome on the peripheral response, and a change in microglial morphology. No changes were observed in female mice treated with antibiotics. Similar results were seen in a separate transgenic mouse model of Alzheimer’s disease. Sisodia’s group is now determining which cytokines and chemokines are important for the decrease in amyloid burden and is looking at antibiotic-dependent changes in metabolites.

Transcriptomic analysis of >500 microglial genes (M GnD gene set) in collaboration with Oleg Butofsky’s group at Harvard University showed that antibiotic treatment affected gene transcription in microglia from male, but not female, mice. Many of the genes affected were related to the TGF-β pathway and in shifting microglial from an activated to a homeostatic state.

Sisodia’s group is interested in understanding the basis for the sex-specific microbiome effects on amyloid burden. While it is unclear whether these effects are relevant to humans, it is known that women have a higher predisposition to Alzheimer’s disease.

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Competing interests

D.M.H. is cofounder of C2N Diagnostics LLC, and serves on scientific advisory boards/consults for Genentech, C2N Diagnostics, Denali, and is an inventor on a patent licensed by Washington University to C2N Diagnostics on the therapeutic use of antitau antibodies; this antitau antibody program was licensed to AbbVie. The Holtzman laboratory receives research grants from the National Institutes of Health, Cure Alzheimer’s Fund, Tau Consortium, the JPB Foundation, and C2N Diagnostics. M.G.T. is coinventor of XPro1595 and a consultant to INmune Bio Inc., the biotechnology company developing XPro1595 for neurological indications.

References

1. Mayeux, R. & Y. Stern. 2012. Epidemiology of Alzheimer disease. Cold Spring Harb. Perspect. Med. 2: a006239.
2. Long, J.M. & D.M. Holtzman. 2019. Alzheimer disease: an update on pathobiology and treatment strategies. Cell 179: 312–339.
3. Musiek, E.S. & D.M. Holtzman. 2015. Three dimensions of the amyloid hypothesis: time, space and “wingmen.” Nat. Neurosci. 18: 800–806.
4. Biogen & Eisai, Co., Ltd. 2019. Biogen plans regulatory filing for aducanumab in Alzheimer’s disease based on new analysis of larger dataset from phase 3 studies. October 22, 2019 Accessed January 5, 2020. http://investors.biogen.com/news-releases/news-release-details/biogen-plans-regulatory-filing-aducanumab-alzheimers-disease.

https://www.nyas.org/events/2019/alzheimers-disease-therapeutics-alternatives-to-amyloid-2019/
5. International Alzheimer’s and Related Dementias Research Portfolio (IADRP). The A3 study: ante-amyloid prevention of Alzheimer’s disease. Accessed December 27, 2019. https://iadrp.nia.nih.gov/project/a3-study-ante-amyloid-prevention-alzheimers-disease.

6. National Institute on Aging (NIA). Anti-amyloid treatment in asymptomatic Alzheimer’s disease (A4). Accessed December 27, 2019. https://www.nia.nih.gov/alzheimers/clinical-trials/anti-amyloid-treatment-asymptomatic-alzheimers-disease-a4.

7. Clinicaltrials.gov. Dominantly inherited Alzheimer network trial: an opportunity to prevent dementia. Accessed December 27, 2019. https://clinicaltrials.gov/ct2/show/NCT01760005.

8. Johnson, K.A., A. Schultz, R.A. Betensky, et al. 2016. Tau positron emission tomographic imaging in aging and early Alzheimer disease. Ann. Neurol. 79: 110–119.

9. Bateman, R.J., C. Xiong, T.L.S. Benzinger, et al. 2012. Clinical and biomarker changes in dominantly inherited Alzheimer’s disease. N. Engl. J. Med. 367: 795–804.

10. Sigurdsson, E.M. 2018. Tau immunotherapies for Alzheimer’s disease and related tauopathies: progress and potential pitfalls. J. Alzheimers Dis. 64: S555–S565.

11. Clinicaltrials.gov. Safety, tolerability, pharmacokinetics, and pharmacodynamics of INONIS-MAPTRx in patients with mild Alzheimer’s disease Accessed December 27, 2019. https://clinicaltrials.gov/ct2/show/NCT03186989.

