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Review Article

The roles of inflammation and immune mechanisms in Alzheimer's disease

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Abstract The Alzheimer's Association's Research roundtable met in April 2015 to explore the role of neuroinflammatory mechanisms in the progression of Alzheimer's disease (AD). The ability of innate immune cells, particularly microglia and astrocytes, to mediate neuroinflammation in AD has been implicated as a significant contributor to disease pathogenesis. Adaptive immunity, which plays an important role in responding to injury and some diseases of the central nervous system, may contribute to neuroinflammation in AD as well. Communication between the central and peripheral immune systems may also be important in AD. An increased understanding of the physiology of the innate immune system may aid the identification of new therapeutic targets or mechanisms. The development of predictive animal models and translatable neuroinflammation biomarkers for AD would also facilitate the advancement of novel treatments for innate immunity. Important challenges impeding the advancement of new therapeutic agents and strategies to overcome them were discussed.

Keywords: Microglia; Astrocyte; Innate immunity; Neuroinflammation; Alzheimer's disease; Adaptive immunity

1. Introduction

When Alois Alzheimer peered through a microscope at histologic sections of Auguste D's brain over a century ago, he saw not only the characteristic amyloid plaques

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and neurofibrillary tangles that have become the hallmarks of Alzheimer's disease (AD), but also glial cells clustered around the plaques [1]. These innate immune cells that mediate neuroinflammation in AD—primarily microglia and astrocytes—are now thought to play an important role in disease pathogenesis, possibly providing novel therapeutic targets that may ultimately be as important as the amyloid and tau proteins that make up the plaques and tangles themselves [2]. Adaptive immunity also plays an essential role in responding to disease or injury in the central nervous system (CNS), although adaptive immune-system driven effects, mediated by T and B cells, appear at present to be far more important in neuroinflammatory diseases such as multiple sclerosis (MS) than in AD, where innate immunity appears to drive neuroinflammation.

In the early 2000s, the finding that individuals receiving various nonsteroidal anti-inflammatory drugs (NSAIDs) for diverse systemic inflammatory disorders had a reduced incidence and prevalence of AD was noted fairly consistently in a number of epidemiologic studies [3]. This observation in part led to trials of NSAIDs in both mild cognitive impairment (MCI) and AD dementia; however, the results of these studies were negative, dampening further investigation of this therapeutic strategy for almost a decade. Interest in the role of neuroinflammation in AD has increased dramatically in recent years, however, driven by important findings in neurobiology and genetics, and the topic has been the focus of several recent international symposia and reviews [2,4,5]. In April 2015, the topic was addressed by the Alzheimer's Association's Research roundtable, a partnership of experts from academia, industry, and regulatory agencies. Participants at the meeting examined what is currently known, including research gaps, therapeutic opportunities, and barriers for clinical development. This article aims to contribute to the evolving understanding of inflammatory and immune mechanisms in AD and their potential as therapeutic targets by summarizing key aspects of those discussions.

2. Cells and mediators of inflammation in neurodegenerative disease

There is increasing appreciation that AD pathogenesis and progression are not a consequence solely of neuronal dysfunction but also involve glia-dependent neuroinflammatory mechanisms. Microglia and astrocytes are the major glial cell types that respond to disease stressors by innate immune responses such as production and release of inflammatory mediators. In addition, perivascular macrophages and peripheral myeloid cell populations that can enter the diseased brain also participate in neuroinflammatory signaling. If not kept in check, these neuroinflammatory responses can contribute to pathology and disease progression. Microglia were historically viewed as mainly protecting the brain from exogenous insults, and astrocytes were seen as primarily providing nutritive and structural support for neurons. However, both cell types are now known to play multiple roles in brain health. During neurodegenerative disease processes their function may be adversely affected through inflammatory signaling responses.

2.1. Microglia

Microglia, derived from primitive hematopoietic cells in the yolk sac, seed the brain during fetal development, expand in numbers dramatically after birth, and are self-renewing throughout adult life. They are the resident phagocytes of the CNS and though sharing many properties with peripheral tissue macrophages and monocytes, they are autonomous from peripheral monocytes, which normally do not enter the brain. Although phagocytosis is perhaps their best known property, recent research has revealed multiple roles and distinct functions for microglia in development and adult life. In development, they remove excess synaptic connections and modulate circuit development—a role that is critical for proper brain development [6].

In the adult brain, microglia play an important role in regulating synaptic plasticity and remodeling neuronal circuits. Another key function of microglia is to act as sentinels, surveilling the parenchyma for danger signals, especially the intrusion of pathogens, and contribute to homeostasis. This capacity is facilitated by their numerous extensions (filopodia) that maintain close contact with neurons, perivascular cells, and astrocytes. They may also participate in neurogenesis and synaptogenesis in brain regions where this occurs, as well as in the removal of debris resulting from (non-neuronal) apoptotic cell death [6]. Importantly for AD, given its preponderant onset in late-life, aging-associated changes in the quality of these functions have been increasingly appreciated as well.

