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Hypothesis

Do Microglia Default on Network Maintenance in Alzheimer's Disease?

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Abstract. Although the cause of Alzheimer's disease (AD) remains unknown, a number of new findings suggest that the immune system may play a critical role in the early stages of the disease. Genome-wide association studies have identified a wide array of risk-associated genes for AD, many of which are associated with abnormal functioning of immune cells. Microglia are the brain's immune cells. They play an important role in maintaining the brain's extracellular environment, including clearance of aggregated proteins such as amyloid-β (Aβ). Recent studies suggest that microglia play a more active role in the brain than initially considered. Specifically, microglia provide trophic support to neurons and also regulate synapses. Microglial regulation of neuronal activity may have important consequences for AD. In this article we review the function of microglia in AD and examine the possible relationship between microglial dysfunction and network abnormalities, which occur very early in disease pathogenesis.

Keywords: Microglia, network abnormalities, neural networks, phagocytosis, synapse pruning

INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disease that primarily affects the regions of the brain that are associated with high functioning. AD is characterized by progressive dementia that begins with mood changes, memory loss, and reduced cognition [1]. The primary pathogenic process in AD is the accumulation of amyloid-β protein (Aβ) [1–3]. Aβ aggregates into extracellular amyloid plaques that are a hallmark pathological feature of the disease. Aβ is cleaved from the larger amyloid-β protein precursor (AβPP) [4–6]. However, it remains unclear why Aβ, a protein fragment normally only present in small amounts within the brain, is able to accumulate in the AD brain and cause toxicity. In a small percentage (5%) of AD sufferers, the cause of the disease is genetic. Inherited mutations within the AβPP gene itself appear to predispose the protein to Aβ production [7]. Mutations within the presenilin 1 and 2 genes, encoding proteins that form part of the secretase complex that cleaves the Aβ peptide from AβPP, also result in inherited AD due to accumulations of Aβ [8–11].

The cause of AD is largely unknown for the remaining 95% of cases of sporadic AD, which typically develops a decade or two later than familial AD [12]. However, the degenerative processes are nearly identical between the two forms of the disease. Therefore, it is reasonable to assume that the underlying disease process is the same between the two forms of the disease. Genetic studies have identified a number of genetic risk factors for AD. An early discovery was that allelic variants of apolipoprotein E (ApoE) carry inherently different risks of AD [13–15]. In particular, the ε4 allele carries a high risk of AD, with risk of disease occurring in a dose-dependent manner based on

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zygosity. The ApoE ε4 allele is associated with increased Aβ aggregation, reduced lipid transport, and reduced receptor-mediated Aβ clearance [16]. Interestingly, ApoE is predominantly expressed by non-neuronal cells—astrocytes and microglia, rather than by neurons [17]. These findings suggested that although clinical AD manifests from neuronal degeneration, other cells of the central nervous system (CNS) may be intimately involved in pathogenesis or disease progression.

More recently, genome-wide association studies (GWAS) have been used to identify a large number of risk genes for AD. In 2013, a mutation in the triggering receptor expressed on myeloid cells 2 (TREM2) was identified [18, 19]. TREM2 is almost exclusively expressed by immune cells within the brain, and mutations to TREM2 are associated with decreased phagocytosis and an increased pro-inflammatory reactive phenotype. Individuals heterozygous for TREM2 mutations have a high risk of developing AD, however the mutation is rare [18, 19]. Additional AD risk factor genes that have been identified include genes associated with lipid processing, endocytosis, and the immune response, which have recently been covered in excellent reviews [20, 21]. The common unifying feature of these immune-associated mutations is that they are proposed to interfere with microglial function, in particular, the efficiency of phagocytosis [20]. Specifically, mutations to complement receptor 1 (CR1) and cluster of differentiation 33 (CD33) can result in reduced activity of the complement system and reduced phagocytosis [22, 23]. Phosphatidylinositol binding clathrin assembly protein (PICALM) and bridging integrator 1 (BIN1) mutations affect clathrin-mediated endocytosis [24, 25] and SORL1 mutations reduce intracellular trafficking of AβPP [26]. The function of some of these proteins in relation to phagocytosis is discussed later in this review. The identification of such a wide array of risk genes associated with reduced immune cell function now leads us to believe that abnormal functioning of immune cells may play a more important role in the early stages of disease than previously considered.