12. Shi, Y., M. Manis, J. Long, et al. 2019. Microglia drive APOE-dependent neurodegeneration in a tauopathy mouse model. J. Exp. Med. 216: 2546–2561.

13. Mancuso, R., G. Fryatt, M. Cleal, et al. 2019. CSF1R inhibitor INJ-40346527 attenuates microglial proliferation and neurodegeneration in P301S mice. Brain J. Neurol. 142: 3243–3264.

14. Kang, J.-E., M.M. Lim, R.J. Bateman, et al. 2009. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science 326: 1005–1007.

15. Holth, J.K., S.K. Fritschi, C. Wang, et al. 2019. The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. Science 363: 880–884.

16. BELSOMRA® (suvorexant) C-IV | Official Website. Accessed January 5, 2020. https://www.belsomra.com/.

17. Janto, K., J.R. Prichard & S. Pusalaavydasagar. 2018. An update on dual orexin receptor antagonists and their potential role in insomnia therapeutics. J. Clin. Sleep Med. 14: 1399–1408.

18. Clavaguera, E., T. Bolmont, R.A. Crowther, et al. 2009. Transmission and spreading of tauopathy in transgenic mouse brain. Nat. Cell Biol. 11: 909–913.

19. de Calignon, A., M. Polydoro, M. Suárez-Calvet, et al. 2012. Propagation of tau pathology in a model of early Alzheimer’s disease. Neuron 73: 685–697.

20. Iba, M., J.L. Guo, J.D. McBride, et al. 2013. Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer’s-like tauopathy. J. Neurosci. 33: 1024–1037.

21. Sanders, D.W., S.K. Kaufman, S.L. DeVos, et al. 2014. Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron 82: 1271–1288.

22. Pooler, A.M., M. Polydoro, E.A. Maury, et al. 2015. Amyloid accelerates tau propagation and toxicity in a model of early Alzheimer’s disease. Acta Neuropathol. Commun. 3: 14.

23. Wegmann, S., R.E. Bennett, L. Delorme, et al. 2019. Experimental evidence for the age dependence of tau protein spread in the brain. Sci. Adv. 5: eaaw6404.

24. Shen, Z., X. Bao & R. Wang. 2018. Clinical PET imaging of microglial activation: implications for microglial therapeutics in Alzheimer’s disease. Front. Aging Neurosci. 10: 314.

25. Jansen, I.E., J.E. Savage, K. Watanabe, et al. 2019. Genomewide meta-analysis identifies new loci and functional pathways influencing Alzheimer’s disease risk. Nat. Genet. 51: 404–413.

26. Goronzy, J.J., G. Li, Z. Yang, et al. 2013. The janus head of T cell aging — autoimmunity and immunodeficiency. Front. Immunol. 4: 131.

27. Chou, R.C., M. Kane, S. Ghimire, et al. 2016. Treatment for rheumatoid arthritis and risk of Alzheimer’s disease: a nested case–control analysis. CNS Drugs 30: 1111–1120.

28. Cavanagh, C. & T.P. Wong. 2018. Preventing synaptic deficits in Alzheimer’s disease by inhibiting tumor necrosis factor alpha signaling. IBRO Rep. 4: 18–21.

29. Cavanagh, C., Y.C. Tse, H.-B. Nguyen, et al. 2016. Inhibiting tumor necrosis factor-α before amyloidosis prevents synaptic deficits in an Alzheimer’s disease model. Neurobiol. Aging 47: 41–49.

30. MacPherson, K.P., P. Sompol, G.T. Kannarakt, et al. 2017. Peripheral administration of the soluble TNF inhibitor XPro1595 modifies brain immune cell profiles, decreases beta-amyloid plaque load, and rescues impaired long-term potentiation in 5xFAD mice. Neurobiol. Dis. 102: 81–95.

31. De Sousa Rodrigues, M.E., M.C. Houser, D.I. Walker, et al. 2019. Targeting soluble tumor necrosis factor as a potential intervention to lower risk for late-onset Alzheimer’s disease associated with obesity, metabolic syndrome, and type 2 diabetes. Alzheimers Res. Ther. 12: 1.