In the face of injury and neurodegenerative disorders, however, microglia assume a radically different phenotype. They are rapidly activated in response to acute injury, for example, trauma or stroke, becoming "nurturers" and "warriors" as well as sentinels [7]. This phenotypic alteration involves both chemical and morphologic changes. Morphologically, filopodia retract and microglia can become actively phagocytic, participating in the resolution of tissue damage. However, process retraction by microglia will eliminate their ability to monitor synaptic activity, thereby compromising the microglial contribution to network homeostasis. The ability of microglia to change their phenotype dramatically on activation has led to their being referred to as a "double-edged sword."

In AD brain, microglia (or peripherally-derived macrophages) have long been noted to cluster around neuritic plaques but appear to have a loss of phagocytic capacity and possibly a gain of toxic function as well [8,9]. It is important to note that AD is a very slow process, with the interval between the onset of amyloid β (Aβ) deposition (the leading hypothesized etiologic culprit) and dementia being approximately 20 years [10]. Also, as far as is known, the pathophysiological processes are endogenous; therefore,

microglial reactions in AD brain are likely quite different than for exogenous insults such as bacterial or viral infections, or even trauma and stroke, which involve the sudden intrusion of cells and chemicals normally not present in the brain. Finally, AD most often develops in late life, and thus its pathophysiology is superimposed on the effects of aging on the brain.

Distinct heterogeneity in microglia/macrophage responses has been shown in early-stage AD brain samples [11]. Wilcock et al. performed qPCR analysis of a group of putative neuroinflammatory markers, using autopsy tissue from clinically characterized early-stage AD brains. They grouped samples into two categories, defined by virtue of showing different expression patterns for these markers. In one group, expression of markers often associated with inflammatory responses was elevated, whereas in the other group, expression of markers categorized in studies of peripheral macrophages as being associated with repair processes, fibrosis, and counter-regulation of inflammatory components was elevated. Using the same assay, they did not see this polarization of neuroinflammatory gene expression in late-stage AD brains. Unbiased, genome-wide expression profiling with extensive bioinformatics analysis of these samples will help to define the tissue response to the AD disease process over time. It can be hypothesized that molecular heterogeneity of the CNS tissue response during AD will enable insights leading to novel target identification for therapeutics.

The recent identification of several genetic risk factors involving proteins associated with microglial function has greatly galvanized interest in the role of these cells in AD. For example, a rare missense mutation in the gene encoding the triggering receptor expressed on myeloid cells 2 (TREM2), which is expressed on microglia and confers an increased risk of AD and other misfolded protein-associated neurodegenerative diseases [12,13]. Studies using direct ribonucleic acid (RNA) sequencing demonstrated that TREM2 down-regulates Aβ-induced microglial phagocytic function and dysregulates these cells' proinflammatory responses [14,15]. Furthermore, the TREM2 protein can be measured as a soluble variant (sTREM2) in the cerebrospinal fluid (CSF), demonstrating recent data that CSF sTREM2 levels are increased in the early symptomatic phase of AD, probably reflecting a corresponding change of the microglia activation status in response to neuronal death [16,17]. The transmembrane protein CD33 is expressed at higher levels in AD microglia compared to age-matched controls. CD33 modulates innate immunity and has been identified as another risk factor for AD (with the risk allele leading to increased CD33 expression in microglia and monocytes), presumably by promoting $A\beta_{42}$ accumulation [18–20]. The ATP-binding cassette transporter A7 (ABCA7) is also highly expressed in microglia and can affect AD pathogenesis [21]. These proteins could be therapeutic targets. For example, lintuzumab, an antibody against CD33, can down-regulate surface expression of CD33 [22].

Other studies using direct RNA sequencing indicate that microglia express a unique set of transcripts distinct from peripheral mononuclear phagocytes. This includes a sensing cluster or "sensome" of transcripts that are differentially regulated during aging [23]. Interestingly, these studies have demonstrated an aging-associated up-regulation of microglial neuroprotective genes, including, but not limited to, *neuregulin and Stat 3*; as well as associated down-regulation of oxidative phosphorylation and neurotoxicity-associated factors. Whether there are regional differences in subsets of microglia that are associated with aging is not known. Also not known is whether transcriptional changes in these and other genes translate into functional outcomes during disease.

