Brain, Behavior, & Immunity - Health 22 (2022) 100462

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Infection and inflammation: New perspectives on Alzheimer's disease

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ARTICLE INFO

Keywords:
Alzheimer's
Inflammation
Infection
Microglia
Inflammasome
Microbe
Fungi
Microbiome
Metabolism

ABSTRACT

Neuroinflammation has been recognized as a component of Alzheimer's Disease (AD) pathology since the original descriptions by Alois Alzheimer and a role for infections in AD pathogenesis has long been hypothesized. More recently, this hypothesis has gained strength as human genetics and experimental data suggest key roles for inflammatory cells in AD pathogenesis. To review this topic, Duke/University of North Carolina (Duke/UNC) Alzheimer's Disease Research Center hosted a virtual symposium: "Infection and Inflammation: New Perspectives on Alzheimer's Disease (AD)." Participants considered current evidence for and against the hypothesis that AD could be caused or exacerbated by infection or commensal microbes. Discussion focused on connecting microglial transcriptional states to functional states, mouse models that better mimic human immunity, the potential involvement of inflammasome signaling, metabolic alterations, self-reactive T cells, gut microbes and fungal infections, and lessons learned from Covid-19 patients with neurologic symptoms. The content presented in the symposium, and major topics raised in discussions are reviewed in this summary of the proceedings.

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https://doi.org/10.1016/j.bbih.2022.100462
Received 3 April 2022; Accepted 10 April 2022
Available online 22 April 2022

Table 1
Critical gaps in our knowledge regarding the potential role of infection or microbes in Alzheimer's disease.

Topic Areas	Knowledge Gaps
Microglia	What are the triggers for microglial state changes? How does microglial *function* differ across various microglial states that have been defined morphologically or by transcriptomic signatures? How do microglial dynamics change with age, or in the setting of chronic inflammation? Which microglial pathways and signals, when manipulated, alter disease-specific outcomes? Are there unique micro(glia) clusters and/or subtypes that preferentially drive processes of neurodegeneration after infection?
Microbiome, Autoimmunity, and Inflammasome	Can generalized inflammation or immune system activation trigger processes that initiate, unmask or worsen neurodegenerative disease? Do sex-specific differences in microbiome drive sex-based differences in AD development and progression? How does the "mycobiome" (fungal) contribute to AD risk? Does "trained immunity" (innate immune memory) contribute to CNS inflammation and AD risk? Do T cell-neuron interactions play a role in the development of AD pathology? How are APOE status and neuronal MHC expression involved in interactions between neurons and T cells? Are specific inflammasome-mediating agents, such as NLRP3 inhibitors or anti-IL1β antibodies or IL1Ra, potential therapeutics for Alzheimer's disease? Does modulation of the human gut microbiome alter AD risk or progression?
Brain metabolism, amyloid, and tau	Do AD-associated changes in brain metabolism precede or follow neuroinflammation, or is the relationship bidirectional? What is the role of microbes in triggering changes in brain metabolism or amyloid production or removal? Does amyloid play an antimicrobial role in human brains? Does neuroinflammation, microglial activation, or microbe exposure alter the progression from amyloid accumulation to tauopathy in AD?
Specific pathogens and AD risk	Does COVID-19 infection impact AD risk or progression? If there are specific pathogens involved in AD development or risk, does the timing, severity, location, or duration of exposure matter? Can meta-scale metabolomics and metagenomic analyses identify individual microbial species, microbial communities, or microbial metabolites that play a role in AD? Even if pathogen exposure is not needed to cause AD pathology, is it possible that some pathogens trigger brain changes (e.g., memory cell formation, complement activation, loss of synapses) that impede resilience to AD, thus worsening cognitive symptoms?
Blood-brain barrier	How might blood-brain barrier dysfunction and neuronal pathways lead to neuroinflammation and cognitive decline? Are there more vulnerable areas of the brain that facilitate microbial entry or other pathologic features following infection, in the context of aging and neurodegeneration? What are the limitations of plasma biomarkers (NfL, sTREM2, S100b etc) as indicators of barrier damage and CNS pathology, and are there more specific approaches that could be developed?

AD = Alzheimer's disease; APOE = apolipoprotein e; CNS = central nervous system; COVID-19 = coronavirus disease of 2019; IL = interleukin; IL1R = interleukin 1 receptor; MHC = major histocompatibility complex; Nfl = neurofilament light chain; NLRP3 = NLR family pyrin domain containing 3; sTREM2 = soluble triggering receptor expressed on myeloid cells 2; s100b = soluble astrocyte marker 100b.

Table 2
Resources needed to advance science on the potential role of infection or microbes in Alzheimer's disease.

Fields of Study	Resource Needs
Genomic/ Proteomic	Human brain tissue linked to data on pathogen exposure Signatures associated with prior infection or exposure (rather than relying on detection of active infection or presence of microbes) Comprehensive libraries of self and non-self antigens and antibodies
Model Systems	Animal models that mimic the immune systems and functions, neurodegenerative patterns, and microbial susceptibility of humans Validated measures in iPSC models of specific brain cell functions that have been linked to AD or neurodegeneration Biomarkers that non-invasively track disease-associated states of microglia, neuronal dysfunction, and blood-brain barrier permeability
Population-based studies	Biological repositories linked with clinical data from diverse populations Longitudinal cohort studies to track changes in microbiome or antibody/antigen profile over the lifespan, linked to disease-specific outcomes Dementia-relevant endpoints in ongoing studies of people who have been exposed to a pathogen (e.g. longitudinal studies of COVID-19 survivors)

AD = Alzheimer's disease; COVID-19 = coronavirus disease of 2019; iPSC = induced pluripotent stem cells.