32. Clinicaltrials.gov. A biomarker-directed study of XPro1595 in patients with mild to moderate Alzheimer’s. Accessed January 5, 2020. https://clinicaltrials.gov/ct2/show/NCT03943264.

33. Bouchon, A., J. Dietrich & M. Colonna. 2000. Cutting edge: inflammatory responses can be triggered by TREM-1, a distincttauprionstrainspropagateincellsandmiceanddefine differenttauopathies. Neuron 33: 4991–4995.

34. Otero, K., I.R. Turnbull, P.L. Poliani, et al. 2009. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and beta-catenin. Nat. Immunol. 10: 734–743.

35. Ulland, T.K., Y. Wang & M. Colonna. 2015. Regulation of microglial survival and proliferation in health and diseases. Semin. Immunol. 27: 410–415.

36. Paloneva, J., T. Manninen, G. Christman, et al. 2015. Regulation of macrophage survival and proliferation in a model of early Alzheimer’s disease. Sci. Rep. 5: 131.

37. Guerreiro, R., A. Wojtas, J. Bras, et al. 2013. TREM2 variants in Alzheimer’s disease. N. Engl. J. Med. 368: 117–127.

38. Jonsson, T., H. Stefansson, S. Steinberg, et al. 2013. Variant of TREM2 associated with the risk of Alzheimer’s disease. N. Engl. J. Med. 368: 107–116.
Alternatives to amyloid for Alzheimer's disease therapies

39. Wang, Y., M. Cell, K. Mallinson, et al. 2015. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell 160: 1061–1071.

40. Wang, Y., T.K. Ulland, J.D. Ulrich, et al. 2016. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. J. Exp. Med. 213: 667–675.

41. Keren-Shaul, H., A. Spinrad, A. Weiner, et al. 2017. A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169: 1276–1290.e17.

42. Zhou, Y., W.M. Song, P.S. Andhey, et al. 2020. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer’s disease. Nat. Med. 26: 131–142.

43. Adsera, C.B., Y.P. Park, W. Meuleman, et al. 2019. Integrative analysis of 10,000 epigenomic maps across 800 samples for regulatory genomics and disease dissection. Cold Spring Harb. Lab. https://doi.org/10.1101/810291.

44. Ernst, J. & M. Kellis. 2015. Large-scale imputation of epigenomic datasets for systematic annotation of diverse human tissues. Nat. Biotechnol. 33: 364–376.

45. Gjoneska, E., A.R. Pfenning, H. Mathys, et al. 2015. Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease. Nature 518: 365–369.

46. Chibnik, L.B., L. Yu, M.L. Eaton, et al. 2015. Alzheimer's loci: epigenetic associations and interaction with genetic factors. Ann. Clin. Transl. Neurol. 2: 636–647.

47. Mathys, H., J. Davila-Velderrain, Z. Peng, et al. 2019. Single-cell transcriptomic analysis of Alzheimer's disease. Nature 570: 332–337.

48. Irwin, R.W. & R.D. Brinton. 2014. Allopregnanolone as regenerative therapeutic for Alzheimer's disease: translational development and clinical promise. Prog. Neurobiol. 113: 40–55.

49. Irwin, R.W., C.M. Solinsky, C.M. Loya, et al. 2015. Allopregnanolone preclinical acute pharmacokinetic and pharmacodynamic studies to predict tolerability and efficacy for Alzheimer's disease. PloS One 10: e0128313.

50. Irwin, R.W., C.M. Solinsky & R.D. Brinton. 2014. Frontiers in therapeutic development of allopregnanolone for Alzheimer's disease and other neurological disorders. Front. Cell. Neurosci. 8: 203.

51. Masiulis, S., R. Desai, T. Uchański, et al. 2019. GABAA receptor signalling mechanisms revealed by structural pharmacology. Nature 565: 454–459.

52. Chen, Z.-W., J.R. Bracamontes, M.M. Budelier, et al. 2019. Multiple functional neurotransmitter binding sites on GABAA receptors. PloS Biol. 17: e3000157.