2.2. Astrocytes

Compared to microglia, much less is known about the role of astrocytes in AD pathogenesis. The most common cell type in the CNS, astrocytes were historically thought to serve essentially a nutritive role and provide structural support and a physical scaffold for neurons. However, astrocytes are now known to play multiple active roles in normal neurophysiology, including significant involvement in neurotransmission, especially glutamatergic transmission. They are themselves excitable, they communicate with neurons by sensing neurotransmitter release and in turn releasing their own signaling molecules (gliotransmitters), and they are intimately associated with synapses physically. These attributes have given rise to the term "tripartite" synapse to reflect the importance of glia in neurotransmission. In addition to intimate contact with neurons, astrocytes also associate with the cerebrovasculature through specialized processes called endfeet. The astrocyte endfeet almost completely ensheath intraparenchymal blood vessels in the brain and are important in the maintenance of ionic and osmotic homeostasis and gliovascular signaling [24,25]. Finally, astrocytes are in close contact with microglia as well, and there is strong evidence for bidirectional signaling between the two cell types.

Like microglia, astrocytes can be activated by various stimuli, and astrocytic activation is increasingly appreciated as an important element of neurodegenerative disorders. Astrocytes also undergo age-related changes, on which the pathophysiologic features of late-life neurodegenerative disorders such as AD are superimposed. Astrocytic involvement in neuroinflammation is characterized by increased cytokine production and the release of signaling molecules that affect neurons either directly or through microglial activation. One possibly important pathway involves NFκB-activated astrocyte release of complement protein C3, which can bind neuronal C3aR and induce neuron damage [26]. This suggests that inhibition of NFκB signaling or neuronal C3aR may be therapeutically beneficial. Another astrocytic signaling molecule of interest is soluble CD40 ligand, which binds to its cognate microglial cell surface receptor. This binding in turn drives

increased production and release of tumor necrosis factor (TNF)-α, an important pro-inflammatory effector molecule with recognized potential to contribute to tissue destruction in AD, as well as possibly other important cytokines [27].

3. Tools to enable discovery and translational research and drug development

3.1. Biomarkers

New tools developed to assess the time course of neuroinflammation across the continuum of the disease have both helped clarify its clinical correlates and provided potential outcome measures for clinical trials. The roundtable discussed several imaging and fluid biomarkers in development as well as the need for additional biomarkers. The 18-kDa translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor, is a mitochondrial protein and a marker of inflammation highly expressed on activated microglia, activated astrocytes, and macrophages, thus making it a putative biomarker for activation of the immune system. A TSPO positron emission tomography (PET) radioligand in development was used to quantify neuroinflammation in volunteers with AD-associated MCI. This study showed that inflammation increases in the cortical regions typically affected by plaque deposition after conversion of MCI to AD [28]; it also showed increased inflammation in AD patients with early age of onset. These results suggest that the ligand may be useful in longitudinal observational and interventional studies in MCI. Current TSPO tracers have various limitations, including genotype-specific properties, nonspecific binding, and high affinity for targets within the cerebrovasculature that prevent access to parenchymal targets of interest. These limitations must be overcome if TSPO PET is to achieve greater utility as a diagnostic, prognostic, or theranostic marker; newer agents are in development that are designed for that goal. Cyclooxygenase (COX), the inhibition of which was the basis for early trials of NSAIDs, is the focus of tracer development aimed at creating isoform-specific (COX-1 and COX-2) agents. Although success in this endeavor will be valuable, radioligands for other markers, such as targets within the signaling cascades for pro- and anti-inflammatory cytokine production and release, as well as for phagocytosis are greatly desired. Astrocyte-specific and microglia-specific tracers would be especially helpful [29].

Another promising biomarker for neuroinflammation in AD is YKL-40 (also known as chitinase 3-like protein 1), which is expressed in astrocytes and present in the CSF. Studies have shown that YKL-40 levels increase in AD and other neurodegenerative diseases even in their early stages, correlate with other markers of neurodegeneration (e.g., total tau and phosphorylated tau [p-tau]) and negatively correlate with measures of cortical thickness in temporal regions of the brain [30,31]. Furthermore, in early AD patients, CSF YKL-40 is related to a structural pattern distinct from that found to be linked to p-tau-associated neurodegeneration presenting a nonlinear corelation with cortical thickness. These results support the presence of concomitant neuroinflammatory and neurodegenerative processes at AD initial clinical stages and suggest that YKL-40 could be useful in tracking inflammatory processes related to AD neurodegeneration [32]. CSF levels of various well-known cytokines have also been explored as biomarkers of neuroinflammation; however, data regarding their variability and change in the course of AD are lacking, as are standardized, validated protocols for sample collection, storage, and quantification. These problems limit the feasibility of using such measures at this time.

There have also been many efforts to identify plasma markers of neuroinflammation. Plasma YKL-40 levels correlated only weakly with CSF levels [33]; the situation was the same for other markers such as plasma progranulin [34] and tau [35]. Neuroinflammatory markers were also included in early plasma proteomic studies using the Luminex xMAP technology in cohorts of reasonable size; however, the findings were inconsistent [36]. More recent plasma proteomic studies in AD have identified a number of markers that appear to be affected by the disease and are also key members of various inflammatory pathways, such as the complement system, toll-like receptor signaling, and intracellular signaling cascades associated with the production and release of pro- and anti-inflammatory cytokines and chemokines [37]. However, the specific markers vary from one cohort to another, suggesting substantial heterogeneity in study populations and assay performance [38]. Nonetheless, these investigations have suggested that further research may reveal plasma biomarkers, or more likely a panel of markers, that could be measured consistently and may be used for diagnosis, prognosis and in clinical trials. Moreover, emerging proteomic technologies such as aptamer-based technologies are providing better coverage of some of these pathways.

