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Introduction- Alzheimer's disease (AD) continues to threaten aged individuals and health care systems around the world. Human beings have been trying to postpone, reduce, or eliminate the primary risk factor for AD, aging, throughout history. Despite this, there is currently only symptomatic treatment for AD and this treatment is limited to only a handful of FDA approved AD drugs.

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Synaptic Pruning in Alzheimer's Disease: Role of the Complement System

Frederic H. Brucato MA [α] & Daniel E. Benjamin PhD [σ]

I. Introduction

Alzheimer's disease (AD) continues to threaten aged individuals and health care systems around the world. Human beings have been trying to postpone, reduce, or eliminate the primary risk factor for AD, aging, throughout history. Despite this, there is currently only symptomatic treatment for AD and this treatment is limited to only a handful of FDA approved AD drugs.

This review will cover:

- AD epidemiology
- Current FDA approved drugs (treat symptomology)
- Mild Cognitive Impairment
- The transition process from MCI to AD
- Genetic and Biologic Markers in AD
- New targets
- The role of neuroinflammation in AD
- Factors and systems that influence inflammation including the complement system
- Complement system's direct involvement in AD including a role in Beta-Amyloid and Tau Pathology
- Complement inhibition in AD modulation and prevention

One in ten people older than 65 currently has AD induced dementia. Recent estimates are that AD will grow to greater than 16 million individuals by the year 2050 [1][2]. There are many risk factors for AD, but clearly, age is the strongest predictor. In 2017 approximately 6.1 Americans had AD or a form of mild cognitive impairment (MCI) that is likely to progress to AD. Also, in 2017, based on amyloidosis, neurodegeneration, or both, 46.7 million Americans exhibited preclinical signs of AD [2]. According to ClinicalTrials.gov, in 2019, there were 2231 trials for Alzheimer's disease that were either recruiting, under way, terminated, or completed. Unfortunately, we still lack drugs that can modify the course of Alzheimer's disease [3].

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The only FDA approved drugs; donepezil, galanthamine, rivastigmine (acetylcholinesterase inhibitors) memantine (N-MDA antagonist), and donepezil/memantine (acetylcholinesterase inhibitor combined with N-MDA antagonist) demonstrate varying efficacy only with symptomatic management. There are recent advances and potential breakthroughs that need more validation but may become significant [4]. A recently discovered APOE double mutation may yield insight into the mechanism of AD. However, this promise remains years away. [5] The current standards of care rely on acetylcholinesterase inhibitors, N-MDA blockers, or a combination of the two. In the 1980's, a loss of cholinergic neurons in the Nucleus Basalis of Meynert region of Alzheimer's brains gave rise to the cholinergic hypothesis. Subsequently, an accumulation of beta-amyloid protein (Aβ), especially in the hippocampus, gave rise to the beta-amyloid hypothesis. This suggested the accumulation of Aβ in the brain was the primary cause of AD. The tau hypothesis followed, which presumed that intracellular accumulations of tau, creating spindle fibers, also contributed to AD [6]. Now the picture is more complicated, but beta -amyloid and tau remain important aspects of AD. The pressure to develop-disease modifying drugs and cures for AD drugs continues to increase [7].

In order to develop effective disease modifying drugs, it is important to better understand: 1) Alzheimer's disease mechanisms such as generation and clearance of beta amyloid, p-tau, the role of APOE4, synaptic maintenance and synaptic elimination or pruning as well as and many other mechanisms 2) biomarkers that identify, predict and /or track progression of AD 3) biological systems that play a role in day-to-day homeostasis and health but also play a role in Alzheimer's disease and 4) the relationship between Mild Cognitive Impairment (MCI) and AD. What causes the progression of MCI to AD, and what prevents the progression? This is a fundamental question we will try to address in this review. The cost of Alzheimer's drug discovery and drug development is substantial. Total national cost of caring for those with Alzheimer's and other dementias is estimated at $277 billion (not including unpaid caregiving) in 2018, of which $186 billion is the cost to Medicare and Medicaid; out-of-pocket costs represent $60 billion of the total payments, while other costs total $30 billion. [8].

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Drugs that have been approved for a different indication may be repurposed for Alzheimer's disease. are highly attractive. These may include medications that are structurally or functionally related to compounds that already have passed phase I safety trials.

II. Complement System in Alzheimer's Disease

There has been much recent interest in the complement system's role in AD. The complement system facilitates the immune system' response to destroy and remove foreign pathogens. It also appears to influence beta-amyloid, tau, and APOE4 interaction in AD [9] [10]). (For complement system review see Fritzinger and Benjamin (2016) [11].

Complement system activation is a precise process, controlled by regulatory proteins found in both plasma and at host cells' surfaces. C3 protein plays a major role in complement activation and control of immune responses. Deficiencies of C3 and so-called early and late complement proteins contribute to the emergence of recurrent bacterial, viral, and fungal infections. Importantly, mannose-binding lectin occurs at low levels. This protein plays a protective role in the early stages of infection as well as controlling inflammation. C3 deficiency is a common cause of human immunodeficiency, observed in microbial infections and autoimmune diseases such as rheumatoid arthritis. However, excessive activation of complement proteins has now been linked to Alzheimer's disease [12], autoimmune diseases, schizophrenia, atypical hemolytic-uremic syndrome, angioedema, macular degeneration, and Crohn's disease [13].

In the case of multiple sclerosis, inflammation is tightly linked with neurodegeneration, and it is the accumulating neurodegeneration that underlies increasing neurological disability in progressive multiple sclerosis (MS). Complement expression can be evaluated by immunocytochemistry and, in situ hybridization causes expression of the transcript for C1qA in neurons and the activation fragment and opsonin C3b-labelled neurons and glia in the MS cortical and deep grey matter. A recent study by Watkins et al. (2016) [14] demonstrated the density of immunostained cells positive for the classical complement pathway protein C1q and the alternative complement pathway activation fragment Bb was significantly increased in cortical grey matter lesions compared to control grey matter. Cells immunostained for the membrane attack complex (MAC) were elevated in cortical lesions, indicating complement activation to completion. Classical (C1-inhibitor) and alternative (factor H) pathway regulator-positive cells were unchanged between MS and controls. Complement anaphylatoxin receptor-bearing microglia in the MS cortex were closely opposed to cortical neurons [14].

Complement immune positive neuron morphology reflects cell stress/damage, suggesting significant neurodegeneration in cortical grey matter lesions. Thus, complement appears activated in MS cortical grey matter lesions where increased complement receptor-positive microglia were found.

The finding that complement proteins are abundant and can play pathological roles in neurological conditions offers potential for therapeutic intervention. Accordingly, frequent studies have explored unique activation pathways, proteases, receptors, complexes, and natural inhibitors of complement to mitigate pathology in acute neurotrauma and chronic neurodegenerative diseases. Brennan et al. (2016) reviewed recent studies that discussed the mechanisms of complement activation in the central nervous system (CNS), and the effects of complement inhibition in cerebral ischemic-reperfusion injury, traumatic brain injury, spinal cord injury, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease [15]. The authors of this particular review provide perspectives on how promising complement-targeted therapeutics could become part of novel and effective future treatment options [15]. In the rat, a single intracerebroventricular injection of neuraminidase from Clostridium perfringens induces ependymal detachment and death. The neuraminidase study implicates critical involvement of the complement system. In this study, complement activation, triggered by neuraminidase, was analyzed by Western blot. Primary cultures of ependymal cells and explants of the septal ventricular wall were assessed in vitro. In these models, ependymal cells were exposed to neuraminidase in the presence or absence of complement, and their viability was assessed by observing cilia or by trypan blue staining. The role of complement in neuraminidase induced ependymal damage was analyzed in vivo in two rat models of complement blockade: systemic inhibition of a C5 blocking antibody and testing in C6-deficient rats [15].

Injecting rats intracerebroventricularly with neuraminidase causes complement membrane attack complex (MAC) to immunolocalize on the ependymal surface [16]. C3 activation fragments were found in serum and cerebrospinal fluid of rats treated with neuraminidase, suggesting that neuraminidase itself activates complement. In ventricular wall explants and isolated ependymal cells, treatment with neuraminidase alone induced ependymal cell death; however, the addition of complement caused increased cell death and disorganization of the ependymal epithelium. Granados-Durán and colleagues (2016) [16] treated rats with anti-C5 or used C6-deficient rats, with intracerebroventricular injection of neuraminidase that resulted in reduced ependymal alterations, compared to

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non-treated or control rats. Immunohistochemistry confirmed the absence of membrane attack complex on the ependymal surfaces of neuraminidase-exposed rats treated with anti-C5 or deficient in C6.

The authors concluded these results demonstrate that the complement system contributes to ependymal damage and death caused by neuraminidase. However, neuraminidase alone can induce moderate ependymal damage without the aid of complement [15] [16].