1. Introduction

This article summarizes topics reviewed at the virtual symposium, "Infection and Inflammation: New Perspectives on Alzheimer's Disease," hosted May 27, 2021 by Duke Health and the Duke/UNC Alzheimer's Disease Research Collaborative. Though ample evidence implicates neuroinflammation and microglia, the brain's resident immune cells, in Alzheimer's disease (AD), an intriguing question is whether microbial infection or exposure could play a causative role in disease onset or progression.

The objective of this "think-tank" style symposium was to review the current state of science on this topic, consider gaps and high-priority questions, and to propose actionable hypotheses and ways to test them.

Once a fringe theory, the possibility of microbial involvement in AD has garnered increasing research interest in recent decades. The number of publications on this topic rose from 601 papers between 1991 and 2000 to 3138 papers between 2011 and 2020, according to a PubMed search for "Alzheimer AND infection." The virtual symposium drew 650 registered participants representing 262 institutions in the United States and internationally. Following an opening session with two introductory lectures, the symposium featured three topic sessions that each included brief (10-min) lectures by leading experts. Lectures were intended to highlight compelling findings or resources available to advance knowledge about the potential role of infection or microbes in AD. Large group discussions and break-out sessions elucidated key questions and needed resources, as summarized in Table 1 and Table 2. Table 3 defines acronyms and abbreviations that were used throughout the presentations and discussion.

2. Introductory lectures by Rudolph Tanzi and alison goate

2.1. The antimicrobial protection hypothesis of Alzheimer's disease

An infectious etiology for AD was first proposed around the same time the disease was initially described by German psychiatrist Alois Alzheimer in 1906. It wasn't until the 1980s that researchers isolated the

Table 3
Key for abbreviations and acronyms.

Acronym/ Abbreviation	Complete Name or explanation
5XFAD	Five familial Alzheimer's Disease mutation (a common mouse model used in AD studies)
Aβ	Amyloid beta
AD	Alzheimer's disease
AG	Arginase
APP	Amyloid Precursor Protein
APPSWE/PS1ΔE9	A transgenic mouse model for studying AD
BZLF1	*Bam*HI Z fragment leftward open reading frame 1 (name of a viral gene/protein of the Epstein-Barr virus)
CD33	Cluster differentiation 33 (a transmembrane marker on myeloid cells)
CNS	Central nervous system
CreERT2	Cre recombinase (Cre) fused to a mutant estrogen ligand-binding domain (ERT2), which can be activated by tamoxifen
CVN-AD	A transgenic mouse model of AD
Covid-19	Coronavirus disease of 2019
CSF	Cerebrospinal fluid
Cx3cR1	C-X3-C Motif Chemokine Receptor 1
GLIPH	Grouping of lymphocyte interactions by partope hotspots
GWAS	Genome wide association studies
HSV	Herpes simplex virus
IFN	Interferon
IL	Interleukin
iMGLs	Induced microglial-like cells
MCC950	An NLRP3 inflammasome inhibitor
MHC 1	Major histocompatibility complex 1
MS	Multiple sclerosis
NLRP3	NLR family pyrin domain containing 3 (name of a protein or gene involved in innate immunity)
NOS	Nitric oxide synthase
OLT1177	An NLRP3 inflammasome inhibitor
OSM	Oncostatin M
PASC	Post-acute sequalae of COVID-19
Pro-IL1	Precursor to interleukin 1
PU.1	A transcription factor protein that binds to a purine-rich sequence called the PU-box
Spl1	Gene that codes for the protein PU.1
TCR	T cell receptor
TdTomato	A brightly red fluorescent protein
Th17 cells	T helper 17 cells
TMEM119	Transmembrane protein 119
TREM2	Triggering receptor expressed on myeloid cells 2 (a transmembrane marker mainly on microglia)
SARS-Cov-2	Severe acute respiratory syndrome coronavirus 2 (virus causing COVID-19)

hallmark pathologies in the brains of people with Alzheimer's — amyloid-β (Aβ) plaques and neurofibrillary tau tangles. In 1987, several labs cloned the gene encoding amyloid precursor protein (APP) and shortly thereafter, scientists identified mutations in APP and other genes, such as presenilin, that cause familial Alzheimer's disease. Those discoveries pointed to amyloid as the initial trigger of disease and prompted research into whether amyloid deposition could lead to tau hyperphosphorylation and tangle formation(Makin, 2018). Dr. Tanzi's lecture highlighted the observed progression of AD pathology from amyloid deposition to tau tangles to activation of immune processes in the brain and posed the question of whether microbe exposure may be implicated at two points in this chain of events. First, it is plausible that microbes or pathogens may play a role in causing or exacerbating the stimulation of the brain's immune system. Second, Dr. Tanzi outlined evidence for an antimicrobial function of amyloid beta, raising the possibility that microbes could be an early trigger for amyloid accumulation.

Most mouse models mimic Alzheimer's-like amyloid accumulation in the brain but do not develop tangles. However, in neural 3-dimensional cell culture models, amyloid-β does induce formation of neurofibrillary tangles from endogenous tau protein(Choi et al., 2014). Further studies in 3-dimensional models showed that higher Aβ42:40 ratios drive tangle formation(Kwak et al., 2020), and it is known that pharmacologic inhibition of Aβ42 with gamma secretase modulators decreases tau pathology.