53. Chen, S., J.M. Wang, R.W. Irwin, et al. 2011. Allopregnanolone promotes regeneration and reduces β-amyloid burden in a preclinical model of Alzheimer's disease. PloS One 6: e24293.

54. Wang, J.M., P.B. Johnston, B.G. Ball, et al. 2005. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. J. Neurosci. 25: 4706–4718.

55. Wang, J.M., C. Singh, L. Liu, et al. 2010. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer’s disease. Proc. Natl. Acad. Sci. USA 107: 6498–6503.

56. Brinton, R.D. 2013. Neurosteroids as regenerative agents in the brain: therapeutic implications. Nat. Rev. Endocrinol. 9: 241–250.

57. Singh, C., L. Liu, J.M. Wang, et al. 2012. Allopregnanolone restores hippocampal-dependent learning and memory and neural progenitor survival in aging 3xTgAD and nonTg mice. Neurobiol. Aging 33: 1493–1506.

58. Sage Therapeutics, Inc. 2019. ZulressoTM (brexanolone) prescribing information 2019. Accessed January 1, 2020. https://assets.sagerx.com/zulresso/prescribing-information.pdf.

59. Clinicaltrials.gov. Allopregnanolone for mild cognitive impairment due to Alzheimer's disease or mild AD. Accessed January 1, 2020. https://clinicaltrials.gov/ct2/show/NCT02221622.

60. Miller, S.L., E. Fenstermacher, J. Bates, et al. 2008. Hippocampal activation in adults with mild cognitive impairment predicts subsequent cognitive decline. J. Neurol. Neurosurg. Psychiatry 79: 630–635.

61. Dickerson, B.C., D.H. Salat, D.N. Greve, et al. 2005. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology 65: 404–411.

62. Bakker, A., M.S. Albert, G. Krauss, et al. 2015. Response of the medial temporal lobe network in amnestic mild cognitive impairment to therapeutic intervention assessed by fMRI and memory task performance. Neuroimage Clin. 7: 688–698.

63. Pasquini, L., F. Rahmani, S. Maleki-Balajoo, et al. Medial temporal lobe disconnection and hyperexcitability across Alzheimer’s disease stages. J. Alzheimers Dis. Rep. 3: 103–112.

64. Wilson, I.A., M. Gallagher, H. Eichenbaum, et al. 2006. Neurocognitive aging: prior memories hinder new hippocampal encoding. Trends Neurosci. 29: 662–670.

65. Koh, M.T., R.P. Haberman, S. Foti, et al. 2010. Treatment strategies targeting excess hippocampal activity benefit aged rats with cognitive impairment. Neuropsychopharmacology 35: 1016–1025.

66. Sanchez, P.E., L. Zhu, L. Verret, et al. 2012. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer’s disease model. Proc. Natl. Acad. Sci. USA 109: E2895–E2903.

67. Levetiracetam. Epilepsy foundation. Accessed January 1, 2020. https://www.epilepsy.com/medications/levetiracetam.

68. Bakker, A., G.L. Krauss, M.S. Albert, et al. 2012. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74: 467–474.

69. Clinicaltrials.gov. Study of AGB101 in mild cognitive impairment due to Alzheimer’s disease. Accessed January 1, 2020. https://clinicaltrials.gov/ct2/show/NCT03486938.

70. Huijbers, W., E.C. Mormino, A.P. Schultz, et al. 2015. Amyloid-β deposition in mild cognitive impairment is associated with increased hippocampal activity, atrophy and clinical progression. Brain J. Neurol. 138: 1023–1035.

71. Maass, A., D. Berron, T.M. Harrison, et al. 2019. Alzheimer's pathology targets distinct memory networks in the ageing brain. Brain J. Neurol. 142: 2492–2509.
1. Alternatives to amyloid for Alzheimer’s disease therapies

Cable et al.

72. Attems, J. & K.A. Jellinger. 2014. The overlap between vascular disease and Alzheimer’s disease—lessons from pathology. BMC Med. 12: 206.

73. Gottesman, R.F., T.H. Mosley, D.S. Knopman, et al. 2019. Association of intracranial atherosclerotic disease with brain β-amyloid deposition: secondary analysis of the ARIC study. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2019.4339.