Other novel approaches include studies of circulating exosomes, which are membranous vesicles that are released by neurons, astrocytes and microglia. In a recent study comparing AD subjects and controls at two time points, neuronal derived plasma exosomes from AD subjects contained elevated levels of markers of amyloid pathology and neurodegeneration ($A\beta_{42}$, total tau, and p-tau,) that nearly perfectly discriminated AD from controls [39]. Intriguingly, in a longitudinal sample, the study also revealed that the elevations were present long before the onset of cognitive impairment, suggesting that exosomes might be a marker of preclinical AD.

Another approach of increasing interest is the use of computational tools, informatics, and large-scale integrated systems biology approaches to identify genetic nodes and networks involved in immune system function. Two examples of the utility of these approaches are (1) identification of an immune-specific and microglia-specific network dominated by genes implicated in phagocytosis and up-regulated in AD [40] and (2) demonstration of the promise of these

multiscale biology approaches to understand the organization, molecular circuits, and dynamics of the immune system and to predict interactions of drugs and immune cells in a system-wide manner [41,42].

3.2. Animal models

Animal models are also essential for novel target exploration, biomarker discovery, and drug development. Much of the research described earlier regarding the subtypes of microglia and their relationship to neuroinflammation and the main AD pathologic hallmarks could only be accomplished in animal studies. Preclinical animal models have been and continue to be important tools for AD drug discovery and for exploration of pathogenic mechanisms [43–45]. Live imaging of microglia in mouse brain has demonstrated the active responses these cells have to neuronal activity [46]. The role of several genes implicated as risk factors for Alzheimer's disease while still unclear suggests an important role for resident microglia in the efficient clearance of Aβ peptide from brain parenchyma [47]. Yet, there is a clear need for more predictive animal models that more accurately reflect human pathogenesis. This represents a particular problem in the study of neuroinflammation and neuroimmunity for several reasons. First, there are substantial differences between the immune systems of rodents and humans. Second, most laboratory animals are raised in pathogen-free conditions, so that they are never exposed to pathogens that are commonly encountered by humans which shape both innate and adaptive immune responses. Finally, aging has not been well addressed using animal models. Although the NIH houses an aged mouse repository, it remains extremely costly to maintain animals to advanced age mirroring the common late-life incidence of AD in humans. A major advance in the past 2 years in regard to generating more predictive models has been the emergence of CRISPR/Cas9 genome editing [48], which has the potential to revolutionize the generation of animal and cell models.

4. Translational research

New translational research approaches are also needed to clarify how aging and chronic systemic disease affect BBB permeability, immune cell profiles, and homeostasis in the CNS. For example, the relationship among astrocytes, microglia, and neurons is complex and context-specific; and other immune cells such as pericytes may also play important roles in maintaining BBB integrity. In addition, peripheral inflammatory cells or mediators can contribute to neuroinflammatory responses, especially in situations of compromised BBB integrity. An improved understanding of these and related processes could enable development of mechanism-based immunomodulatory therapies to restore immune cell homeostasis and delay/attenuate synaptic dysfunction in AD.

Research on IL-12 and IL-23 represents an example of how a translational program could advance both our understanding of neuroinflammation in AD and therapeutics targeting it. It is known that CSF levels of IL-12 and IL-23 are increased in AD. Studies in AD mouse models indicate that these immune mediators are released by activated microglia and that blocking their signaling reduces cerebral amyloid burden [49]. Inhibitors of IL-12 and IL-23 have already been tested in clinical trials for other diseases and thus could be available for testing in AD [5]. Another example of a productive translational approach currently under investigation involves leveraging research on neuroinflammation in Parkinson's disease (PD) to advance understanding of this process in AD. Tansey et al. hypothesized that neutralizing soluble TNF-α could reduce neuronal damage and loss. Testing this strategy in a rat model of PD, they showed that XPro1595, a dominant-negative TNF-α inhibitor selective for the soluble form of the cytokine reduced the numbers of microglia and astrocytes and attenuated the loss of dopamine neurons [50]. They also tested this strategy in a transgenic AD mouse model, where they showed that XPro1595 modulated immune cell populations in both the CNS and periphery and improved electrophysiological measures and synaptic plasticity.