The Aβ42:40 ratio also increases with age, and in most AD-associated genetic mutations. It is well accepted that amyloid and tau pathology appear in the brain decades before a person develops symptoms of Alzheimer's disease (Long and Holtzman, 2019).

While early-onset familial AD genes implicate amyloid in the early stages of disease, more recent genome-wide association studies suggest that synaptic function, microglia, and innate immune processes are also important in the disease process(Bertram and Tanzi, 2019; Prokopenko et al., 2021). Brain imaging studies support a progression of amyloid accumulation to tau accumulation to immune activation, though some individuals can accumulate substantial amounts of Aβ and tau pathology without developing dementia (Sperling et al., 2019).

Experiments in AD mouse models also align with the idea that the innate immune system plays a role as the disease progresses. When CD33, an inhibitory myeloid cell receptor, is knocked out in 5xFAD mice, Aβ plaque burden decreases and cognition improves(Griciuc et al., 2019). However, the opposite effect is seen in 5xFAD mice that lack TREM2, a protein that activates myeloid cells(Griciuc et al., 2019). These findings suggest that some degree of myeloid activation is adaptive and potentially neuroprotective.

Together, the observations in human brain scans and mice suggest that plaques and tangles can initiate the disease process but downstream events — namely, cytokine release and inflammation — may be required to kill neurons and induce symptoms.

As scientists puzzled over the innate immune involvement, the late Robert Moir worked with Rudolph Tanzi and colleagues on a set of experiments that support the idea that amyloid-β acts as an antimicrobial peptide. Their studies show that *Salmonella enterica* bacteria and herpes viruses can rapidly trigger seeding of Aβ into oligomers and fibrils (Eimer et al., 2018), and that Aβ protects against fungal and bacterial infections in mouse, worm and cell culture AD models (Kumar et al., 2016). Furthermore, herpes virus-infected neurons go on to form tau tangles and release pro-inflammatory cytokines (unpublished).

One caveat with those experiments is that *Salmonella* and herpes infections were performed in 5xFAD mice, an AD model that expresses whopping amounts of Aβ. Ongoing experiments are testing whether these pathogens can seed Aβ oligomers and fibrils in human APP knock-in mice with more physiological levels of amyloid.

Meanwhile, the findings in 5xFAD mice have prompted further investigation into which microbes, if any, could be driving AD pathology. Thus far, unpublished metagenomic analyses of tissue samples from human brain banks have detected small increases in the levels of three periodontal bacterial species but no significant differences in the levels of any virus, including herpes simplex virus 1 (HSV1), in AD versus control brains.

2.2. *Alzheimer's disease genetics implicate efferocytosis in microglia*

Genomewide association studies (GWAS) conducted in European populations since the 1990s have identified 40 loci associated with AD risk (Kunkle et al., 2019). However, this does not equate to 40 AD genes. Identifying causal variants requires susceptibility loci to be mapped to specific genes through functional genomics studies. Nott et al. analyzed chromatin and promoter activity in cell nuclei from human brains and found that unlike psychiatric disorders whose gene loci show broad effects in multiple cell types, AD risk variants are specifically enriched in microglial enhancers (Nott et al., 2019).

A set of recent studies implicate dysfunction of the endolysosomal system in myeloid cells (not neurons) in the etiology of AD(Novikova et al., 2021). The endolysosomal system is important in endocytosis, phagocytosis, and efferocytosis (the process by which apoptotic cells are phagocytosed and cleared)(Hipolito et al., 2019; Martin et al., 2014). Dr. Goate's team has combined information gained from GWAS about AD risk loci, which often code enhancer proteins in monocytes, macrophages and microglia, with analysis of myeloid epigenomic and

transcriptomic datasets in order to nominate candidate genes that were the likely targets of the enhancers. The objective of this work is to shed light on the causal pathways associated with AD genetic susceptibility, an approach that could be important in future work to elucidate the potential role of microbe exposure in immune activation or phagocytosis/efferocytosis in AD.

One protective allele associates with delayed AD onset and lower expression of the gene *SPI1*, which encodes PU.1, a transcription factor critical for myeloid cell development and function. Huang et al. modulated PU.1 expression in BV2 mouse microglial cells and showed this correspondingly affected the expression of other AD risk genes with PU.1 binding sites (Huang et al., 2017). Furthermore, PU.1 genetic knockdown shares a transcriptional signature with disease-associated microglia.

Another set of experiments analyzed amyloid pathology in 5xFAD mice with two *SPI1* copies or a single copy of *SPI1* expressed in microglia. In these mice, lower SPI1 seems to correlate with more compact plaques and greater recruitment of microglia to plaques (P. Ayata & A. Schaefer, unpublished).

All told, studies that integrate AD GWAS data with myeloid epigenomic annotations, chromatin interactions and expression of quantitative trait loci point toward microglial-mediated efferocytosis — the process by which apoptotic cells are removed by phagocytic cells — as a causal pathway (Novikova et al., 2021).