It also appears that mitochondrial function and neuroinflammation are related. Neuroinflammation causes over-activation of microglia, which in turn causes increases in pro-inflammatory cytokines, processes that are hallmarks of AD [17] [18] [12]. Complement's role in normal brain development and neuropathology has not been traditionally appreciated. Complement protects the host against infection. Thus, improved function was not always predicted. Complement has been implicated in depression, epilepsy, demyelination and dementia. Complement's role in inflammation is complicated, with respect to these diseases. Activation of complement pathways may actually accentuate development of AD, bringing into focus the possibility that complement inhibition may be a viable approach to AD treatment [9] [12]. Complement's role in modulating synapse density in AD may also be a critical part of disease progression. C3 deficient mice apparently are unable to remove synapses from damaged neurons as efficiently as control mice. Reducing C3 in mice increased numbers of synapses, and improved cognitive performance according to Berg et al. (2012) [19]. While this result is somewhat counterintuitive, because C3 deficient mice don't clear or eliminate damaged neurons, it is supported by the fact that the known age-associated synaptic loss in hippocampus is also reduced in C3 deficient mice. Reducing age-associated synaptic loss is associated with better learning and memory. The implication clearly becomes complement may work against robust synaptic health in aging and may play a critical role in AD. To take this thought one step further, complement inhibition could be a viable strategy for aging and AD. Axotomized spinal motoneurons lacking C3 caused reduced removal of synaptic terminals, suggesting an important role for C3 in AD [19].

In addition, mice lacking C3 showed altered cognitive performance and synaptic function in the hippocampus. [20]. Similarly, mice lacking C3 do not show typical age-related hippocampal decline [21].

Complement C7 appears to have a role as a novel gene in AD. In a recent study by Zhang et al., (2019) [22], whole-exome sequencing of Han Chinese patients with familial and/or early-onset Alzheimer's disease was conducted. The exome was independently validated, imaged and characterized. Investigators identified an exome-wide significant rare missense variant rs3792646 (p.K420Q) in the C7 gene. Investigators validated the association in different cohorts and a combined sample (1615 cases and 2832 controls). The risk allele was associated with reduced hippocampal volume and impaired working memory performance younger adults. This risk allele may be associated with early onset AD. Overexpression of p.K420Q altered cell viability, activation of the immune system and affected β -amyloid processing. The mutant p.K420Q inhibited excitatory synaptic transmission in pyramidal cells, in an electrophysiogical assay. This result further supports the idea that C7 is a novel risk gene in AD in the Han Chinese population [22].

Accumulated beta-amyloid peptides in AD brains activate the classical C pathway by binding to a collagen-like domain (CLF) within C1q. This pathway is activated by synthetic analogues of beta-amyloid peptides, beta 1-42 and beta 1-40, bound to C1q. Beta 1-42 bound more effectively to C1q than beta 1-40. C pathway activation impacted beta1-42 more so than did beta 1-40. This C-activating capacity appears correlated with the assembly of the beta 1-42 into aggregates and/or macromolecular fibrils. While these studies are not recent, they are important to mention because they helped establish the connection between beta amyloid, inflammation and complement, especially the classical C pathway [23].

Beta-amyloid peptide is cleared from periphery by a complement-mediated mechanism that appears to be deficient in Alzheimer's disease. The mechanism should be enhanced by beta-amyloid antibodies that form immune complexes (ICs) with Aβ and therefore may be relevant to current beta-amyloid immunotherapy approaches. Targeting peripheral mechanisms that may facilitate beta-amyloid clearance has the potential as immunotherapy to treat Alzheimer's disease [24].

Alzheimer's disease appears to be associated with brain inflammation. Activated microglia are associated with brain lesions, which in turn, may also be involved with brain inflammation. Anti-inflammatory treatment may protect against AD, possibly through beta-amyloid mediated activation of the complement system. The activated complement system has anti-inflammatory properties [25] and is highly involved in immune system homeostasis [26].

It is known that complement protein C5a binds to and inhibits the receptor C5aR1. C5a inhibits pathology and AD cognitive deficits in AD mouse models. However, to be sure C5a acts via C5aR1 inhibition, C5aR1 deficient mice were generated, and compared to wild type mice plaque load and behavioral assessments such as novel object recognition (NOR), hpc dependent and independent versions and object location memory (OLM), hpc dependent. [27]

It is known that C5aR1 is expressed primarily on myeloid lineage cells, secondarily on brain and endothelial cells. Thus, gene expression was compared at 2, 5 ,7, and 10 months, across each genotype. [27]

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As stated, to accomplish this, C5aR1 knockout mice were crossed to the Arctic AD mouse. Hernandez et al. (2017) [27] found C5aR1 deficient mice did not show behavior deficits at 10 months, although amyloid plaque load was not altered. Of interest, there were no CCR2+ monocytes/macrophages near the plaques in the Arctic brain with or without C5aR1. To underscore this finding, the Arctic C5aR1KO mice showed a reduction of hippocampal neuron complexity and improved behavioral deficit. [27]

RNA-sequence analysis showed inflammation related genes were differentially expressed and expression was increased in the Arctic mice relative to wild type. Expression was decreased in the Arctic/C5aR1KO relative to Arctic. In addition, phagosomal-lysosomal gene expression was increased in the Arctic mice relative to wild-type but this increase was even more prominent in the Arctic/C5aR1KO mice. In the Artic mice, aging was associated with reduced neuronal hippocampal complexity. This effect at 10 months was correlated to the observed behavioral deficit. The reduction of neuronal complexity in hippocampus and the behavioral deficit were both rescued in Arctic/C5aR1KO.

Neurofibrillary tangles aggregated from hyperphosphorylated tau protein cause significant pathology in AD. Complement C3 (or C3a) is linked to AD pathology. An important question is whether C3a or the C3a receptor is specifically tied to tau phosphorylation. In a recent study by Hu et al. (2019) [28], investigators found that exposing SH-SY5Y cells to okadaic acid (OA) decreased cell viability and induced tau hyperphosphorylation. The C3a receptor antagonist SB290157 blocked these effects. In addition, SB290157 blocked the action of glycogen synthase kinase 3β (GSK3β) but did not affect protein phosphatase 2A C subunit (PP2Ac) and cyclin-dependent kinases 5 (CDK5). The authors concluded findings indicate the unique role C3a receptor plays in regulating tau phosphorylation via GSK3β signaling pathways and highlight C3a receptor as a potential target for treating AD [28].

Complement pathway overactivation can lead to neuronal damage in various neurological diseases. Although AD is characterized by β-amyloid plaques and tau tangles, previous work examining complement has largely focused on amyloidosis models. Wu et al. (2019) [29] find glial cells show increased expression of classical complement components and the central component C3 in mouse models of amyloidosis (PS2APP). More pronounced is tauopathy (TauP301S). Blocking complement function by deleting C3 rescues plaque-associated synapse loss in PS2APP mice and reduces neuron loss and brain atrophy in TauP301S mice. These changes were confirmed by improved neurophysiological and behavioral measurements. The authors state, C3 protein is elevated in AD patient brains, including at synapses. Processing of C3 is increased in AD patient CSF and correlate with tau. These results demonstrate that complement activation contributes to neurodegeneration caused by tau pathology and suggest that blocking C3 function might be protective in AD and other tauopathies [29].

Synapse loss and Tau pathology are hallmarks of Alzheimer's disease (AD) and other tauopathies, but how Tau pathology causes synapse loss is unclear. This study used unbiased proteomic analysis of postsynaptic densities (PSDs) in Tau- P301S transgenic mice to identify Tau-dependent alterations in synapses prior to overt neurodegeneration. Multiple proteins and pathways were altered in Tau- P301S PSDs, including depletion of a set of GTPase-regulatory proteins that leads to actin cytoskeletal defects and loss of dendritic spines. A striking accumulation of complement C1q in the PSDs of Tau-P301S mice and AD patients was observed. At synapses, C1q perisynaptic membranes accumulated in correlation with phospho-Tau and was associated with augmented microglial engulfment of synapses and decline of synapse density. A C1q-blocking antibody inhibited microglial synapse removal in cultured neurons and in Tau-P301S mice, rescuing synapse density. Thus, inhibiting complement-mediated synapse removal by microglia could be a potential therapeutic target for Tau-associated neurodegeneration [30].