74. Nation, D.A., M.D. Sweeney, A. Montagne, et al. 2019. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat. Med. 25: 270–276.

75. Iturria-Medina, Y., R.C. Sotero, P.J. Toussaint, et al. 2016. Early role of vascular dysregulation on late-onset Alzheimer’s disease based on multifactorial data-driven analysis. Nat. Commun. 7: 11934.

76. Singh-Manoux, A., D. Gimeno, M. Kivimaki, et al. 2008. Low HDL cholesterol is a risk factor for deficit and decline in memory in midlife: the Whitehall II study. Arterioscler. Thromb. Vasc. Biol. 28: 1556–1562.

77. Kuriyama, M., K. Takahashi, T. Yamano, et al. 1994. Low levels of serum apolipoprotein A-I and A II in senile dementia. Jpn. J. Psychiatry Neurol. 48: 589–593.

78. Reitz, C., M.-X. Tang, N. Schupf, et al. 2016. Association of higher levels of high-density lipoprotein cholesterol in elderly individuals and lower risk of late-onset Alzheimer disease. Arch. Neurol. 67: 1491–1497.

79. Bates, K.A., H.R. Sohrabi, S.R. Rainey-Smith, et al. 2017. Serum high-density lipoprotein is associated with better cognitive function in a cross-sectional study of aging women. Int. J. Neurosci. 127: 243–252.

80. van Exel, E., A.J.M. de Craen, J. Gussekloo, et al. 2002. Association between high-density lipoprotein and cognitive impairment in the oldest old. Ann. Neurol. 51: 716–721.

81. Lefterov, I., N.F. Fitz, A.A. Cronican, et al. 2010. Apolipoprotein A-I deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1DeltaE9 mice. J. Biol. Chem. 285: 36945–36957.

82. Robert, J., E.B. Button, B. Yuen, et al. 2017. Clearance of beta-amyloid is facilitated by apolipoprotein E and circulating high-density lipoproteins in bioengineered human vessels. eLife 6. https://doi.org/10.7554/eLife.29595.

83. Robert, J., E.B. Button, S. Stukas, et al. 2017. High-density lipoproteins suppress Αβ-induced PBMC adhesion to human endothelial cells in bioengineered vessels and in monoculture. Mol. Neurodegener. 12: 60.

84. Morton, A.M., M. Koch, C.O. Mendivil, et al. 2018. Apolipoproteins E and CIII interact to regulate HDL metabolism and coronary heart disease risk. JCI Insight 3. https://doi.org/10.1172/jci.insight.98045.

85. Burgess, J.N., A.B. Pant, L.H. Kasper, et al. 2017. CD4+ T cells from multiple sclerosis patients respond to a commensal-derived antigen. Ann. Clin. Transl. Neurol. 4: 825–829.

86. Wang, Y., S. Begum-Haque, K.M. Telesford, et al. 2014. A commensal bacterial product elicits and modulates migratory capacity of CD39(+)-CD4 T regulatory subsets in the suppression of neuroinflammation. Gut Microbes 5: 552–561.

87. Burgess, J.N., A.B. Pant, L.H. Kasper, et al. 2016. Reconstituted microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat. Med. 22: 516–523.

88. Sampson, T.R., J.W. Debelius, T. Thron, et al. 2016. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell 167: 1469–1480.e12.

89. Erny, D., A.L. Hrabe de Angelis, D. Jaitin, et al. 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18: 965–977.

90. Minter, M.R., C. Zhang, V. Leone, et al. 2016. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer’s disease. Sci. Rep. 6: 30028.

91. Minter, M.R., R. Hinterleitner, M. Meisel, et al. 2017. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1ΔE9 murine model of Alzheimer’s disease. Sci. Rep. 7: 10411.

92. Dodiya, H.B., T. Kuntz, S.M. Shaik, et al. 2019. Sex-specific effects of microbiome perturbations on cerebral Αβ amyloidosis and microglia phenotypes. J. Exp. Med. 216: 1542–1560.

93. Fisher, D.W., D.A. Bennett & H. Dong. 2018. Sexual dimorphism in predisposition to Alzheimer’s disease. Neurobiol. Aging 70: 308–324.