3. Session 1: role of the CNS resident immune response in Alzheimer's disease

3.1. Speakers: beth stevens, carol colton, Joseph El Khoury

3.1.1. Mapping microglia states and (Dys)function in Alzheimer's disease

Human genetic analyses implicate microglia in AD and other neurodegenerative diseases, but these immune cells have diverse roles in the brain that change dynamically in health and disease(Salter and Stevens, 2017). For example, under various circumstances microglia can become more inflammatory or more phagocytic. Moreover, inflammation can be beneficial or detrimental, depending on context. The same is true for phagocytosis: clearing Aβ plaques or dead cells is generally helpful, yet clearing healthy synapses is harmful.

Using transcriptional single-cell sorting to map immune populations in wild-type and AD transgenic mouse brains, Keren-Shaul et al. identified a novel subtype of disease-associated microglia (DAM) (Keren--Shaul et al., 2017). Using genes that denote this state, researchers can localize DAM using *in situ* hybridization and other approaches. An important challenge is connecting these morphological and transcriptional states to specific functions. This ongoing work will be critical for understanding how to therapeutically target specific disease-relevant subsets of microglia.

To model DAM and other microglial states, Stevens and colleagues are growing human induced microglial-like cells (iMGLs) in monocultures and exposing them to brain-relevant challenges such as apoptotic neurons, amyloid or myelin debris. Their early data suggest that TREM2-deficient iMGLs have impaired phagocytosis and DAM production (unpublished). This model system can also be used to read out inflammation, migration and other microglial functions — in hopes of identifying new biomarkers to track specific disease-associated states.

3.1.2. Role of innate immune system and neuroinflammation in influencing brain metabolism in AD

The brain contains a specialized tissue environment whose amino acid contents differ from plasma and other tissue compartments due largely to the blood brain barrier. Early studies using mass spectrometry showed that metabolism of the amino acid arginine is significantly altered in AD brains (Bergin et al., 2018; Liu et al., 2014). Arginine metabolism is associated with the activity of two critical immune-regulated enzymes — nitric oxide synthase 2 (NOS2) and arginase 1,2 (AG1,2), and brains of people with AD exhibit lower NOS activity and higher arginase activity and higher levels of arginine in some regions, compared to age-matched controls(Liu et al., 2014). Growing evidence supports a metabolic role for innate immune activation in AD whereby NOS2 and AG1,2 compete for arginine in response to AD-mediated activation of immunity, thereby altering the profile of critical metabolites in vulnerable brain regions (Badea et al., 2019).

To better emulate human (rather than rodent) immune responses, Colton and colleagues have created an AD mouse model (CVN-AD) that expresses the human amyloid beta protein precursor in mice that lack mouse NOS2 and/or express only human NOS2 (Bryan et al., 2021; Colton et al., 2014; Hoos et al., 2013; Kan et al., 2015). Proteomic analyses show that metabolic pathways in mouse strains using this approach align well with pathological changes in human AD brains (Badea et al., 2016; Bryan et al., 2021; Hoos et al., 2013). Future pre-clinical work that investigates the potential role of microbes or pathogens in AD pathogenesis may benefit from the use of animal models with "humanized" immune or metabolic systems.

To better understand how arginine is used in the brain in CVN-AD mice and the potential impact of immune-regulated metabolic shifts, a metabolite tracing protocol has been developed using heavy labelled isotopes (Adams et al., 2021). This analysis, which detects metabolic shifts caused by the disease process, has confirmed that the AD phenotypes in the brains of CVN-AD mice are associated with high levels of arginine flux through an arginase 1-related pathway (unpublished data). Dr. Colton's team is designing studies to interrogate changes in other metabolic pathways that are interconnected to arginase activation, including methylation-based pathways that may also play key roles in the brain's metabolic outcomes in people with AD.

3.1.3. Novel animal model in the study of microglia and HSV-6

Using Transmembrane protein 119 (Tmem119) — a protein highly expressed in microglia of the brain but not of the periphery — Joseph El Khoury and colleagues have created a new mouse pipeline for studying microglia in the healthy, injured and diseased CNS. This pipeline allows researchers to isolate and study microglia *in vitro*, visualize them *in vivo*, study them in various disease models and target them *in vivo* for genetic manipulation — all in the same mouse (Hickman, unpublished).

The mice have TdTomato and CreERT2 introduced in tandem into the Tmem119 gene. Microglia from these mice have transcriptomes that are indistinguishable from wild-type microglia. Tmem119 is expressed in the Tmem119-TdTomato-CreERT2 mice at similar levels as the receptor protein Cx3Cr1 — which is important because other mouse models for studying microglia have genes knocked into the Cx3Cr1 locus.

Using two-photon microscopy, microglia in Tmem119-TdTomato-CreERT2 mice can be visualized *in vivo* moving toward sites of laser-induced injury. Similarly, Tmem119-TdTomato-CreERT2 microglia can be seen accumulating at injury sites in a cortical impact model of traumatic brain injury.

Most relevant to Alzheimer's disease, the sites and extent of Aβ deposition were similar in 5xFAD and Tmem119-TdTomato-CreERT2 mice bred onto the 5xFAD background. In contrast, other microglial mouse models show a significant reduction in amyloid plaques compared to 5xFAD mice.

To study the role of CNS infections — particularly herpes viruses — in AD, Tmem119-TdTomato-CreERT2-5xFAD mice were bred to mice that express human CD46 to facilitate viral infections (unpublished). This new mouse line can now be used to study the role of viral infection in microglial activation and amyloid progression.