III. Genetic and Biological Markers in Alzheimer's Disease

Evidence indicates the APPSwDI/Nos2-/- (CVN-AD) mouse model replicates multiple AD pathologies [31]. Badea et al. (2016) identified multivariate biomarkers that appear to predict cognitive decline. One of these biomarkers is the fornix. In vivo and ex vivo magnetic resonance imaging (MRI) reveals CVN-AD mice replicate the hippocampal atrophy (6%), characteristic of human AD. It has been shown the fornix is 23% smaller in these mice. This is important anatomically because the fornix connects the septum, hippocampus, and hypothalamus. Ultrastructural analysis has shown the fornix has reduced axonal density (47% fewer), axonal degeneration (13% larger axons), and abnormal myelination (1.5% smaller g-ratios) in these mice. CD68 staining showed that white matter pathology might not be the cause, instead could be secondary to neuronal degeneration. Alternatively, the authors state it could be due to direct microglial attack. Thus, the fornix provides multiple biomarkers to characterize circuit disruption in a mouse model of Alzheimer's disease [31]. Deposition of tau and beta-amyloid in the brain yields biomarkers that may be valuable. The core cerebrospinal fluid (CSF) AD biomarkers amyloid peptide 1–42 (Aβ 1–42), total tau (t-tau) and phosphorylated tau 181 (p-tau 181) show good

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diagnostic sensitivity and specificity. [32] Regardless, more biomarkers that can help preclinical diagnosis or facilitate tracking disease progression are needed. Activation of the complement system, occurs at very early stages in the AD brain. Therefore, CSF levels of complement proteins could be linked to cognitive and structural changes in AD and may provide diagnostic and prognostic value.[32] xMAP® technology has been used to measure complement 3 (C3) and factor H (FH) in the CSF of human controls (CN), mild cognitive impairment (MCI) and AD subjects of the AD Neuroimaging Initiative (ADNI). [32] The association between CSF biomarkers and different outcome measures were analyzed using Cox proportional hazard models (conversion from MCI to AD), logistic regression models (classification of clinical groups) and mixed-effects models adjusted for age, gender, education, t-tau/Beta-Amyloid1–42 and APOE 4 presence (baseline and longitudinal association between biomarkers and cognitive scores). Although no association was found between the complement proteins and clinical diagnosis or cognitive measures in this particular study, lower levels of C3 and FH were associated with faster cognitive decline in MCI subjects as measured by the AD Assessment Scale-cognitive subscale (ADAS-Cog) test according to study authors. FH levels were associated with larger lateral ventricular volume ($p = 0.024$), indicating potential brain atrophy. Toledo et al. (2014) conclude C3 and FH are not good diagnostic biomarkers of AD but might have modest potential as prognostic biomarkers and therapeutic targets in cognitively impaired patients. Low levels of cerebrospinal fluid complement 3 and factor H predict faster cognitive decline in mild cognitive impairment [32]. Patients diagnosed with MCI may exhibit significant behavioral and psychological signs and symptoms (BPS), symptoms also frequently observed in patients with Alzheimer's disease (AD). A recent study by Pocnet et al (2015) [33] evaluated the extent and variability of BPS in MCI vs AD, with the intent of providing an additional marker that may predict conversion from MCI to AD. Global cognitive performance, BPS, and ADL were assessed using validated clinical methods at baseline and at two-year follow-up in 46 MCI patients, 54 AD subjects and 64 controls. The BPS variability over the follow-up period was more pronounced in the MCI group than in patients with AD: some BPS improved, others occur developed or worsened, while others still remain unchanged.[33] Changes in BPS were associated with rapid deterioration of the global cognitive level in MCI patients. In particular, an increase of euphoria, eating disorders, and aberrant motor behavior, as well as worsened sleep quality, predicted a decline in cognitive functioning. Results from this study confirm MCI patients have a higher variability of BPS over time compared to AD patients. In addition, there is evidence of associations between specific BPS and cognitive decline in the MCI group associated with a potential risk of conversion for individuals with amnestic MCI to AD [33].

Another study, which evaluated potential novel protein biomarkers for MCI progression to AD, found that Chromogranin A, secretogranin II, neurexin 3, and neuropentraxin 1 were elevated in MCI patients and MCI patients progressing towards AD. Duits and colleagues (2018) [34] concluded that these proteins which are involved in vesicular transport and synaptic stability may participate in early phases of the AD pathophysiological cascade [34]. Cognitive and functional decline in beta-amyloid positive preclinical AD patients has been compared to prodromal AD subjects (beta-amyloid positive, MCI) patients. These subjects were compared to MCI patients with no existing Beta-amyloid status. Patients were followed for an average of 4 years, and a maximum of 10 years. Preclinical AD subjects showed steeper declines in brain metabolism than beta-amyloid negative progressors. Insel et al. (2017) [35] found preclinical AD subjects also showed elevated rates of white matter hyperintensity and increased CSF phosphorylated tau levels at baseline. In this particular study, Aβ-negative progressors displayed greater baseline frequency of depressive symptoms. [35].

Evidence of blood-based biomarkers for cognitive decline in aging, (MCI) and (AD) has been sparse. Cumulative evidence suggests that apolipoproteins, complement system, and transthyretin are involved in AD pathogenesis by sequestration of beta amyloid. However, no clinical study assesses the utility of "sequester proteins" in risk assessment and/or diagnosis of MCI and AD.

Serum levels of sequester proteins and their clinical potential in cognitive decline assessment were analyzed by a recent longitudinal and cross-sectional study by Uchida and colleagues [36]. A combination of apolipoprotein A1, complement C3, and transthyretin appear to be involved in AD possibly by sequestration of beta-amyloid. These proteins also appear to differentiate MCI subjects from healthy controls. The authors conclude a set of sequester proteins could be blood-based biomarkers for assessment of early stages of cognitive decline [36].

Previous studies have shown that beta amyloid peptide (AB) is cleared by a complement-mediated process from the peripheral circulation, and that this process is deficient in Alzheimer's disease. The process may be enhanced by beta-amyloid antibodies that form immune complexes (ICs) with beta amyloid. In turn, providing improvements to current beta amyloid immunotherapy approaches. Recent studies demonstrated complement-mediated capture of beta amyloid-antibody immune complexes compared with beta-amyloid alone in both erythrocytes and THP1-derived macrophages. [24] Beta amyloid antibodies dramatically increased complement activation and

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opsonization of beta amyloid followed by enhanced beta amyloid capture by human erythrocytes and macrophages. The present study strongly suggests that peripheral mechanisms are relevant to beta amyloid immunotherapy. Findings are also consistent with enhanced peripheral clearance of intravenously administered beta-amyloid antibody immune complexes in nonhuman primates. [24].

IV. MCI Conversion to AD

Alzheimer's disease (AD) is characterized by the deposition of tau and amyloid in the brain. While core cerebrospinal fluid (CSF) AD biomarkers beta amyloid peptide 1–42, total tau (t-tau) and phosphorylated tau 181 (p-tau 181) show good diagnostic sensitivity and specificity, these biomarkers alone don't adequately address preclinical diagnosis or disease progression. Complement system-initiated inflammation occurs at very early stages in the AD brain. Therefore, complement proteins found in CSF, could be linked to cognitive and structural changes in AD and may have diagnostic and prognostic value.

As stated previously, Toledo et al. (2014) determined compliment factors 3 & H may not be suitable markers for identifying AD, but these factors may potentially predict rapidity of MCI individuals cognitive decline [37].

MCI represents an early stage of developing cognitive impairment, however there is some consensus that not all MCI patients necessarily progress to AD. Patients diagnosed with MCI do not meet the criteria for dementia as their cognitive abilities and activity levels exceed those of demented individuals. Minor changes in instrumental activities of daily living (ADL) may occur. In some cases, they may exhibit significant behavioral and psychological signs and symptoms (BPS), also frequently observed in patients with Alzheimer's disease (AD). In this study, investigators evaluated the extent to which specific BPS are associated with cognitive decline in participants with MCI or AD. 164 participants were categorized; 46 patients with amnestic (single or multi-domain) MCI, 54 patients with AD, and 64 control participants without cognitive disorders. Global cognitive performance, BPS, and ADL were assessed using validated clinical methods at baseline and at two-year follow-up. The BPS variability over the follow-up period was more pronounced in the MCI group than in patients with AD: some BPS improve, others occur newly or worsen, while others still remain unchanged. Moreover, specific changes in BPS were associated with a rapid deterioration of the global cognitive level in MCI patients. In particular, an increase of euphoria, eating disorders, and aberrant motor behavior, as well as worsened sleep quality, predicted a decline in cognitive functioning.

Findings confirm a higher variability of BPS over time in the MCI group than in AD patients. Pocnet and colleagues [33] state this could be due to differences in baselines as some in the MCI group may have been only marginally impaired. Results provide evidence of associations between specific BPS and cognitive decline in the MCI group that might suggest a risk of conversion of individuals with amnestic MCI to AD [33].

Identification of specific tests providing a high certainty for stable MCI and factors that precipitate instability of MCI could provide greater sensitivity towards detecting and following progression of AD [38].