Although genome-wide association studies (GWASs) have identified a number of genetic variants associated with AD, such as *TREM2*, GWAS does not provide information on the causal single-nucleotide polymorphism or the gene that is affected. However, conjoint GWAS and protein expression studies have provided insight about how various genes and gene products affect the function of monocytes in AD models and subjects. For example, such studies have confirmed patterns of gene expression modulation unique to monocytes and T cells with AD and PD loci compared to patterns in other diseases such as MS. Specifically, gene expression is modulated in monocytes but not T cells at AD and PD loci, whereas the opposite is true in MS and rheumatoid arthritis [51].

Targeted analysis of the *TREM* locus, combined with endophenotyping in several large cohorts of elderly individuals, has revealed additional details about the functional consequences of different gene variants that confer either increased risk or protection against plaques and cognitive decline [52]. Similar studies focused on other loci have also provided insight into the interaction of CD33, TREM2, TREM1, and other proteins, which determine whether monocytes will have a risk or protective phenotype. Eventually, these studies could provide clarity about how best to modulate monocytes therapeutically.

5. Path toward novel treatments

5.1. Innate immunity—increase or decrease?

Despite substantial progress in the field, uncertainty remains regarding the central question of whether

interventions should aim to increase or decrease innate immunity. A number of research findings argue in favor of increasing innate immunity by activating microglia:

- Some microglia activation responses, including those induced by TLR activation, complement activation, or cytokine overproduction, can lead to reduced amyloid plaque deposition in amyloid depositing mice [53].
- Mice lacking the microglial receptor CX3CR1, which have altered microglia activation because of the disruption of CX3CL1-CX3CR1 signaling between microglia and neurons, show reduced Aβ deposition [54].
- Mice lacking the microglial CCR2 chemokine receptor show impaired microglial function, decreased clearance of Aβ, and accelerated progression of AD [55].

Support for decreasing innate immunity includes data showing that

- Experimental manipulations that increase microglia activation, such as LPS administration, IL-1β overexpression, and deletion of CX3CL1, leads to exacerbation of tau pathology in tau-depositing AD mouse models [53,56–58].
- Immune reactions regulate amino acid catabolism and reduce the production of arginine in microglia, which results in neuronal death [59].
- Chronic elimination of microglia in AD model mice rescues dendritic spine loss, prevents neuronal loss, and improves cognitive performance in contextual memory tests [60].

These examples illustrate that there is not a simple answer to whether innate immunity should be increased or decreased. The previous classification of inflammatory responses of microglia and/or macrophages into M1 (classical inflammatory activation, secretion of proinflammatory cytokines, and other tissue damaging molecules) and M2 (alternative activation associated with tissue repair and inflammatory resolution) is now known to be too simplistic. These cells can assume a broad spectrum and complexity of inflammatory phenotypes that are influenced by a number of factors, including the activating stimuli, the timing, the cell types involved, genetic components, and the microenvironment [2,4]. These will be key issues to address in any therapeutic strategies targeting neuroinflammation.

The idea of beneficial versus detrimental microglia responses is also evolving. It is becoming clear that inflammatory responses in the CNS are often accompanied by up-regulation of molecules that suppress or resolve neuroinflammation. There appears to be a complex interplay among multiple cell types in the AD brain that can change the balance between proinflammatory mediators and neuroinflammatory modulators; this imbalance results in either neuroprotective or neurotoxic consequences [61,62]. Adding to the degree of complexity are recent reports that cytokines typically thought of as pro-inflammatory (TNFα, IL-6) or anti-inflammatory (IL-10, TGF-β) can have dichotomous roles depending on concentration, target cell, receptor subtype, and disease stage. For example, studies have demonstrated beneficial effects of blocking anti-inflammatory IL-10 [63,64] or TGF-β [65] signaling in AD mouse models. These data suggest that therapeutic approaches aimed at rebalancing innate immunity to a healthy state, typically approached by selective suppression of pro-inflammatory responses, should also explore the effectiveness of blocking key anti-inflammatory cytokine action.

And what is the impact of age? Few mouse studies have considered the effect of age on innate immune activity; yet, in older humans, microglia rendered dystrophic and dysfunctional by aging-related processes may contribute to neurodegeneration, and there is evidence that microglia undergo priming with normal aging, predisposing them to exaggerated responses to pro-inflammatory stimuli. Indeed, mouse models have generated a great deal of confusion and contradictory data with regard to understanding neuroinflammation in AD and how to modulate it to improve clinical outcome by transforming microglia from a neuroinflammatory to neuroprotective phenotype.