4. Session 2: circulating immune responses in the development of AD

4.1. Speakers: jenny ting, michael heneka, robyn klein, David Gate

4.1.1. The inflammasome and Alzheimer's disease

Inflammasomes are cytoplasmic multiprotein complexes of the innate immune system that are implicated in various neurologic disease states including traumatic brain injury, stroke, multiple sclerosis and Alzheimer's disease(Zheng et al., 2020). Inflammasomes activate inflammation through a signaling cascade triggered by pathogens and damage-associated host proteins. Pro-inflammatory cytokines are regulated by the enzyme caspase-1, which becomes activated in the final stage of inflammasome-induced molecular events, resulting in the cleavage of pro-IL1β and pro-IL18 to their mature forms(Zheng et al., 2020). Additionally, gasdermin D is cleaved by caspase-1, resulting in an N-terminal fragment that forms membrane pores, resulting in cytokine release and eventually pyroptosis (cell death) (Broz and Dixit, 2016).

Earlier work by Dr. Heneka's team shows that the NLRP3 inflammasome in microglia contributes to AD (Heneka et al., 2013; Venegas et al., 2017). This was evidenced by elevated levels of cleaved caspase-1 in the brains of patients suffering from AD or mild cognitive impairment, compared with controls, as well as in the APP/PS1 AD mouse model (Heneka et al., 2013). In these mice with familial AD mutations, NLRP3 or caspase-1 genetic deficiency reduced Aβ deposition and protected against memory loss(Heneka et al., 2013). Not only is the NLRP3 inflammasome essential for the development and progression of Aβ pathology in AD mice, but studies in Tau22 mice show that NLRP3 is necessary for tau hyperphosphorylation and aggregation (Ising et al., 2019). In addition to AD, NLRP3 and other inflammasomes have been implicated in other central nervous system disease or disease models, including multiple sclerosis (Gris et al., 2010; Inoue et al., 2012), demyelination and traumatic brain injury (Adamczak et al., 2014; de Rivero Vaccari et al., 2009; Irrera et al., 2017; Ismael et al., 2018; Jha et al., 2010).

The identification of the NLRP3 protein as an ATPase points to therapeutic potential because enzymes make attractive targets for drug discovery. Repeated treatment with the NLRP3 inflammasome inhibitor MCC950 relieved problems with long-term potentiation in a rat model of AD amyloidosis (Qi et al., 2018). Another NLRP3 inhibitor, OLT1177, protected against functional deficits and spinal cord demyelination in a mouse model of experimental autoimmune encephalomyelitis (Sánchez-Fernández et al., 2019). Thus, whether or not infectious agents may serve as the trigger to activate an inflammasome, there is great interest in brain permeable NLRP3 inhibitors or broad inflammasome inhibitors as neuroprotectors.

4.1.2. How anti-viral immune responses in the CNS might trigger pathological forgetting

Dr. Klein's lab has developed *in vivo* models to study immune mechanisms that underlie cognitive impairment after infection by arthropod-borne viruses (eg *Flaviviridae, Togaviridae*) or neurovirulent respiratory viruses (eg *Orthomyxoviridae, Coronaviridae*). These viruses trigger flu-like acute illnesses followed by chronic illness that can result in cognitive and motor deficits(Klein et al., 2019).

Studies from this lab have shown that T cells persisting in the brain after infections become memory cells that produce IFNγ, which activates microglia and triggers complement-mediated synapse elimination leading to spatial learning defects (Garber et al., 2019). In this setting, cytokines such as IL-1β can reprogram neural stem cells to tone down the generation of new neurons and instead create astrocytes(Lin et al., 2019).

IL-1 plays a key role in these processes, as mice that lack the IL-1 receptor IL-1R1 show normal neurogenesis, recover presynaptic termini and resist spatial learning defects (Garber et al., 2018). Spatial learning defects are also relieved when mice are treated with the IL-1R antagonist anakinra(Garber et al., 2018).

To further examine the contribution of IL-1, Dr. Klein's team is now utilizing floxed mice, in which gene sequences can be selectively activated or deleted in certain conditions or cell types, to selectively delete IL-1R1 in neural stem cells, astrocytes, microglia or neurons. Tamoxifen was given prior to infection to avoid potential confounding effects of IL-1R deletion from activated astrocytes. Data presented demonstrated that neural stem cells were the target of IL-1, with deletion of IL-R1 in these cells leading to reversal of astrogenesis, return of neurogenesis, synapse repair and cognitive recovery after West Nile Virus infection (unpublished).

These results raise the possibility that host-pathogen interactions could underlie the development of AD and other dementias and suggest that it could be possible to repurpose anti-IFN or anti-IL-1R1 receptor drugs as therapeutics in these settings.

4.1.3. Intrathecal immunity in Alzheimer's disease

The idea that infectious agents could cause AD was pioneered more than a century ago by Czech physician Oskar Fischer, a contemporary of Alois Alzheimer. To investigate this notion, Gate et al. used histology and single-cell genomics to analyze the central and peripheral immune system of AD patients versus healthy individuals. In the brains of people with AD, T cells appeared to enter the brain via the cerebral vasculature and were associated with cerebral amyloid angiopathy blood vessels (Gate et al., 2020). The team also detected $CD8^{+}$ T cells adjacent to neuronal processes, within Aβ plaques and in association with microglia. In cerebrospinal fluid analyses, T cells from AD patients were more clonally expanded compared with T cells from healthy individuals, suggesting an antigen-specific immune response in AD(Gate et al., 2020).