Ellendt and colleagues (2016) tested 130 participants annually using a test battery that included measures of memory, language, executive functions, intelligence and dementia screening tests. Exclusion criteria at baseline included severe cognitive deficits such as diagnosis of dementia, psychiatric or neurological disease. Regression and Receiver Operating Characteristic (ROC) curve analysis was used to identify potential predictors for stability or instability of MCI-diagnosis. Age, IQ and APOE status were evaluated using of test performance tests and group membership. MCI (49%) was observed at baseline with a reversion rate of 18% after two years. Stability of MCI was related to (VLMT: delayed recall, CERAD: recall drawings, CERAD: Boston Naming Test, Benton Visual Retention Test: number of mistakes). Conversion to MCI is associated with language functions. Reversion to 'normal' was primarily predicted by single domain impairment. There was no significant influence of variables such as demographic, medical or genetic. The results of this study underscore the role of repeated measurements of functional neuropsychological predictors and the need for better diagnostic reliability. In cases of high uncertainty, close monitoring over time is mandatory to more closely estimate outcome. [38]

In another study attempting to define the relationship between MCI and AD, investigators developed a multivariate model for predicting MCI-to-dementia progression at the individual patient level. [39] Using baseline data from 259 MCI patients and a probabilistic pattern classification approach, Korolev et. al. (2016) trained a classifier to distinguish between patients who did or did not progress to AD-type dementia during over a three-year period. More than 750 variables across four data sources were evaluated as potential progression predictors. Data included risk factors, cognitive and functional assessments, structural magnetic resonance imaging (MRI) data, and plasma proteomic data. Predictive utility was cross validated.

Cognitive and functional markers most strongly predicted progression while plasma proteomic markers did not predict as well [39]. The best performing model predicted at 80% using a combination of cognitive/functional markers and morphometric MRI measures. Predictors of progression included scores on the Alzheimer's Disease Assessment Scale, Rey Auditory Verbal Learning Test, and Functional Activities

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Questionnaire, as well as volume/cortical thickness of three brain regions (left hippocampus, middle temporal gyrus, and inferior parietal cortex). The study authors state calibration analysis revealed that the model is capable of generating probabilistic predictions that reliably reflect the actual risk of progression. Finally, the authors found that the predictive accuracy of the model varied with patient demographic, genetic, and clinical characteristics and could be further improved by taking into account the confidence of the predictions. In this case, we see the development of an accurate prognostic model for predicting MCI-to-dementia progression over a three-year period. The model utilizes available, cost-effective, non-invasive markers and can be used to improve patient selection in clinical trials and identify high-risk MCI patients for early treatment [39].

Microarray screening in human dentate gyrus, using entorhinal cortex expression levels, has been used to differentiate age-related memory loss from AD. Using this technique, Kandle and colleagues (2013) have shown an aged related decline in the histone acetylation regulatory molecule RbAp48. This deficiency occurs in both human and mice that age normally [40]. This provides more evidence that MCI may not be simply early stage AD and may not convert to AD in all cases, even if given enough time.

Morgan et. al (2019) reviewed 53 plasma proteins obtained from control, MCI and AD groups [41]. Ten of these showed significant differences between groups. Using pairwise comparisons of AD vs CTL, they found increased C4 and eotaxin-1, decreased sCR1, C5, and CRP and for MCI vs CTL they found increased FH, C3, and MCP-1, decreased C5 and MIP-1b. For the AD vs MCI comparison, they found increased eotaxin-1 and MIP-1b, decreased FI, C3, CRP, MCP-1. These findings increase the knowledge about potentially useful biomarkers that may predict conversion to MCI and AD.

Another recent study by Helgadottir et al. (2019) evaluated CD55 and its upstream transcription factors in the temporal cortex of a Late Onset Alzheimer's disease (LOAD) patient compared to an early onset (EOAD) patient [42]. To date, sequencing has focused primarily on germline mutations. Improved technology has created opportunities to study somatic mutations in brain tissue that shows pathology. This current study used ultra-deep sequencing on brain and blood from early-onset AD (EOAD) and late-onset AD (LOAD) patients and non-AD individuals (n = 16). 2.86 Mb of genomic areas that have been associated with AD, were targeted. This included 28 genes and upstream and downstream regulatory areas. Bioinformatics filtering identified 11 somatic single nucleotide variants in temporal cortex of AD patients. In contrast, there were none in the controls. In a LOAD patient, one variant was present at 0.4% allele frequency in temporal cortex. This variant was predicted to affect transcription factor binding sites upstream of the CD55 gene, contributing to AD pathogenesis by affecting the complement system. These results suggest that future studies targeting larger portions of the genome may increase understanding for the molecular basis of both EOAD and LOAD [42].

Another recent study by Han and colleagues (2018) tying the complement system to AD was designed to identify and characterize novel AD drug target genes. This study employed a combinatorial approach for the first time to discover AD drug targets. Investigators did this by considering ontology inference and network analysis. Potential AD drug target genes were discovered by integrating information from multiple popular databases (TTD, Drug Bank, Pharm GKB, AlzGene, and BioGRID). Enrichment analyses of the identified drug targets genes based on nine well-known pathway-related databases were conducted.

Eighteen potential drug target genes were identified, and thirteen of them had been reported to be closely associated with AD. Enrichment analyses of these identified drug target genes, based on nine pathway-related databases, revealed that four of those identified drug target genes are involved in the classical complement pathway and the process of presenting antigens [43].

Results suggested the combinatorial approach, and the remaining five new targets could enrich our understanding of AD pathogenesis and drug discovery. Moreover, this study supported validity of the combinatorial approach integrating ontology inference with network analysis in the discovery of novel drug target for neurological diseases [43].

V. Microglia, Astrocytes and Mitochondria

Cyclophilin D (CypD) is a mitochondria-specific cyclophilin that plays a pivotal role in the formation of the mitochondrial permeability transition pore (mPTP). The formation and opening of the mPTP disrupts mitochondrial homeostasis, causes mitochondrial dysfunction and eventually leads to cell death. Several recent studies have found that CypD promotes the formation of the mPTP upon binding to Aβ peptides inside brain mitochondria, suggesting that neuronal CypD has a potential to be a promising therapeutic target for Alzheimer's disease [44].

In this study, researchers generated an energy-based pharmacophore model by using the crystal structure of CypD-cyclosporine A (CsA) complex and performed virtual screening of the ChemDiv database, which yielded forty-five potential hit compounds with novel scaffolds. Investigators tested compounds using mitochondrial functional assays in neuronal cells and identified fifteen compounds with excellent protective effects against Aβ-induced mitochondrial dysfunction. To validate whether these effects were derived from

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binding to CypD, surface plasmon resonance (SPR)-based direct binding assays with selected compounds were done. Investigators discovered compound 29 was found to have the equilibrium dissociation constants (KD) value of 88.2 nM. This binding affinity value and biological activity corresponds to the predicted binding mode. The authors conclude that this study offers new insights into the rational design of small molecule CypD inhibitors and provides a promising lead for future therapeutic development [44].

In addition to amyloid-beta plaque and tau neurofibrillary tangle deposition, neuroinflammation is considered a key feature of Alzheimer's disease pathology. Inflammation in Alzheimer's disease is characterized by the presence of reactive astrocytes and activated microglia surrounding amyloid plaques, implicating their role in disease pathogenesis. Microglia in the healthy adult mouse depends on colony-stimulating factor 1 receptor (CSF1R) signaling for survival, and pharmacological inhibition of this receptor results in rapid elimination of nearly all of the microglia in the central nervous system. In this study by Spangenberg & colleagues (2016) [45], investigators wished to determine if chronically activated microglia in the Alzheimer's disease brain are also dependent on CSF1R signaling, and if so, how microglial cells contribute to disease pathogenesis. Ten-month-old 5xfAD mice were treated with a selective CSF1R inhibitor for 1 month. This resulted in elimination of approximately 80% of microglia. Chronic microglial elimination did not alter amyloid-beta levels or plaque load; however, elimination did reduce dendritic spine loss and prevent neuronal loss in 5xfAD mice, as well as reduce overall neuroinflammation. Importantly, behavioral testing revealed improvements in contextual memory. Collectively, these results demonstrate that microglia contribute to neuronal loss, as well as memory impairments in 5xfAD mice, but do not mediate or protect from amyloid pathology [45]. Activated microglia are classified into two specific states: classically activated (M1) and alternatively activated (M2) subtypes. Polarization of M1/M2 phenotype plays an important role in Alzheimer's disease (AD). However, the mechanisms regulating this process remain unclear. In this study, investigators tried to determine the role of milk fat globule epidermal growth factor 8 (MFG-E8). MFG-E8 is a unique protein which can bind to microglia and regulate its inflammatory responses. It is speculated that MFG-E8 may play a role in the balance of microglial polarization. Here, fibril amyloid beta-42 was used in vitro to stimulate mouse primary microglial cultures. Study authors found M1 marker expression, along with retained M2 marker production. It was determined that MFG-E8 pretreatment reversed the increased trend of M1 markers and the decreased expression of M2 markers, which were induced by Aβ 42. Moreover, MFG-E8 effects could be effectively blocked by an MFG E8 antibody. Further analysis on the signaling pathways showed that NF-"B upregulation and Akt downregulation in microglial cultures were observed after Aβ 42 incubation. The alteration of these pathways could also be reversed by MFG-E8 [45]. Next, investigators evaluated the effects of NF-"B and PI3K-Akt on M1/M2 alteration using their specific inhibitors. Pyrrolidine dithiocarbamate, a NF-"B inhibitor, inhibited M1 marker expression; moreover, LY294002, an Akt inhibitor, enhanced M1 marker expression. It appears MFG-E8 plays a regulatory role of microglia M1/M2 alteration providing a basis for understanding the potential role of microglia activation in AD [46].