5.2. *Targeting adaptive immunity*

Under normal conditions, cells of the adaptive immune system are not found in substantial numbers in the brain. However, significant infiltration of peripheral immune cells occurs in neuroinflammatory conditions, and the responses of infiltrating cells can have a substantial impact on the neuroinflammatory environment and AD pathology progression [66–68]. Therefore, the role of adaptive immune cell responses and whether there are temporal and spatial therapeutic windows for effective intervention are areas of increasing interest. This interest is especially important given the prevalence of therapeutic approaches using Aβ immunization that engage the adaptive immune system. Increased infiltration of monocytes/macrophages, lymphocytes, and T cells into the brain occurs with age and is seen in AD mouse models and human AD brain. Whether the consequences are detrimental or beneficial is complex. The role of adaptive immunity appears to be influenced by a number of factors, including the stage of disease progression, the repertoire of inflammatory insults, and the ratio of specific immune cells types. Using T cells as an example, infiltrating Th1 cells were historically considered to be associated with increased inflammation, impaired synaptic plasticity and cognitive function, and amyloid accumulation, whereas Th2 cells and Tregs were usually associated with reduced inflammation, improved cognitive performance, and lower amyloid burden. However, this is certainly an oversimplification, as there are a number of reports that indicate both positive and negative effects of all of these T cell subtypes in AD. For example, infiltrating CD4+ T cells have been reported to produce interferon-gamma, promote microglia activation and increase amyloid burden [66]. In contrast, it has been suggested that infiltrating CD4+ T cells and peripheral blood-derived

monocytes are recruited to the brain to modify destructive local inflammatory responses, and that AD represents an imbalance in this protective mechanism [68].

Even without cell infiltration, the presence of systemic inflammation is communicated to the brain. Systemic inflammation induced by a variety of stimuli has been reported to lead to microglia priming, increased production of proinflammatory molecules in the brain, and acceleration of disease progression in AD animal models [69]. Recognition of the cross-talk between systemic and central inflammation has important implications for AD therapeutic strategies. However, translation of these conceptual advances to the clinic will require a fuller untangling of the molecular and bidirectional communication pathways between the adaptive and innate immune systems. These studies must consider the diverse nature of inflammatory responses at different stages of disease progression and the distinct temporal and spatial contributions of specific inflammatory responses so that optimal preventive or therapeutic strategies can be developed to maintain or restore immune balance.

5.3. Lessons from trials of anti-inflammatory drugs

The roundtable reviewed the findings of the trials of NSAIDs and other anti-inflammatory agents conducted almost two decades ago, in an effort to appreciate lessons from these negative trials that might enhance the chance of success for studies anticipated to be conducted with agents specifically targeting innate immunity. Among the factors that could account for the negative results of the early trials are inappropriate disease stage and inappropriate targets. Most of the early trials were conducted in MCI or AD dementia, so it is possible that such agents could be efficacious only as preventive strategies. One trial (ADAPT) enrolled an earlier-stage sample and tested the effects of the nonselective COX inhibitor naproxen and the selective COX-2 inhibitor celecoxib on cognitive change in noncognitively impaired elderly with a family history of AD-like dementia. Although a 2011 follow-up study of the ADAPT trial suggested possible protective effects of naproxen in cognitively intact subjects, a later 2013 follow-up study showed no effect in delaying time to AD diagnosis [2]. It is thus also possible that COX is not a key mediator of neuroinflammation in AD. Notably, the early trials did not have the benefit of two factors discussed above that are likely to be critical for the success of future trials, namely, the need for prior positive data in appropriate animal models and the availability of relevant biomarkers.

Several recent or planned investigational efforts aim to treat neurodegenerative disease by modulating immunity and neuroinflammation. Table 1 lists clinical trials of selected Alzheimer's drug candidates with anti-inflammatory activity.

In PD, both innate and adaptive immunity have been shown to affect disease progression. Using a mouse model of PD, researchers have explored an approach that both attenuates neuroinflammation and induces neuroprotection [70] by shifting the balance of anti-inflammatory regulatory T cells (T_{reg}) and neurodestructive effector T cells (T_{eff}).

Another approach, being pursued by CereSpir, attempts to simultaneously stimulate microglial phagocytic activity and blunt the production and release of cytokines that damage tissue by leveraging astrocyte-microglia communication. CereSpir's compound, CSP-1103, is a small molecule derived from a NSAID scaffold but with COX-1 inhibition substantially reduced and COX-2 inhibition entirely removed. It has shown beneficial effects on amyloid plaques and several biomarkers of neuroinflammation in transgenic mouse models as well as good safety and tolerability in phase 1 and 2a studies [71]. An adaptive phase 2b/3 trial of CSP-1103 in MCI due to AD is being planned.

Retinoid X receptor (RXR) agonists have also been under investigation for the treatment of AD. Retinoids modulate many cellular functions including immune responses [72]. RXR agonists heterodimerize with retinoic acid receptors (RARs) to regulate gene expression [73]. Studies have suggested that PPARγ binds with RXR to induce APOE-HDL synthesis and subsequent soluble Aβ degradation [74]. Bexarotene is an RXR agonist that has been reported in several studies to increase APOE expression and microglial phagocytosis, reduce soluble Aβ levels, and improve cognition [75]; however, other studies suggest no beneficial effects of bexarotene in AD model mice [76].

Finally, drugs that affect microglial priming and systemic inflammation, such as the TNF-α blocker etanercept, are being investigated for use in AD. In a randomized, placebo-controlled, double-blind phase 2 study in mild to moderate AD, etanercept was well tolerated and showed some trends toward cognitive, functional, and behavioral benefits [77].