To determine the antigens driving this clonal expansion, the researchers PCR-amplified the T-cell receptor (TCR) genes of single cells. They then used a machine learning method known as GLIPH to identify candidate TCRs that were shared between AD subjects' individual T cells. Screening for reactivity of candidate TCRs to a major histocompatibility complex (MHC)-I peptide library revealed a clonal AD TCR specific to BZLF1, an Epstein-Barr virus protein(Gate et al., 2020). These findings suggest that T cell interactions with MHC I-expressing neurons, potentially mediated by Epstein-Barr virus exposure, may play a role in AD pathology.

In the context of a recent analysis showing that neuronal ApoE regulates MHC I expression (Zalocusky et al., 2021), the Gate et al. findings suggest that T-cell interactions with MHC I-expressing neurons could lead to neurodegeneration. To study this possibility, the team is using patient-derived T cell and neuron cultures to model T cell-neuron interactions in an *in vitro* antigen presentation assay.

5. Session 3: pathogens and microbiome in the development of Alzheimer's disease

5.1. Speakers: rima Kaddurah-Daouk, sangram sisodia, mari shinohara, serena spudich

5.1.1. Gut-brain chemical axis in Alzheimer's disease

The trillions of microbes making up the gut microbiome influence metabolic and immunological states and thereby play critical roles in human health and disease(Rooks and Garrett, 2016). Bidirectional biochemical communication between the gut and brain has been demonstrated mechanistically in animal models and suggested by human association studies as contributing to neurodegenerative and psychiatric diseases(Needham et al., 2020). Yet the details, and especially causation, in humans remain poorly defined. The Alzheimer Gut Microbiome Project (AGMP) — an initiative funded by the National Institute on Aging to define the biochemical axis of communication between the gut microbiome and brain — seeks to define links between gut metabolites, AD phenotypes and brain imaging changes.

Bile acids, products of cholesterol metabolism in the liver, get further metabolized by gut microbes. In an analysis of 1464 older adults enrolled in the AD Neuroimaging Initiative (ADNI) and in a separate set of serum samples in the Rush Religious Orders and Memory and Aging Project (ROSMAP), AD patients had lower serum concentrations of a primary bile acid (cholic acid) and increased levels of the bacterially produced deoxycholic acid, compared with cognitively normal individuals (MahmoudianDehkordi et al., 2019). Increased levels of cytotoxic deoxycholic acid in blood correlated with cognitive decline and biomarkers of Alzheimer's disease. High levels of bacterially-produced, secondary bile acids in human brains correlated with poor cognition and degeneration(MahmoudianDehkordi et al., 2019).

Baloni et al. analyzed transcriptome data from 2114 postmortem brain samples from ROSMAP, Mayo Clinic and the Mount Sinai Brain Bank. They reconstructed brain region-specific metabolic networks and found that taurine transport, bile acid synthesis and cholesterol metabolism differed in AD patients versus healthy individuals (Baloni et al., 2019). Some bile acids measured in brain tissue cannot be explained by the presence of enzymes responsible for their synthesis, raising the possibility that they could originate from the gut microbiome and get transported into the brain (Baloni et al., 2019). These findings motivate further research exploring a possible connection between bile acid metabolism and cognitive decline in AD.

5.1.2. Sex-specific alterations in neuroinflammation and amyloid deposition by the microbiome in animal models

Previous research has shown that CNS microglia from mice raised in germ-free conditions or treated with antibiotics show striking changes in morphology and transcriptional profiles, compared to microglia from control mice (Erny et al., 2015). Consistent with these findings, evidence from GWAS implicated the innate immune system in late-onset AD (Bertram and Tanzi, 2019; Efthymiou and Goate, 2017).

To test if the gut microbiome modulates neuroinflammation to ultimately influence amyloid deposition, Minter et al. treated APPSWE/PS1ΔE9 mice with high-dose antibiotics and found substantial changes in gut microbial composition and diversity. The altered gut microbiome was associated with decreased Aβ plaque load and increased levels of soluble Aβ (Minter et al., 2016).

Follow-up experiments that included additional AD mouse models yielded a surprise: the microbiome-associated impact on amyloidosis was specific to male animals. Antibiotic-treated females showed no changes in Aβ deposition or microglial phenotypes (Dodiya et al., 2019). In male AD mice that did exhibit such changes, transplants of fecal microbiota from non-antibiotic-treated males partially restored the Aβ pathology and microglial morphology, indicating a causal role for the microbiome(Dodiya et al., 2019). The sex-related differences parallel trends in human AD, where women who are heterozygous for ApoE4, a protein expressed at high levels in microglia, show faster decline and greater cognitive deterioration than age-matched heterozygous men (Ungar et al., 2014).

Ongoing and future experiments will characterize the microglial transcriptome in germ-free AD mice to explore potential pathways and mechanisms underlying the impact of gut microbes on inflammation and amyloidosis. Other analyses — perhaps using ovariectomized mice or animals deficient in follicle-stimulating hormone receptor — will assess the impact of sex hormones on sex-specific effects.

5.1.3. Fungal infections and fungal receptors in AD: lessons from research on multiple sclerosis

Fungi have been detected in brain tissue from AD patients (Alonso et al., 2018), and next-generation sequencing has identified the fungal species as *Alternaria, Botrytis, Candida, Cladosporium* and *Malassezia* (Alonso et al., 2017).