Recent genetic evidence from Czirr et al. (2017) [47] supports a link between microglia and the complement system in Alzheimer's disease (AD). Here, investigators uncovered a novel role for the microglial complement receptor 3 (CR3) in the regulation of soluble beta-amyloid clearance, independent of phagocytosis. Unexpectedly, ablation of CR3 in human amyloid precursor protein-transgenic mice results in decreased, rather than increased, beta-amyloid accumulation. In line with these findings, cultured microglia lacking CR3 are more efficient than wild type at degrading extracellular beta amyloid by secreting enzymatic factors, including tissue plasminogen activator. Furthermore, in-vivo microdialysis showed a small molecule modulator of CR3 reduces soluble Aβ levels and Aβ half-life in brain interstitial fluid (ISF), These results suggest that CR3 limits beta amyloid clearance from the (ISF) illustrating a novel role for CR3 and microglia in brain Aβ metabolism and defining a potential new therapeutic target in AD [47].

Neuroinflammation is clearly associated with AD pathology, however its role in disease progression is unclear. The authors that review this topic state evidence suggests that Aβ complexes interact with microglial and astrocytic pattern recognition receptors that initiate immunity. This process involves secretion of pro-inflammatory cytokines, chemokines and generation of reactive oxygen species that, in excess, drive a dysregulated immune response that contributes to neurodegeneration. A neuroinflammatory response involving microglial activity, enhanced astrocyte reactivity and elevated pro-inflammatory cytokine and chemokine load has long been implicated in AD and proposed to facilitate neurodegeneration. [48].

Inflammatory components related to AD neuroinflammation include brain microglia and astrocytes, the complement system, as well as cytokines and chemokines. Cytokines play a key role in inflammatory and anti-inflammatory processes in AD. An important factor initiating the inflammatory process is the overexpression of interleukin (IL)-1. Other important cytokines in neuroinflammation are IL-6 and tumor necrosis factor (TNF)-α. By contrast, other cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4, IL-10, and

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transforming growth factor (TGF)-β can suppress both proinflammatory cytokine production and their action, subsequently protecting the brain. Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) decreases AD risk. according to basic research findings. Unfortunately, clinical trials of NSAIDs in AD patients have not provided much insight. Proinflammatory responses may be attenuated by polyphenols. Polyphenol supplementation may provide an alternative to treating Aβ [49].

Microglia modulate synaptogenesis and help repair damage from injury, by restoring neuronal connections. They also release cytokines, which in turn modulate synaptic transmission and potentially restore damaged dendritic spines. This suggests that the microglia could play a prominent role in learning and memory [50]. Thus, the compliment system appears to play many roles in the healthy and diseased brain. Recent evidence shows the complement system is involved with much more than just inflammation but appears to be is involved with regulation of synapse population, during development, normal aging and disease states such as Alzheimer's disease and schizophrenia. It has been shown that complement is directly involved with synapse tagging and elimination or synaptic pruning. In a mouse model of glaucoma, Stevens et al. (2007) demonstrated the complement protein C1q, is expressed by postnatal neurons in response to immature astrocytes [51 52].

The complement cascade provides protection from infection as well as destructive inflammation, as stated above. Complement participates in elimination of neuronal synapses which is essential for proper development. However, elimination of synapses can be detrimental during aging and disease. C1q, required for several of these complement-mediated activities. It is present in the neuropil, microglia, and a subset of interneurons in the brain. [53]

To identify the source(s) of C1q in the brain, investigators selectively inactivated the C1qa gene in the microglia or Thy-1+ neurons in both wild type mice and a mouse model of Alzheimer's disease (AD). C1q synthesis was assessed by immunohistochemistry, QPCR, and western blot analysis.

While C1q expression in the brain was unaffected after inactivation of C1qa in Thy-1+ neurons, the brains of C1qaFL/FL: Cx3cr1CreERT2 mice in which C1qa was ablated in microglia lacked C1q with the exception of limited C1q in subsets of interneurons. The study authors stated "this loss of C1q was surprising since it occurred even in the absence of tamoxifen by 1 month of age". This demonstrated that Cre activity is tamoxifen-independent in microglia in Cx3cr1CreERT2/WganJ mice. C1q expression in C1qaFL/FL: Cx3cr1CreERT2/WganJ mice continued to decline through aging and in AD model mice. No difference in C1q was detected in the liver or kidney from C1qaFL/FL: Cx3cr1CreERT2/WganJ mice relative to controls, and C1qaFL/FL: Cx3cr1CreERT2/WganJ mice had minimal, reduction in plasma C1q.

It was concluded that microglia, but not neurons or peripheral sources, are the dominant source of C1q in the brain [53]. While demonstrating that the Cx3cr1CreERT2/WganJ mice cannot be used for adult-induced deletion of microglia genes the model described enables further investigation of physiological roles of C1q in the brain and identification of therapeutic targets for the selective control of complement-mediated activities contributing to neurodegenerative disorders [53].

A prominent attribute of AD pathogenesis is neuroinflammation. Over-expression of complement proteins co-localizes with neurofibrillary tangles, thereby indicating that a complement system may be involved in neuroinflammation. The authors of the current study suggest this demonstrates, using a microglial cell line, complement activation influences neuroinflammation.

The authors determined the expression levels of the pro-inflammatory factor's TNF-α, IL-1β, and IL-6 and explored whether the neuroinflammatory response, caused by AB 42 treatment of BV-2 cells, was mediated by JAK/ STAT3 signaling [54].

C5a had an enhanced effect on the neural cell viability of BV-2 cells treated with Aβ42. In addition, C5a increased the Aβ-induced neuro-inflammatory response, and these effects were blocked by the C5aR antagonist, PMX205. The neuroinflammatory responses induced by Aβ and C5a were mediated through JAK/STAT3 signaling. By blocking this pathway with an antagonist, AG490, the expression of TNF-α, IL-1β, and IL-6 was also blocked. Thus, the complement protein C5a could exaggerate the Aβ-induced neuroinflammatory response in microglia. The study authors conclude C5aR may be a potential therapeutic tool for AD treatment.[54]

The neurogenic process, consisting of the proliferation, differentiation and maturation of neural stem cells (NSC), is regulated via epigenetic mechanisms by controlling the expression of specific sets of genes. This topic is reviewed by Li et al. (2016), [55]. They reiterate the pathology of AD, due to impairments in epigenetic mechanisms, the generation of neurons from NSCs is damaged, which exacerbates the loss of neurons and the deficits in learning and memory function associated with AD. Based on neurogenesis, a number of therapeutic strategies have shown capability in promoting neuronal generation to compensate for the neurons lost in AD, thereby improving cognitive function through epigenetic modifications. This provides potential for the treatment of AD by stimulating neurogenesis using epigenetic strategies. The epigenetics of AD and adult neurogenesis may provide therapeutic strategies for AD [55].

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Alzheimer's disease markers beta-amyloid plaques and neurofibrillary tangles composed of Aβ peptides and abnormally hyperphosphorylated tau protein are tightly correlated with AD. However, synaptic loss may be a better correlate of cognitive impairment in AD than beta-amyloid or tau pathologies. Thus, one strategy for AD is to shift the balance from neurodegeneration to neuroregeneration and synaptic repair. Kazim & Iqbal (2016) state "neurotrophic factors, by virtue of their neurogenic and neurotrophic activities, have potential for the treatment of AD". But therapeutic use of recombinant neurotrophic factors is limited because of the unfavorable pharmacokinetic properties, poor blood-brain barrier (BBB) permeability, and adverse effects. Neurotrophic factor small-molecule mimetics, offer a potential strategy to improve these limitations and have shown promise in preclinical studies. Neurotrophic factor small-molecule mimetics do show promise for AD drug development [56].

The ciliary neurotrophic factor (CNTF) small-molecule peptide mimetic, Peptide 021 (P021) has also received attention as a potential AD therapeutic. P021 is a neurogenic and neurotrophic compound which enhances dentate gyrus neurogenesis and memory processes via inhibiting leukemia inhibitory factor (LIF) signaling pathway and increasing brain-derived neurotrophic factor (BDNF) expression. It inhibits tau abnormal hyperphosphorylation by enhancing BDNF mediated decrease in glycogen synthase kinase-3 (GSK-3B, major tau kinase) activity. P021 is a small molecular weight, BBB permeable compound with suitable pharmacokinetics for oral administration. It also lacks adverse effects associated with the native CNTF or BDNF molecule. P021 has shown beneficial therapeutic effect in several preclinical studies and has emerged as a promising compound for AD drug development [56].