MICROGLIA

Microglia are the immune cells of the CNS and account for approximately 10% of the CNS cell population, with regional variation in density [27, 28]. During embryonic development, microglia originate from yolk sac progenitor cells that migrate into the developing CNS during early embryogenesis [29,30]. Following construction of the blood-brain barrier (BBB), microglia are renewed by local turnover [31]. In the healthy brain, microglia actively support neurons through the release of insulin-like growth factor 1, nerve growth factor, ciliary neurotrophic factor, and brain-derived neurotrophic factor (BDNF) [32–34]. Microglia also provide indirect support to neurons by clearance of debris to maintain the extracellular environment, and phagocytosis of apoptotic cells to facilitate neurogenesis [35, 36]. In the adult brain, microglia coordinate much of their activity with astrocytes and activate in response to similar stimuli [37, 38]. Dysfunctional signaling between microglia and astrocytes often results in chronic inflammation, a characteristic of many neurodegenerative diseases [39, 40].

Historically, it has been thought that microglia 'rest' when not responding to inflammatory stimuli or damage [41, 42]. However, this notion is being increasingly recognized as inaccurate [43]. When not involved in active inflammatory signaling, microglia constantly patrol the neuropil by extension and retraction of their finely branched processes [44]. Microglial activation is often broadly classified into two states; pro-inflammatory (M1) or anti-inflammatory (M2) [36, 45], based on similar phenotypes in peripheral macrophages [46]. M1 activated microglia are characterized by increased expression of pro-inflammatory mediators and cytokines, including inducible nitric oxide synthase, tumor necrosis factor-α, and interleukin-1β, often under the control of the transcription factor nuclear factor-κB [45]. Pro-inflammatory microglia rapidly retract their processes and adopt an amoeboid morphology and often migrate closer to the site of injury [47]. Anti-inflammatory M2 activation of microglia, often referred to as alternative activation, represents the other side of microglial behavior. Anti-inflammatory activation is characterized by increased expression of cytokines including arginase 1 and interleukin-10, and is associated with increased ramification of processes [45]. The polarization of microglia into M1 or M2 throughout the brain is well characterized, especially in neurodegenerative diseases [48]. In the AD brain, microglia expressing markers of M1 activation are typically localized to brain regions such as the hippocampus that are most heavily affected in the disease [49]. However, it is important to note that M1 and M2 classifications of microglia may over-simplify microglial

phenotypes and may only represent the extremes of microglial activation [50]. It has been more recently proposed that microglia likely occupy a continuum between these phenotypes [39, 51].

Do microglia have multiple roles in AD?

Classical pro-inflammatory activation of microglia has long been associated with AD [39, 49]. Samples taken from late-stage AD brains contain characteristic signs of inflammation, including amoeboid morphology of microglia, high levels of pro-inflammatory cytokines in the cerebrospinal fluid, and evidence of neuronal damage due to chronic exposure to pro-inflammatory cytokines and oxidative stress [52, 53]. The cause of this inflammation may be in response to direct toxicity of Aβ to neurons resulting in activation of nearby microglia and astrocytes [53, 54]. However, Aβ may also induce inflammatory activation of microglia and astrocytes. Activated immune cells are typically present surrounding amyloid plaques [55–57], with such peri-plaque cells exhibiting strong evidence of pro-inflammatory activation [56, 58–60]. The presence of undigested Aβ particles within these activated microglia may suggest that the Aβ peptide itself is a pro-inflammatory signal for microglia [61–64]. *In vitro* experiments provide supporting evidence for the *in vivo* studies, with Aβ promoting pro-inflammatory microglial activation [65, 66], and also acting as a potent chemotactic signal [67].

However, it is important to note that although widespread inflammation is characteristic of late-stage AD, it remains unclear what role inflammation could play in early stages of the disease. Some evidence suggests that reducing inflammation through the long-term use of some non-steroidal anti-inflammatory drugs (NSAIDs) can reduce the risk of AD [68]. However, these findings have not yet been verified in clinical trials [69, 70]. Little is understood about how NSAIDs and related compounds affect the delicate balance of pro- versus anti-inflammatory microglial activity within the brain. Although there is considerable evidence to suggest that chronic inflammation may contribute to pathology in the later stages of AD, it is important to note that inflammation normally only represents a small aspect of microglial function. The non-inflammatory functions of microglia may play a more important role in early disease; specifically, microglial functions relating to maintenance of the CNS.