Pattern recognition receptors regulate innate immune responses that can mitigate or exacerbate diseases of the central nervous system. Among pattern recognition receptors, the C-type lectin receptor Dectin-1 has been studied as a critical receptor to detect fungi. In AD, disease-associated microglia (DAM) highly express Dectin-1(Deerhake and Shinohara, 2021).

Normally, Dectin-1 signaling in myeloid cells induces antifungal proinflammatory responses through a pathway involving the signaling molecule Card9(Jia et al., 2014). Furthermore, Dectin-1-stimulated myeloid cells interact with $CD4^+$ T cells and polarize them toward the T-helper 17 (Th17) state, which is typically good for fighting fungal infection but exacerbates autoimmune inflammation(Deerhake et al., 2021).

Unexpectedly, in the experimental autoimmune encephalomyelitis model of multiple sclerosis (MS), Dectin-1 signaling in myeloid cells limited neuroinflammation and encephalomyelitis severity (Deerhake et al., 2021). Dectin-1 signaling through a Card9-independent pathway turns on expression of Oncostatin M (OSM), which plays a neuroprotective role when detected by astrocytes(Deerhake et al., 2021). This mechanism of beneficial myeloid cell-astrocyte communication seems to operate in CNS autoimmunity, but its relevance to AD is not yet known. Future studies are needed to assess the role of microglial Dectin-1 in AD, probe possible contributions from the gut "mycobiome" and ultimately determine what influence, if any, fungal infection could have before or after AD onset.

5.1.4. What role(s) might COVID-19 infection play in AD development?

Covid-19 patients show a spectrum of neurological manifestations including stroke, seizure, neuromuscular disorders and encephalopathy. Understanding how SARS-CoV-2 affects the brain could give insight into how infections could potentially trigger long-term neurological effects including AD(Zubair et al., 2020).

Pathological analyses of postmortem samples from people who died of Covid-19 have detected limited viral infection in the brain. In one study, immunohistochemistry failed to detect SARS-CoV-2 in neurons, glia, endothelium or immune cells (Solomon et al., 2020). Another analysis detected SARS-CoV-2 protein or RNA in only about a third of samples, yet found evidence of widespread microglial activation and $CD8^+$ T cell infiltration in the brain (Matschke et al., 2020).

Studies of hospitalized Covid-19 patients with neurologic symptoms have found unusual patterns of CSF biomarkers that suggest that immune responses, rather than SARS-CoV-2 itself, may be the underlying driver of CNS manifestations(Jarius et al., 2022). In addition to increased cytokines, Song et al. found a higher frequency of B cells and SARS-CoV-2 antibodies, some of which could be autoreactive, in CSF of people with acute Covid-19 and neurologic syndromes (Song et al., 2021).

Reports of cognitive impairment, anxiety, depression and other neurologic problems in people who have recovered from acute Covid-19 are a growing concern. The National Institutes of Health held a workshop in December 2020 about these issues, which are collectively referred to as "Long COVID" or Post-Acute Sequelae of SARS-CoV-2 infection (PASC). In February 2021 the NIH launched an initiative to research underlying causes and treatments for people who suffer lingering symptoms of Covid-19, most of whom are young and were never hospitalized for Covid-19(NIH). These individuals would not have been exposed to the same anti-thrombotic and anti-inflammatory therapies that are commonly used when COVID-19 is managed in the hospital.

Based on preliminary findings, brain imaging and cerebrospinal fluid and blood measures are normal in most participants with neuropsychiatric manifestations of PASC (unpublished). Curiously, auto-neuronal antibodies were found in the CSF of a 30-year-old male COVID-19 patient whose abrupt onset psychosis did not respond to anti-psychotic therapy but improved with intravenous immunoglobulins (McAlpine et al., 2021). Long-term observation of neurologic and neuropathologic outcomes in patients who recover from COVID-19 may provide insight into frequency and mechanisms of infectious and immune-mediated