The practical pharmacogenetics of AD is limited to acetylcholinesterase inhibitors (AChEIs) and memantine. However, pharmacogenetic procedures should be applied to novel strategies in neurotransmitter regulators, anti-Aβ treatments, anti-tau treatments, pleiotropic products, epigenetic drugs and combination therapies. Over 60% of AD patients pathologies demand additional treatments which increase the likelihood of drug-drug interactions. Lipid metabolism dysfunction is common to AD neurodegeneration. The therapeutic response to hypolipidemic compounds is influenced by the APOE and CYP genotypes. It is paramount that the development of novel compounds and the use of combination/multifactorial treatments avoids adverse drug reactions and optimizes therapeutic potential [57].

There has been little success targeting the neurodegenerative aspect of AD. This failure has created interest in neuroregeneration and neural stem cells (NSCs) regeneration. Small molecules offer much potential to manipulate NSCs, and provides therapeutic tools that may prove very useful. Classically, these molecules have been generated either by target-based or phenotypic approaches. To circumvent specific liabilities, development of nanomedicines may offer a viable alternative.

Recent examples that could accelerate development of neuroregenerative drugs against Alzheimer's disease are reviewed by Uliassi et al. (2017) [58].

Novel approaches to AD therapy also include Rho-associated protein kinase (ROCK), a serine-threonine kinase originally identified as a crucial regulator factin cytoskeleton. Recent studies have defined ROCK as a critical component of diverse neuronal signaling pathways. Inhibition of ROCK causes several biological events such as increase of neurite outgrowth, axonal regeneration, and activation of prosurvival Akt. ROCK is a promising therapeutic target for the treatment of neurodegenerative disorders including Alzheimer disease, Parkinson's disease, Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis [59].

VI. Synaptic Density, Tagging and Pruning

Complement protein C1q is localized to synapses in the postnatal CNS and the retina [51]. Importantly, C1q and (downstream) C3 deficient mice express major deficits in CNS synaptic elimination as shown by excess retinal innervation. C1q is localized to synapses during synaptic pruning that occurs in the developing retina and brain. Further experiments by the Steven's lab (Shi et al. 2015) on aged C3 deficient (KO) mice found an additional role of C3 for synaptic maintenance. In hippocampal region CA3, the investigators found aged wild type (WT) mice had showed synaptic loss while the C3 KO animals did not have such loss. In aged WT mice, synaptic loss in CA3 was followed by neuronal loss. Electrophysiology and behavior studies reinforce the loss of CA3 synapses in aged WT mice. [60] [21].

Steven's lab found, relative to aged WT animals, aged C3 KO mice exhibited enhanced LTP, suggesting greater synaptic activity and connectivity. In addition, aged WT mice differed in learning and memory as well as behavior. WT mice were more anxious than C3 KO mice on the plus maze. Finally, CA3 KO mice demonstrated better memory in the water T-maze than aged WT mice, but only upon a test of reversal learning. C3 KO mice, in addition, showed superior context fear conditioning memory, a hippocampal dependent task, during normal aging. In this study, it appears that complement protein C3 or any downstream signaling, may be harmful to synapses in specific brain regions [21].

It is well established that synapse loss is observed in Alzheimer's disease and corresponds to

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cognitive decline. In an Alzheimer's disease mouse model that incorporates beta-amyloid injections to model the disease, C1q is increased and is associated with synapses before amyloid plaques develop, [61]. Inhibition of C1q or C3 reduces both microglia and early synapse loss. In order for beta-amyloid to create toxicity, C1q must be present. Activation errors of the complement dependent pathway and microglia may mediate synapse loss in Alzheimer's disease. Additional studies have identified other complement proteins as mediators of synaptic elimination. Complement protein C4A and C4B have also been linked to synaptic elimination or pruning [62].

Increased complement protein C4 has been observed in previous studies. C4 has two isoforms which are encoded by genes C4A and C4B. AD patients tested for these isoforms, show increased C4A and C4B copy numbers and increased C4 protein expression. This observation suggests C4A and C4B may be possible risk factors for AD [63]. In addition to mediating synapse elimination in the developing CNS, C1q has also been found to increase dramatically (300-fold) in the aged mouse and human [65].The localization of C1q to synapses observed by Steven's et al. (2007) has also been observed by Stephan et al. (2013) [64].

Inhibition of the complement pathway via viable complement inhibitors may offer a new strategy to attack Alzheimer's disease. The complement system's role in learning and memory is becoming a topic of much interest. Irradiation of the hippocampal granule cell layer, attenuates neural progenitor differentiation, presumably due to induced inflammation. To investigate the roles of C3, young C3 -/- mice were subject to irradiation and compared to wild type mice. Once recovered, the C3-/- mice showed 55% more microglia, and tended to demonstrate more proliferating cells in the GCL than WT mice. These results apparently influenced future learning capacity as adult C3-/- mice showed better place learning than WT. Further experiments by the Steven's lab on aged C3 deficient (KO) mice found an additional role of C3 for synaptic maintenance [21].

Understanding spine dynamics further increases understanding of AD cognitive impairment and dementia. Recent studies tracking both spines and synaptic markers in vivo reveal that 20% of spines lack PSD-95 and are short lived. Although they account for most spine dynamics, Berry & Nedvivi (2017) [65] state their remodeling is unlikely to impact long-term network structure. The synaptic tagging and capture (STC) hypothesis has opened new areas of research on how activity-dependent gene products may interact with potentiated synapses maintain long-lasting synaptic plasticity. One candidate in this process is Arc/arg3.1, initially assumed to participate in STC processes during LTP. Accumulating evidence indicates that Arc/arg3.1 might rather contribute in weakening of synaptic weights than in their strengthening [65].

Long-lasting forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) underlie learning and memory. Although Arc/arg3.1 was initially assumed to participate in the STC processes during LTP, accumulating evidence indicates Arc/arg3.1 might weaken rather than strengthen synaptic weights [66].

In particular, analyses of Arc/Arg3.1 protein dynamics and function in the dendrites after plasticity-inducing stimuli have revealed a novel form of inactivity-dependent redistribution of synaptic weights, known as "inverse synaptic tagging." The original synaptic tagging and inverse synaptic tagging likely co- exist and are mutually non-exclusive mechanisms, which may help coordinate the redistribution of synaptic weights and promote the enhancement and maintenance of their contrast between potentiated and non-potentiated synapses during the late phase of long-term synaptic plasticity. Arc/Arg3.1, an immediate early gene product which is captured and preferentially targeted to non-potentiated synapses, may provide insight into synaptic tagging and AD according to Okumo et al. (2017) [66].

Recently, it has been shown that the G9a/GLP complex promotes long-term potentiation (LTP) and its associative mechanisms such as synaptic tagging and capture (STC) [67]. The mechanics of this process are not understood. Regulation of G9a/GLP complex by inhibiting its catalytic activity reverses the amyloid-β oligomer-induced deficits in late-LTP and STC. The authors of this study suggest this reversal is achieved by releasing the transcription repression of the brain-derived neurotrophic factor (BDNF) gene. Catalytic inhibition of the G9a/GLP complex leads to BDNF expression upregulation in brain slices treated with oAβ. This inhibition of the G9a/GLP complex ensures the availability of BDNF that subsequently binds its receptor tyrosine kinase B (TrkB) and maintains the late-LTP. Furthermore, the capture of BDNF by weakly activated synapses re-establishes STC. Sharma et al. (2017) [67] conclude reinstatement of functional plasticity and associativity in AD-like conditions provide the first evidence for the role of G9a/GLP complex in AD. Investigators propose G9a/GLP complex as the possible target for preventing oAβ-induced plasticity deficits in hippocampal neurons [67].

Another method for modulating long-lasting forms of memory and synaptic plasticity is by suppressing microRNA-mediated translational silencing at activated synapses through translin/trax. Mice that lack translin/trax have defective synaptic tagging. An absence of translin/trax prevents post learning upregulation of the protein ARCR1C. In mice lacking translin/trax, long term memory deficits are also induced by inhibiting ARCR1 [68]. Recent reports indicate adeno-associated virus (AAV1 and AAV9) exhibit

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anterograde transsynaptic spread properties. AAV1-Cre from transduced presynaptic neurons effectively and specifically drove CRE-dependent transgene expression in selected postsynaptic neuronal targets, and thus allowed the tracing and functional manipulation of axonal projections from the latter input-defined neuronal population. Application of this tool in superior colliculus (SC) revealed that SC neuron subpopulations receiving corticocollicular projections from auditory and visual cortex specifically, drove flight and freezing, two different types of defense behavior, respectively.[69] Such anterograde transsynaptic tagging is thus useful for forward screening of distinct functional neural pathways embedded in complex brain circuits [68][69].

Recent research indicates that a novel class of signaling molecules, the inositol pyrophosphates, act as energy sensors. These signaling molecules can alter the balance between mitochondrial oxidative phosphorylation and glycolytic flux, affecting ATP at a cellular level. Neuronal inositol pyrophosphate synthesis relies on the activity of the neuron enriched inositol hexakisphosphate kinase 3 (IP6K3) enzyme. In this particular study, investigators tried to verify an involvement of inositol pyrophosphate signaling in neurodegenerative disorders, by tagging single nucleotide polymorphism (SNP) analysis of the IP6K3 gene in patients with familial and sporadic late onset Alzheimer's disease (LOAD). Two SNPs in the 5'-flanking promoter region of the IP6K3 gene were associated with sporadic LOAD. Assessing the functionality of the two polymorphisms by luciferase assay revealed that one of them (rs28607030) affects IP6K3 promoter activity. In this case the activity of the G allele increased. As the same allele may reduce disease risk, it may be related to upregulation of IP6K3 expression, consequently increasing inositol pyrophosphate synthesis. The authors of the study conclude this is the first evidence of genetic variability in the IP6K3 gene altering LOAD pathogenesis [70].