A number of practical implications of the early studies and more recent trials should be considered in planning future trials with agents that target innate immunity, including the need to determine the optimal treatment duration and the appropriate approach to diverse patient characteristics that could affect therapeutic efficacy. The latter include body weight/glucose intolerance, the microbiome, comorbid illnesses, especially systemic inflammatory disorders, and the impact of anti-inflammatory medications used for such illnesses. All these factors have either demonstrated or theoretical potential to affect CNS innate immunity, thus possibly affecting the results of agents to be tested for AD.

5.4. Barriers and challenges to clinical development

Several impediments to clinical development of treatments for neuroinflammation in AD must be overcome for the potential benefit of this therapeutic strategy to be realized. Besides those revealed by the failure of early clinical trials discussed above, one substantial obstacle is funding. Some older agents currently approved for other indications might be repurposed for AD, an example being RXR agonists. The bexarotene study, although failing to show benefits, illustrates the ability to translate the results of animal

Table 1
Clinical trials of selected Alzheimer's drug candidates with anti-inflammatory activity

Candidate	ClinicalTrials.gov Identifier(s)	Clinical phase	Summary
Albumin and Immunoglobulin	NCT01561053	III	Naturally occurring antibodies with anti-inflammatory and immunomodulating properties.
ALZT-OP1	NCT02547818, NCT02482324	III	A combination drug therapy consisting of two previously approved drugs. Cromolyn inhibits beta-amyloid peptide polymerization, and lowers cytokine production while Ibuprofen inhibits the neuroinflammatory response.
Atomoxetine (Strattera)	NCT01522404, NCT00191009	II	Atomoxetine is a norepinephrine uptake inhibitor approved for ADHD. Eli Lilly conducted a 6-month phase 2/3 trial to evaluate the effectiveness of atomoxetine in 92 subjects with mild to moderate AD. Atomoxetine was reported to be generally safe; however, it did not benefit cognition in these patients.
Bexarotene (Targretin)	NCT01782742, NCT02061878	II	Bexarotene is approved for the treatment of some cancers. It is an RXR agonist that has been reported in several studies to increase APOE expression and microglial phagocytosis, reduce soluble Aβ levels, and improve cognition [54].
Cerefolin NAC (CFLN)	NCT01745198, NCT00597376	Not active	Homocysteinemia is associated with increased risk for AD, coronary artery disease, and stroke. Cerefolin or Cerefolin NAC (CFLN) has been shown to lower homocysteine levels.
CSP-1103 (CHF 5074)	NCT01303744, NCT01258452, NCT01203384, NCT00954252	II	Selectively reduces pro-inflammatory activities of microglial cells while increasing their ability to remove neurotoxic amyloid beta aggregates.
Curcumin	NCT01811381, NCT00164749, NCT00099710, NCT01383161	II	Plant extract, dietary supplement; thought to have anti-inflammatory and antioxidant properties, and to bind and clear amyloid.
Etanercept (Enbrel)	NCT01068353, NCT01716637, NCT00203359, NCT00203320	II	TNF-α blocker. In phase II, etanercept was well tolerated and showed some trends toward cognitive, functional, and behavioral benefits [56].
GC 021109	NCT02254369, NCT02386306	I	GC 021109 reportedly binds the microglial P2Y6 receptor and to stimulate both microglial phagocytosis and inhibit microglial release of pro-inflammatory cytokines such as IL-12.
Genistein	NCT01982578	II	Genistein is an isoflavone that is present in soybeans and other plants. It acts as a PPARγ agonist with antioxidant and anti-inflammatory properties.
Lipoic acid/Omega-3	NCT01780974, NCT01058941,	II	Lipoic acid is proposed to have anti-inflammatory and neuroprotective properties. A trial is recruiting exploring the effects of lipoic acid in combination with Omega-3 for Alzheimer's prevention.
Minocycline	NCT01463384	II	Minocycline is a tetracycline antibiotic, which crosses the blood-brain barrier. In some animal models, minocycline reduces the Aß accumulation, the levels of pro-inflammatory mediators and the activation of microglia. A small phase II trial with 13 participants with MCI and AD was completed in 2014 where subjects were administered 50-mg minocycline twice daily for 6 months. No significant safety issues were reported.
Nabilone (Cesamet)	NCT02351882	III	A Δ9-tetrahydrocannabinol (THC) analogue, potentially with neuroprotective and anti-inflammatory properties. It is FDA approved as a pain killer and anti-nausea agent for people receiving cancer treatment.
Pioglitazone	NCT01931566, NCT02284906, NCT00982202	III	Pioglitazone is a PPARγ agonists approved as a once-daily treatment of type 2 diabetes. PPARγ activation has been shown to modulate the microglial response to amyloid deposition in such a way that it increases Aβ phagocytosis and decreases cytokine release.
Sargramostim (Leukine)	NCT01409915, NCT02667496	II	A recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) that functions as an immunostimulator and is approved for the treatment of cancer. In Alzheimer's, it may increase the phagocytosis of pathogenic protein deposits by bone marrow–derived macrophages or brain-resident microglia and may also stimulate other neuroprotective innate immunity processes.
Simvastatin (Zocor)	NCT01439555, NCT00842920, NCT00053599, NCT01142336, NCT00486044, NCT00939822, NCT00303277	II	Simvastatin is approved to lower blood cholesterol levels by inhibiting HMG-CoA reductase (a statin drug). Two trials are ongoing in Alzheimer's with simvastatin alone or in combination with L-arginine and tetrahydrobiopterin.
VX-745	NCT02423200, NCT02423122	II	VX-745 is a blood-brain barrier penetrant, selective p38 MAPKα inhibitor.