Phagocytosis: A vital role of microglia that may be lost in AD

Phagocytosis is a complex process involving the recognition, engulfment, and degradation of particles larger than 0.5 μm [71]. Although most cell types have the capacity to phagocytose, it is normally the role of highly specialized cells, predominantly those of the immune cell lineage [72]. Within the brain, microglia perform the bulk of phagocytosis, although astrocytes also contribute [37]. Many peripheral phagocytic cells have a preferred target for phagocytosis; however, microglia are most like their peripheral cousins, macrophages, and readily ingest a wide array of structures, including dead cells, invading pathogens and extracellular proteins [45].

Microglial phagocytosis is triggered by a number of stimuli, for which microglia express a wide variety of receptors. Release of adenosine triphosphate (ATP) from apoptotic or damaged cells is a potent signal for microglial phagocytosis, triggering the purinergic receptor $P2Y_2$ [73]. Similarly, fractalkine, recognized by the microglial receptor CX3CR1, is released by damaged cells and promotes microglial phagocytosis [74]. Microglia also express a wide array of 'scavenging receptors', defined by having a relatively broad range of ligand targets [75], and receptors for various components of the complement system, including complement C1q and complement C3, the receptor for which is known by many names including CR3, CD11b, MAC1, and integrin α_m [36]. A number of scavenging receptors have been proposed to interact with Aβ peptides, including receptor for advanced glycation products, macrophage scavenger receptor with collagenous structure, scavenger receptor A-1 (SCARA-1), SCARB-1 and SCARB-2/CD36 [76–80]. However, it is important to note that these receptors also interact with a broad range of other phagocytic ligands, suggesting that microglia detect Aβ in a non-specific manner.

Phagocytosis of Aβ by immune cells, including microglia, is proposed to assist with clearance of Aβ from the brain [81]. Other mechanisms of Aβ clearance include extracellular proteolysis by Aβ-degrading enzyme and neprilysin, astrocyte-mediated interstitial bulk-flow [81], and potentially direct clearance into the lymphatic system [82]. Microglia in AD appear to have a reduced capacity for Aβ clearance which is likely to result in additional accumulation of Aβ [83]. The TREM2 mutation that increases the risk of AD reduces the normal inhibitory effect of TREM2 on microglial

activation and thereby increases microglial pro-inflammatory signaling [84, 85]. This leads to reduced Aβ uptake. Similarly, mutations in complement receptor CR1 and CD33 are associated with an increased risk of AD [86, 87]. These mutations are proposed to cause a loss of receptor activity, thereby reducing phagocytosis of their respective ligands. Other putative genetic risk alleles for AD include the lipid transporter ATP-binding cassette subfamily A member 7 (ABCA7) and interleukin-1 receptor accessory protein (IL-1RAP) [88, 89]. Mutations in ABCA7 can cause a loss of receptor activity, resulting in reduced microglial phagocytic function [88, 90]. Similarly, an intronic single-nucleotide polymorphism identified in IL-1RAP is proposed to reduce expression of IL-1RAP, resulting in reduced microglial activation and phagocytosis [89]. The activity of microglia in the CNS is affected by a wide range of stimuli and is also dependent on the composition and function of surface receptors. These results highlight how minor changes to a number of microglial receptors have major implications in the context of disease and lead us to consider how these changes to microglial function increase the risk of AD, a disease that is characterized not only by accumulation of Aβ, but also synapse loss and neuronal dysfunction.

SYNAPTIC PRUNING: MICROGLIA CAN REGULATE NETWORK ACTIVITY

Recently, a new function has been proposed for microglia. A number of studies have provided evidence that microglia prune synapses throughout life. Microglia are known to remove extraneous synapses during development to ensure that only meaningful connections remain [43]. It was, however, thought that differentiated astrocytes performed the majority of synaptic pruning in the adult brain [91]. The discovery that microglial processes are constantly active within the brain and are often positioned near synapses raised the question of whether microglial synaptic pruning continued throughout life [44, 47, 92–94]. This question was answered in 2014 in a study that demonstrated that microglia do prune synapses into adulthood, and that this activity is important for normal brain function [95]. These findings supported those found a year earlier in a study reporting that ablation of microglia from brain slices increases synapse density and results in abnormal firing of hippocampal neurons [96].