Research implicates the classical complement cascade in normal brain development and in disease. Complement proteins C1q, C3, and C4 participate in synapse elimination, tagging inappropriate synaptic connections between neurons for removal by phagocytic microglia. Neurodevelopmental disorders, such as schizophrenia and autism, are thought to be caused by an imbalance in synaptic pruning, and recent studies suggest that dysregulation of complement could promote this synaptic pruning imbalance [71].Moreover, in the mature brain, if complement is mistakenly activated to stimulate synapse loss, neurodegenerative diseases may result. Similar pathways can also be activated in response to inflammation, as in West Nile Virus infection or in lupus, where peripheral inflammation can promote microglia-mediated synapse loss. Whether synapse loss in disease is a true reactivation of developmental synaptic pruning programs remains unclear; nonetheless, complement proteins represent potential therapeutic targets for both neurodevelopmental and neurodegenerative diseases. Inhibition of the complement system, at specific neurodegenerative stages, could prove to be a viable therapy for AD and schizophrenia [71].

Microglia are glial cells in the central nervous system (CNS) that have well-known roles in neuronal immune function, responding to infections and brain injury and influencing the progress of neurodegenerative disorders. Microglia expend considerable energy continuously making contacts with pre- and postsynaptic elements of neural circuits. Pruning of synapses may be equivocal to “fine-tuning” of neural circuits. Further dysfunction of such a homeostatic role of microglia could be a primary cause of neuronal disease. As such, neuronal functions including cognition, personality, and information processing are affected by immune status. Understanding interactions between microglia and synapses, the possible cellular and molecular mechanisms that mediate such contacts could be of great value towards understanding neurodegenerative diseases such as AD and schizophrenia [72]. Amyloid protein precursor (APP) is involved in synaptic formation and function. In the human and rodent cingulate cortex, APP is preferentially located in the presynaptic active zone, indicating subsynaptic APP distribution is conserved across species and brain regions. Synaptic APP immunoreactivity decreases in aged cortical samples in deceased males (20-80 years of age). In contrast, the synaptic levels of "alpha-secretase (ADAM10) and beta-secretase (BACE1) did not significantly change. Decreased APP levels may be related to reduced allostasis of synapses in the aged brain and the greater susceptibility of neurodegenerative disorders [73]. Actin-regulating proteins are essential in regulating the shape of dendritic spines, which are sites of neuronal communication. Age related neurodegeneration is attributed to, in part, cofilin and related actin-regulating proteins. The analysis of cofilin motility in dendritic spines using fluorescence video-microscopy may help us understand synaptic functions. To date, the flow of cofilin has not been analyzed by automatic means. Dendrite Protein Analysis (Dendrite PA), a novel automated pattern recognition software may help analyze protein trafficking in neurons [74]. Using spatiotemporal information present in multichannel fluorescence videos, the Dendrite PA generates a temporal maximum intensity projection that enhances the signal-to-noise ratio of important biological structures, segments and tracks dendritic spines, estimates the density of proteins in spines, and analyzes the flux of proteins through the dendrite/spine boundary. According to On et al. (2017), the motion of a dendritic spine is used to generate spine energy images, which are then used to automatically classify the shape of

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common dendritic spines such as stubby, mushroom, or thin. By tracking dendritic spines over time and using their intensity profiles, the system can analyze the flux patterns of cofilin and other fluorescently stained proteins. The cofilin flux patterns correlate with the dynamic changes in dendritic spine shapes. Results also have shown that the activation of cofilin using genetic manipulations leads to immature spines while its inhibition results in an increase in mature spines [74].

Understanding synaptic protein turnover is not only important for determining fundamental aspects of learning and memory, but also has direct implication for understanding pathological conditions like aging, neurodegenerative diseases, and psychiatric disorders. Proteins involved in synaptic transmission and synaptic plasticity are typically concentrated at synapses of neurons and thus appear as puncta (clusters) in immunofluorescence microscopy images. Quantitative measurement of the changes in puncta density, intensity, and sizes of specific proteins provides valuable information on their function circuit development, synaptic plasticity, and synaptopathy. Puncta quantification is time and labor intensive. Recently a software tool has been described that is designed for the rapid semi-automatic detection and quantification of synaptic protein puncta from 2D immunofluorescence images generated by confocal laser scanning microscopy. The software, dubbed as SynPAnal (for Synaptic Puncta Analysis), streamlines data quantification for puncta density and average intensity, thereby increases data analysis throughput compared to a manual method. SynPAnal is stand-alone software written using the JAVA programming language, and thus is portable and platform-free. This new tool has the potential to greatly accelerate understanding of synaptic dynamics in aging and AD [75].

It is well known that chronic stress can induce maladaptive neurophysiological changes, ultimately leading to cognitive impairment. Senescence-accelerated mouse prone 8 (SAMP8) is a naturally occurring animal model that is useful for investigating the neurological mechanisms of chronic stress and Alzheimer's disease.

In this study SAMP8 mice were exposed to unpredictable chronic mild stress (UCMS) for 4 weeks. Then, these mice performed the Morris Water Maze (MWM) test to assess the effect of UCMS on learning and memory. The effects of UCMS on cognition in mice, were evaluated by measuring changes in postsynaptic density 95 (PSD95) and synaptophysin (SYN) proteins, known to be essential for synaptic plasticity.

The Morris water maze experiment revealed that the cognitive ability of the SAMP8 mice decreased with brain aging, and that chronic stress aggravated this cognitive deficit.

Decreased cognition and synaptic plasticity are related to aging, an unsurprising effect. However chronic stress aggravated this cognitive deficit while decreasing SYN and PSD95 expression in the SAMP8 mice. Neurological mechanisms of chronic stress on cognition might be associated with a decrease in hippocampal SYN and PSD95 expression, which may make the SAMP8 mice a valuable model for studying the relationship between aging, synaptic plasticity and stress [76].

Wang et al (2016) focused on how dihydrotestosterone (DHT) regulates synaptic plasticity in the hippocampus of mild cognitive impairment male senescence-accelerated mouse prone 8 (SAMP8) mice. Five-month-old SAMP8 mice were divided into control castrated and castrated-DHT groups, in which the mice were castrated and treated with physiological doses of DHT for a period of 2 months. To determine the regulatory mechanisms of DHT in the cognitive capacity, the effects of DHT on the morphology of the synapse and the expression of synaptic marker proteins in the hippocampus were investigated using immunohistochemistry, qPCR and western blot analysis. The results showed that the expression of cAMP-response element binding protein (CREB), postsynaptic density protein 95 (PSD95), synaptophysin (SYN) and developmentally regulated brain protein (Drebrin) was reduced in the castrated group compared to the control group. However, DHT promoted the expression of CREB, PSD95, SYN and Drebrin in the hippocampus of the castrated-DHT group. Thus, androgen depletion impaired the synaptic plasticity in the hippocampus of SAMP8 and accelerated the development of (AD)-like neuropathology, suggesting that a similar mechanism may underlie the increased risk for AD in men with low testosterone. In addition, DHT regulated synaptic plasticity in the hippocampus of mild cognitive impairment (MCI) SAMP8 mice and delayed the progression of disease to Alzheimer's dementia. The study authors conclude androgen-based hormone therapy is a potentially useful strategy for preventing the progression of MCI in aging men. Androgens enhance synaptic markers (SYN, PSD95, and Drebrin), activate CREB, modulate the fundamental biology of synaptic structure, and lead to the structural changes of plasticity in the hippocampus, all of which result in improved cognitive function [77].