studies into a small proof-of-concept study in humans for a relatively small sum of money. In addition, two other RXR agonists are currently in development.

It is also generally appreciated that funding limitations constrain drug discovery and development efforts for therapeutic strategies outside the mainstream amyloid and tau hypotheses. This state of affairs is particularly problematic for academic groups and small biotech firms striving to advance treatments for neuroinflammation. Despite rapid and dramatic advances in the technology and methodology for target identification and evaluation, this work and the early stages of drug discovery for promising hits and leads arising from it remain long, complicated, and uncertain.

Another obstacle to clinical development of anti-inflammatory treatments for AD is the lack of high-quality observational data for biomarkers of interest in well characterized samples that could support the use of such measures for either enrichment purposes or as indicators of target engagement measures. Some roundtable participants suggested that to gain a clearer picture of neuroinflammation across the continuum of the disease, relevant biomarkers should be evaluated longitudinally in observational studies such as ADNI. In this regard, there is increasing interest in developing and validating neuroimaging agents that reflect specific aspects of neuroinflammation, for use in diagnosis, monitoring treatment effects, and guiding development of new therapies [78]. An exploration of the mechanisms underlying cognitive resilience was also suggested as essential in understanding the role of neuroinflammation and immune mechanisms.

6. Conclusions and key areas for future research

Although a number of epidemiologic studies have linked anti-inflammatory treatment to a lower AD risk, prospective clinical trials with NSAIDs and some other types of anti-inflammatory agents have so far failed to demonstrate efficacy. The reasons for these failures have yet to be fully elucidated, but the rapid pace of basic science research on mechanisms underlying the derangement of the innate immune system in AD augurs the need for key information to provide a clearer understanding in coming years.

A very important area for future research is to define the molecular characteristics and significance of neuroinflammation at different stages of AD. Some evidence supports the notion that the inflammatory response of both astrocytes and microglia may peak during the beginning of the symptomatology, that is, during MCI. Clarifying this will be crucial to development programs for new agents.

It is increasingly clear that innate immunity is pathogenetically important in AD and possibly even central to disease progression; however, proof of a role for adaptive immunity remains limited. Recent evidence suggests that in response to Aβ vaccination, products of adaptive immunity (B cells and immunoglobulins directed against Aβ) may turn microglia into Aβ fighters but this requires more study.

It is also important to remember that AD is associated with both a loss of physiological function and a potential gain of toxic function. Effective treatment will require restoring cells to health, not simply dampening down deleterious processes. In this regard, it is notable that strategies to restore the deficient phagocytic capacity of microglia have been much less explored to date compared to research targeting cytokine reduction.

In general, roundtable participants agreed that the roles of neuroinflammation and the innate immune system in aging and AD are far more complicated than initially thought. There are a number of important aspects of the immune system that have yet to be adequately investigated, as well as genetic and epigenetic mechanisms that are likely to play important roles. The influence of neuropsychiatric disturbances such as depression, agitation, and psychosis that frequently complicate AD dementia may also be important to evaluate, as research is demonstrating inflammatory mechanisms in primary psychiatric disorders such as major depression and schizophrenia [79]. Improved fluid and imaging biomarkers, banking of biospecimens, and endophenotyping of the "neuroinflammation state" of the brain before and during disease will be required to design trials that will demonstrate efficacy in appropriate individuals and thus optimally advance effective anti-inflammatory treatments.

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RESEARCH IN CONTEXT

1. Systematic review: We reviewed the literature of recent work exploring the important roles of inflammation and immune mechanisms on Alzheimer's disease (AD).
2. Interpretation: The present article posits that innate immune cells, particularly microglia and astrocytes, mediate neuroinflammation in AD, which is a significant contributor to disease pathogenesis. Adaptive immunity may also contribute to neuroinflammation in AD.
3. Future directions: A better understanding of the complexity of neuroinflammation and the innate immune system in aging and AD is needed to discover new targets and biomarkers that will lead to novel therapeutic approaches.

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