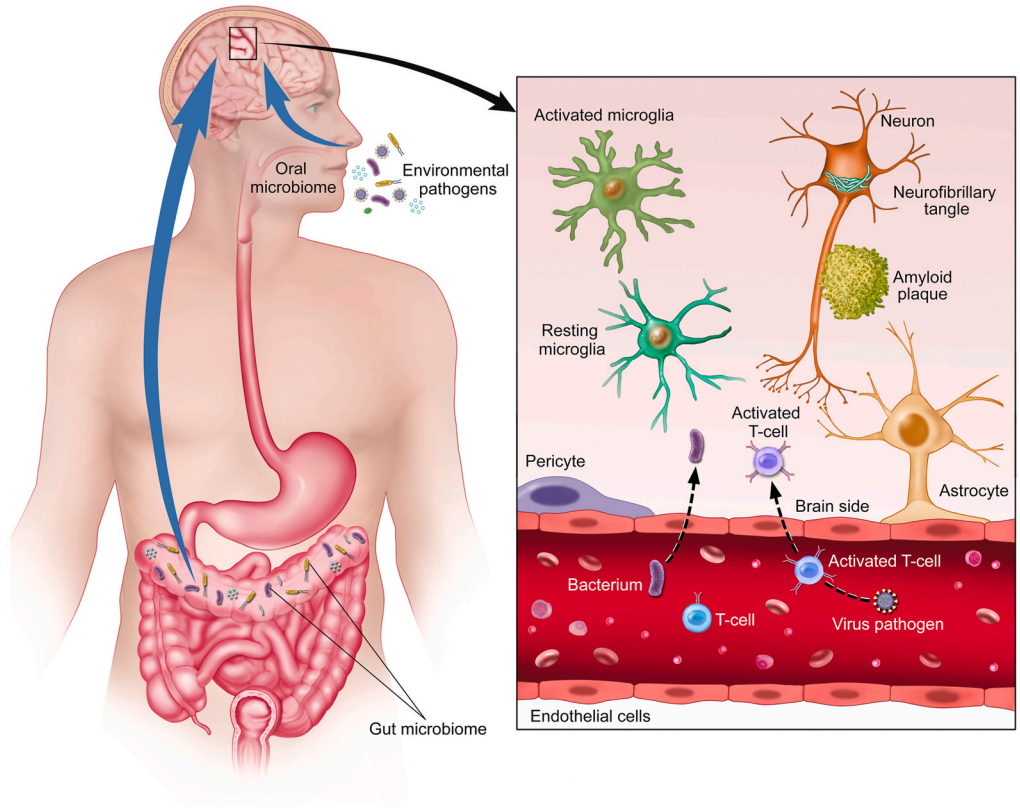

Fig. 1. Legend: Potential Mechanisms by which Microbes May Drive Onset or Progression of Alzheimer's Disease (AD). Humans are continuously exposed to microbial agents, including viruses, bacteria, and fungi, which exist in the environment as well as in normal microbiomes in the body. This symposium reviewed multiple hypotheses related to how microbes, whether or not they act as infection-causing pathogens, may contribute to AD. Microbes in the gut metabolize bile acids, which are thought to contribute to biochemical communication between the gut and brain and may influence immunological states and amyloid deposition in the brain. When microbes of any type gain access to the blood, they may exert changes on the brain through the blood brain barrier. If microbes directly cross the barrier, they may stimulate amyloid deposition in the brain, considering evidence that amyloid has antimicrobial properties. Microbes in the brain could also cause or exacerbate microglial activation or other immunological responses known to be implicated in AD. Microbes in the bloodstream may also exert immune reactions in the brain by activating T cells or perivascular myeloid cells.

neurodegeneration relevant to AD.

6. Conclusions

In summary, this conference examined and discussed evidence for and against the hypothesis that infectious agents play a role in the development or progression of Alzheimer's disease. While the "germ hypothesis" has been neither proven nor disproven, it is plausible that microbes play a causative role in either the early stages of AD pathology by triggering extra-neuronal Aβ accumulation through an antimicrobial response, or in the later stages of AD by exacerbating immune activation in the central nervous system, or both. Functional states and pathways involving microglia, the primary innate immune cells of the brain, are a prime area of investigation. Microglial activation and other inflammatory changes in the brain can certainly occur in the absence of microbial exposure, but researchers are investigating how infection by specific pathogens or exposure to ubiquitous microbes can trigger immune responses leading to long-term neurological changes. Ongoing experiments are assessing how changes in the gut or oral microbiome (including "mycobiome") may evoke changes in brain metabolism or immunological activity, ultimately affecting AD onset or progression. Changes in the permeability or immunoreactivity of the blood-brain barrier are also of interest. While there is some evidence linking microbes in the CNS to AD-relevant changes, microbes do not need to cross the barrier to exert changes in the brain, as CNS changes also occur due to activated T cells or other immune mediators that cross the barrier. Fig. 1 summarizes various hypotheses related to the potential role of microbes and pathogens in AD. This symposium identified high priority questions (Table 1) and resources needed (Table 2) to advance knowledge related to the role of infections or microbes in AD, a highly important topic given its potential to open untapped opportunities for prevention and treatment.

Acknowledgments

The symposium was made possible, in part, by generous support from Dr. Leslie Norins and Mrs. Rainey Norins. Supported by grants from NIH (P30 AG072958-01; R38-AG065762-01; P30-AG034424 to HEW); Cure Alzheimer's Fund to RT and S Sisodia; JPB Foundation to RT; Open Philanthropy Project and Good VenturesFoundation to S Sisodia; NIH (R01 NS120417-01) and National Multiple Sclerosis Society (RG-1801-30040) to MLS; NIH (R35NS122310) to RK; NIH (R01-AI029564) to JPT; NIH (U01AG058635) to AG; NIH (R01 AG057895-01) to CC; NIH (NS112458-01A1) to DG; NIA grants 1U19AG063744, RF1 AG058942, 1RF1AG058942, R01 AG059093 and R01 AG057452 to RKD; Alzheimer Disease Metabolomics Consortium which is a part of NIA's national initiatives AMP-AD and M^{2}OVE-AD (R01 AG046171, RF1 AG051550, and 3U01 AG024904-09S4).

DG is a co-inventor on a patent application related to this work. Patent STDU2-36496/US-1/PRO is for compositions and methods for measuring T cell markers associated with Alzheimer's disease.

MH serves as an advisory board member at IFM Therapeutics, Alector and Tiaki, and received honoraria for oral presentations from Novartis, Roche and Biogen.

RKD is an inventor on key patents in metabolomics, including applications for Alzheimer's disease.

RT reports consulting fees from MarvelBiome, AZTherapies, Promis and Chromadex, and financial relationships with Amylyx, React Neuro, Cognitive Clarity, DRADS Capital, Neurogenetic Pharmaceuticals.

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