Mitochondrial dysfunction, oxidative stress and beta-amyloid formation are believed to contribute to to neuronal and synaptic degeneration underlying cognitive decline in Alzheimer's disease (AD). The senescence-accelerated mouse-prone 8 (SAMP8) mice are well characterized aging models for mechanistic and translational research for AD. The present study by Jia et al. (2016) [77] characterized mitochondrial and synaptic alterations in SAMP8 mice relative to SAMR1 control mice. This study explored the protective effect of the small molecule peptide SS31, a cell membrane penetrant antioxidant, on mitochondrial and synaptic

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protein integrity as well as cognitive performance. Electron microscopic analysis determined mitochondrial/synaptic deterioration in 10 months old SAMP8 relative to SAMR1 mice. SAMP8 changes following 8 weeks treatment with SS31 (5 mg/kg/day, i.p.) Hippocampal lysates in SAMP8 mice relative to SAMR1 revealed elevation of beta amyloid 42, mitochondrial fission protein (DLP1, Fis1) and matrix protein cyclophilin D (CypD). In addition, lysates showed reductions of mitochondrial fusion protein (Mfn2) and synaptic (i.e., synaptophysin, postsynaptic density protein 95 and growth associated protein 43) proteins [78]. These altered protein expressions in the SAMP8 mouse brain were restored with the SS31 treatment. Moreover, the SS31 treatment rescued learning and memory deficits detected in10 month-old SAMP8 mice. Study authors conclude these findings suggest that this mitochondria-targeting antioxidant peptide may be of potential utility for AD therapy, including a possible lowering of central AB levels and protection of mitochondrial homeostasis and synaptic integrity, which may help slow down cognitive decline [78]. Neurexin1 (Nrxn1) and Neuroligin3 (Nlgn3) are cell adhesion proteins, which are important to age-related synaptic plasticity decline. However, the expression of these proteins during aging has not been thoroughly analyzed. In the study by Kumar and Thakur (2015) investigators measured the age-related changes in the expression of these proteins in cerebral cortex and hippocampus of 10-, 30-, 50-, and 80-week-old male mice. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis indicated that messenger RNA (mRNA) level of Nrxn1 and Nlgn3 significantly increased from 10 to 30 weeks and then decreased at 50 weeks in both the regions. However, in 80-week-old mice, Nrxn1 and Nlgn3 were further downregulated in cerebral cortex while Nrxn1 was downregulated and Nlgn3 was upregulated in hippocampus. These findings were corroborated by immunoblotting and immunofluorescence results. When the expression of Nrxn1 and Nlgn3 was correlated with presynaptic density marker synaptophysin, it was found that synaptophysin protein expression in cerebral cortex was high at 10 weeks and decreased gradually up to 80 weeks. In hippocampus, it decreased until 50 weeks and then increased remarkably at 80 weeks. Furthermore, Pearson's correlation analysis showed that synaptophysin had a strong association with Nrxn1 and Nlgn3 in cerebral cortex and with Nlgn3 in hippocampus. The findings showed that Nrxn1 and Nlgn3 are differentially expressed in cerebral cortex and hippocampus which might be responsible for alterations in synaptic plasticity during aging. These finding warrant continued Nrxn1, Nlgn3 research in aged cerebral cortex and hippocampus [79].

Major histocompatibility complex class I (MHCI) proteins may modulate synaptogenesis, synaptic plasticity, and memory consolidation during development. Ultrastructural analyses revealed a decrease in spine head diameter and post synaptic density (PSD) area, as well as an increase in overall synapse density, and non-perforated, small spines [80].

There is increasing evidence for the role of the major histocompatibility complex class I (MHCI), a protein complex best known for antigen presentation and immunological surveillance in the adaptive immune system, in a second function within the central nervous system (CNS). Lazarczyk et al. (2016) stated "Originally, the brain was considered to be 'immunologically privileged', with low expression of MHCI unless evoked in response to traumatic injury or functional impairment in learning and memory". We now know MHCI is expressed on neurons during development and early adulthood in brain regions including the neocortex, hippocampus, spinal motoneurons, and substantia nigra. In the developing CNS, MHCI has been shown to modulate synaptic plasticity, axonal and dendritic morphogenesis, and neuronal polarity all functions that are completely distinct from its role in the peripheral immune system [80].

In rat hippocampus, increasing neuronal expression of MHCI and the following associated proteins: such as β2-microglobulin (β2M), transporter associated with antigen processing (TAP), paired immunoglobulin-like receptor B (PirB), and killer cell lectin-like receptor (Klra; also known as Ly49) causes an association with cognitively impaired as well as cognitively intact aged rats compared to adult rats. However, MHCI expression in humans appears to be significantly increased in cognitively intact oldest-old (≥87 years of age) individuals and decreased in cognitively impaired oldest-old, relative to younger-old (≤86 years of age) cognitively intact individuals. Moreover, recent genome association studies found alleles of human leukocyte antigen A, one of the three human MHCI genes, associated with increased risk of Alzheimer's disease. Lazarczyk concludes, "depending on the species and the cognitive tasks assessed, an age-related increase in MHCI is important in regulating and preserving cognitive function and thus may be a crucial mechanism for maintaining memory function associated with successful aging". As variability in neuronal and spine morphology has been associated with memory formation and cognitive function, MHCI may regulate memory and cognition through the formation and/or elimination of synapses, similar to its developmental function. MHCI is known to modulate excitatory glutamate receptor function. Altered activity of these receptors has been linked to dendritic spine clustering and their expression at the synapse correlated with age-related cognitive decline [80].

Several neuropsychiatric disorders are associated with cognitive and social dysfunction. Post-mortem studies of patients with schizophrenia by

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Piskorowski et al. (2016) [81] have revealed specific changes in Area CA2, a hippocampal recently found to be critical for social memory formation. To examine how Area CA2 is altered in psychiatric illness, investigators used the Df(16)A+/− mouse model of the 22q1 microdeletion, a genetic risk factor for developing several neuropsychiatric disorders, including schizophrenia. Several age-dependent CA2 alterations were reported: a decrease in the density of parvalbumin-stained interneurons, a reduction in the amount of feed-forward inhibition and a change in CA2 pyramidal neuron intrinsic properties. Results show that Area CA2 is less plastic in Df(16)A+/mi ce, making it nearly impossible to evoke action potential firing in CA2 pyramidal neurons and Df(16)A+/− mice display impaired social cognition, providing a potential mechanism and a neural substrate for this impairment in psychiatric disorders [81].

Also in hippocampus, Mulholland et al (2018) [83] have recently shown that Donepezil changes dendritic spine density and morphology in alcohol exposed adolescent rats. When these alcohol exposed rats were treated with Donepezil as adults, dendritic spine alterations and epigenetic modifications were reversed. This raises the prospect that AD patients with a history of alcohol use and/or abuse may respond differently to their non-drinking AD cohorts and these differences are due to dendritic spine morphology [82].

Synapse density is reduced in postmortem cortical tissue from schizophrenia patients, which indicates increased synapse elimination takes place. In this important study by Sellgren & Gracias (2019) [83], investigators used a reprogrammed in vitro model of microglia-mediated synapse engulfment. They demonstrated increased synapse elimination in patient-derived neural cultures and isolated synaptosomes. This excessive synaptic pruning reflects abnormalities in both microglia-like cells and synaptic structures. Schizophrenia risk-associated variants within the human complement component 4 locus are associated with increased neuronal complement deposition and synapse uptake. This observation, however, does not completely explain the increase in synapse uptake. Additionally, the antibiotic minocycline reduces microglia-mediated synapse uptake in vitro and is associated with a decrease in schizophrenia risk compared to other antibiotics in a cohort of young adults. The authors conclude preventing excessive pruning may be one strategy for delaying or preventing the onset of schizophrenia in high-risk individuals [83]. The importance of this insight cannot be understated. Synapse development, growth and elimination are dynamic processes that continue throughout life. Synaptic elimination, or pruning, is important for removing weak, damaged or unnecessary synapses from the brain. Synaptic elimination is modulated by neuronal activity. Recently, the classical complement cascade has been implicated in promoting synaptic pruning. Specifically, microglial cells recognize activated complement component 3 (C3) bound to synapses targeted for elimination, thus facilitating their removal.

The authors point out as this is a highly relevant process for adequate neuronal functioning, disruptions or exacerbations in synaptic pruning could lead to severe circuitry alterations that could underlie neuropathological alterations typical of neurological and neuropsychiatric disorders. This, as has been previously alluded to, raises the possibility that excessive synaptic elimination in AD may involve or be associated with compliment proteins. In turn, this process of complement mediated pruning could involve microglia activity. Further studies are likely to continue connecting the role of the complement cascade and C3 to AD dynamics. This raises the possibility that a complement-based therapy could be developed as a new target for AD [83] [84]. This review covered the following topics:

- AD epidemiology
- Current FDA approved drugs (treat symptomology)
- Mild Cognitive Impairment
- Conversion of MCI to AD
- Genetic and Biologic Markers in AD
- New targets
- The role of neuroinflammation in AD
- Factors and systems that influence inflammation including the complement system
- Complement system's direct involvement in AD including a role in Beta-Amyloid and Tau Pathology
- Complement inhibition in AD modulation and prevention

We began by discussing AD demographics, growth curves and current cost of the disease. While many clinical trials are underway, with many different targets, we underscored the fact that currently there are only a handful of FDA approved drugs, and none address prevention, just symptomatic treatment. We addressed MCI and conversion of MCI to AD, relevant biological and genetic markers that may predict conversion to AD and help define AD itself. These biological and genetic markers may provide new drug or treatment targets in the future. We then changed our focus to inflammation in AD and the role that the complement cascade plays in inflammation, thus setting the stage for complement's potential role in AD. It is known that complement also plays a role in beta-amyloid and tau pathology, thus increasing the potential influence of complement to AD. Finally, we discussed complement inhibition specifically and AD modulation and prevention.

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