Astrocytes have long been known to have important roles in synaptic maintenance and are considered to be as much a part of the synapse as pre- and post-synaptic neurons. Hence the term 'tripartite synapse' was coined for the structure [97]. Microglia are increasingly considered to be equally important at the synaptic structure, forming a key component of a 'quadripartite synapse', comprising pre- and post-synaptic neurons, astrocytes, and microglia [98]. Microglial synaptic pruning is proposed to occur alongside astrocytic synaptic pruning and occurs in response to astrocyte-derived signals including transforming growth factor-β and the complement proteins C1q and C3 [98–101].

Microglia prune synapses as an extension of normal phagocytosis. Synapses to be cleared are tagged with complement proteins [101], expressed by the neurons under the direction of astrocytes [99]. Microglial synaptic pruning is dependent on neuronal activity [101]. Active synapses are protected by expression of C1q on the membrane, whereas quiet synapses express C3 that triggers C3 cleavage and binding of C3 to CR3 on microglia. CR3 activation results in microglial phagocytosis of the synapse [99, 101]. Deletion of complement C1q or C3 in mice reduces synaptic loss during postnatal development, resulting in increased synaptic density within the cortex and hippocampus [102, 103], an effect that lasts with aging [104]. Conversely, activation of CR3 during inflammation and hypoxia induces long-term depression in the hippocampus [105]. These findings highlight the importance of complement regulation for normal synaptic maintenance; however there are additional mechanisms that may also be utilized by microglia to regulate synapses. Neuronally-derived fractalkine appears to regulate synaptic strength independently of electrical activity. Selective activation of the fractalkine receptor CX3CR1 increases synaptic strength [106], whereas deficiency in CX3CR1 results in impaired long-term potentiation and reduced hippocampal synaptic plasticity [107].

Multiple elements of the complement cascade are expressed in AD brains [108]. The complement system has also been implicated in AD because a number of risk mutations involve complement genes, including CR1 and CD33. Current findings suggest that CR1 mutations are associated with reduced inhibition of phagocytosis [109]. Reducing C3 in AD transgenic mice reduces microgliosis and increases cerebral Aβ load [110]. Elevated complement is also a feature of epilepsy, Huntington's disease, Parkinson's disease, and multiple sclerosis [111, 112].

Although the use of the complement system appears to be the primary mechanism for synaptic pruning, other signaling pathways have also been described. Neuronal expression of PirB, a major histocompatibility class I receptor, controls synaptic plasticity by promoting synaptic pruning by microglia [113, 114]. Interestingly, Aβ is proposed to activate PirB resulting in increased synaptic pruning [115], an interaction that may be responsive to pharmacological intervention. Furthermore, microglial release of BDNF promotes synaptic pruning through the induction of tyrosine receptor kinase B autophosphorylation and downstream signaling [116]. Specific depletion of BDNF expression in microglia results in learning and memory impairments [116]. Circadian expression of cathepsin S, a microglial-specific lysosomal cysteine protease, is associated with modulation of cortical neuron activity [117]. Increased cathepsin S secretion by microglia during the awake phase of mice (dark hours) reduces synaptic neurotransmission by cortical neurons to strengthen synapses [117]. Ablation of cathepsin S induces hyperexcitability and loss of coordinated neuronal activity. A common thread to these findings is that disruption of microglial signaling either through deletion of microglia or impairment of key receptors, results in similar functional deficits. In particular, microglial abnormalities dramatically impair normal hippocampal function, which may have direct consequences for the coordination of neuronal activity such as during memory consolidation.

Altered microglial behavior may underlie altered neuronal firing in AD

Although we lack information about how microglia may affect network function in early AD, there is evidence to suggest that altering microglial function has direct consequences for neuronal activity (Fig. 1). Targeted deletion of key microglial receptors reduces the capacity of the mice to learn tasks, specifically those involving hippocampal learning [107, 116]. Reduced developmental synaptic pruning in mice results in overall decreased functional connectivity in the brain and autism-like behaviors including deficits in social interaction and increased repetitive activity [118]. These behavioral effects occur due to reduced functional brain connectivity and weak synaptic neurotransmission as a result of reduced microglial pruning of synapses. In hippocampal tissue slices, depletion of microglia is followed by increased synaptic density and excitatory postsynaptic currents, reversible upon replenishment of cultures with microglia [96].

Altering microglial activity by exposure to pro-inflammatory stimuli results in changes to synaptic activity. Pro-inflammatory activation of microglia with lipopolysaccharide (LPS) in hippocampal slice preparations results in a rapid increase in excitatory postsynaptic currents in neurons, mediated by astrocytic activation [38]. Inflammation has also been shown to trigger a regional-specific increase in neuronal spines, specifically thin dendritic spines that are associated with plasticity [119]. Microglia

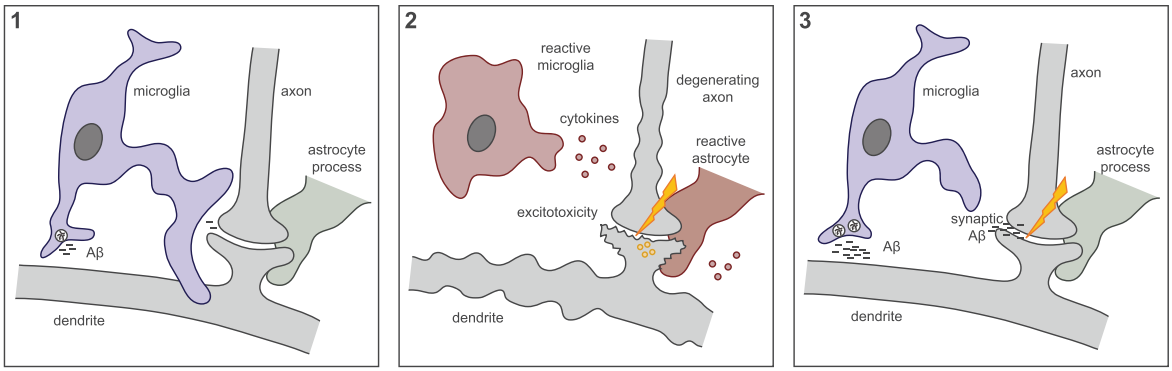

Fig. 1. Increased microglial reactivity or increased protein clearance may reduce microglial synaptic maintenance. (1) Microglia monitor and prune CNS synapses throughout life in conjunction with peri-synaptic astrocytes. Microglia also phagocytose small amounts of extracellular protein such as Aβ, although it is not yet known whether microglia perform both phagocytic tasks simultaneously. Dysfunctional microglial activity at the synapse may produce one of the two following scenarios. (2) Increased pro-inflammatory activation of microglia results in increased production of inflammatory cytokines, co-activating peri-synaptic astrocytes, resulting in neuronal excitability and degeneration. (3) Alternatively, increased demands on microglial phagocytosis, such as elevated Aβ production, may reduce synaptic maintenance and result in increased Aβ at the synapse.

also increase release of BDNF following LPS exposure [120]. Activation of microglial CR3 during hypoxia has been shown to increase hippocampal long-term depression via nicotinamide adenine dinucleotide phosphate (NADPH) signaling and internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) GluR2 subunits [105]. These findings suggest that activation of microglia can alter both synapses and neuronal activity.

Altered neuronal activity is an early phenomenon in AD

Increasingly, altered network activity is considered a feature of AD, specifically, early dysfunction within the default mode network (DMN). The DMN coordinates introspective thought such as day-dreaming and self-referential thought and is normally deactivated during focused thought on external tasks [121, 122]. Imaging of white matter using diffusion tensor imaging [123] or measurement of neuronal activity through glucose metabolism [124] demonstrates that the DMN is consistently hypoactive in AD. Such DMN hypoactivity may represent a diagnostic marker for AD [125]. As AD worsens, network abnormalities progress through to interconnected regions including the hippocampus, dorsal attention network, salience network, sensorimotor network, and executive control network [126]. These network abnormalities closely correlate with increasing symptom severity [126]. A small number of studies have demonstrated that these regions that are specifically affected in AD are also characterized by an increase in activated microglia [127, 128]. It must be noted that some of the changes in neuronal activity that are seen in AD may be due to synaptic toxicity of Aβ [129]. Aβ has been demonstrated to cause damage to synapses and predispose neurons to hyperexcitability and excitotoxicity [129, 130]. Specifically, Aβ binds to subunits of AMPA and *N*-methyl-D-aspartate (NMDA) excitatory receptors and causes increased calcium influx into the neuron [131–133]. Aβ may reduce inhibitory synapses [134], and may directly perturb astrocyte-neuron signaling, on which neurons are typically heavily reliant [135].

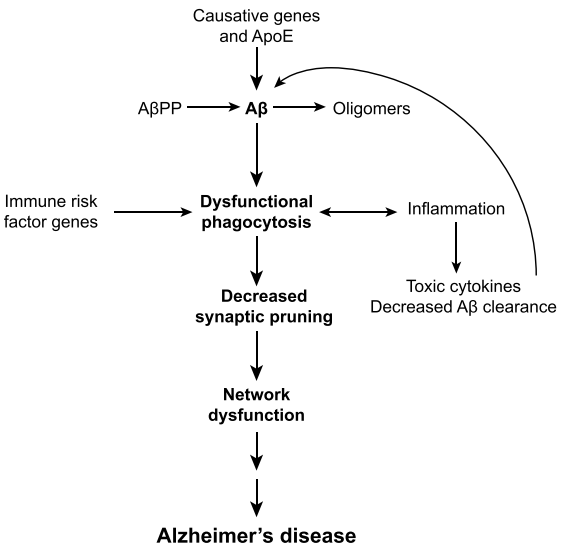

Fig. 2. A model for microglial dysfunction that occurs downstream of Aβ, resulting in network dysfunction progressing to AD.

The cause of DMN hypoactivity in AD is not yet clear; however studies performed in cohorts that are genetically predisposed to AD suggest that DMN hypoactivity is preceded by a period of hyperactivity and increased functional connectivity [123, 136], often manifesting as an absence of normal DMN deactivation during external tasks [137–140]. DMN hyperactivity may interfere with hippocampal memory encoding, leading to the memory deficits that are present in mild cognitive impairment [141, 142]. It has been proposed that hippocampal hyperexcitability in AD may develop as a protective mechanism against increased input from the DMN [142–144]. As AD progresses, the initial hyperexcitability of the DMN and hippocampus may result in hypoactivity due to exhaustion of compensatory mechanisms [123, 136]. Evidence from both transgenic AD mice and longitudinal human studies supports an exhaustion model of hyperactivation leading to later hypoactivation [143, 145–147]. Interestingly, a number of studies report a lower incidence of AD among those who regularly practice meditation which specifically 'calms' the DMN [148].

CONCLUSIONS

Our understanding of AD as a disease is changing. Historically considered to be primarily a disease of neuronal degeneration, this neurocentric view has widened to encompass non-neuronal cells such as astrocytes into our understanding of the disease process and pathogenesis. A proposed model for microglia in AD is shown in Fig. 2. Microglia perform a wide range of functions in the CNS and

although this includes induction of an inflammatory reaction in response to damage, they also have critical roles for maintaining normal function in the brain. Recent evidence shows that microglia regulate neuronal activity through synaptic pruning throughout life as an extension on their normal phagocytosis behavior. The discovery of a large number of AD risk genes associated with reduced immune cell function suggests that perturbed microglial phagocytosis could lead to AD. In our model, altered microglial phagocytosis of synapses results in network dysfunction and onset of AD, occurring downstream of Aβ.

The immune system and microglia represent a novel target for intervention in AD. Importantly, a large number of anti-inflammatory drugs are already in use for other conditions. What is important to know at this stage is exactly how to best target immune cell function. The studies outlined here provide evidence that an indiscriminate dampening down of all microglial activity may result in a worse outcome for individuals by suppressing normal microglial regulatory functions. We currently do not know whether future microglial-based therapies should focus on reducing chronic inflammation or conversely, whether they should be aimed at boosting microglial phagocytosis. It is also likely that future treatment strategies may use a combination of approaches to target Aβ, immune cell phagocytosis and network activity. An increasing view in the AD field is that any drug or therapy needs to be provided very early in the disease process to maximize its beneficial effects. Although we are currently unable to effectively target those at risk of AD at such an early stage, advances in neuroimaging for subtle changes in network activity, or in assays for immune cell function, may provide new avenues for identification of early damage and risk of disease.

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