State of the art

Is Alzheimer disease a failure of mobilizing immune defense? Lessons from cognitively fit oldest-old

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Multifaceted evidence supports the hypothesis that inflammatory-immune mechanisms contribute to Alzheimer disease (AD) neuropathology and genetic association of several immune specific genes (*TREM2, CR1,* and *CD33*) suggests that maladaptive immune responses may be pivotal drivers of AD pathogenesis. We reviewed microglia-related data from postmortem AD studies and examined supporting evidence from AD animal models to answer the following questions: i) What is the temporal sequence of immune activation in AD progression and what is its impact on cognition? ii) Are there discordant, "primed," microglia responses in AD vs successful cognitive aging? iii) Does central nervous system (CNS) repair in aging depend on recruitment of the elements of cellular adaptive immune response such as effector T cells, and can the recruitment of systemic immune cells ameliorate AD neuropathology? iv) How effective are the immune-system-based therapeutic approaches currently employed for the treatment of AD?

Integration of the immune response in the central nervous system

Vigilant immune defenses against infection and injury ensure the endurance of the organism by setting in motion a discrete, localized inflammatory response to thwart a variety of pathogenic and pathophysiological threats. These responses must be tuned and scaled precisely, because insufficiency or excesses of responses such as prolonged inflammation can cause morbidity and mortality, shortening lifespan and compromising health. Homeostasis and health are restored when inflammation is resolved by rapid and reversible anti-inflammatory responses. Derailed resolution of inflammation is accompanied by impaired antigen clearance, persistence of inflammatory triggers, activated macrophages, and the development of maladaptive immunity.[1]

The mammalian nervous system integrates inflammatory responses by gathering information about invasive events, mobilizing defenses, and restoring homeostasis, and creates immune memory to improve likelihoods of survival. Microglia, derived from primitive myeloid progenitors that populate the developing brain during mid-embryogenesis,[2] are the resident immune/inflammatory cells of central nervous system (CNS). Additional subtypes of macrophages (reviewed in ref 3) are positioned at brain boundaries and show diverse transcriptional profiles.[4] These cell populations include perivascular, subdural meningeal, and choroid plexus macrophages which are distinct from parenchymal microglia and are likely derived from hematopoietic precursors during development.[4] Throughout this review we will discuss brain injury responses of adult microglia, the functions of which may be inherently different from the role(s)

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of microglia during prenatal periods when microglia regulate the wiring of brain circuits, influence the outgrowth of dopaminergic axons, impact the laminar positioning of subsets of neocortical interneurons,[5] and effect functional connectivity.[6]

Under homeostatic conditions microglia are ramified cells with multiple branches and processes. Microglia extend processes to contact neurons, macroglia (astrocytes and oligodendrocytes), and blood vessels and constantly monitor and remodel the functional state of synapses.[7] Highly activated microglia transform from a ramified to an ameboid shape in response to injuries or inflammatory stimuli and are characterized by enlarged cell bodies and shortened processes with restricted coverage. Ameboid morphology is associated with phagocytosis and proinflammatory functions. Additional differently shaped activated microglia are recognized (eg, bipolar, rod-shaped etc[8]) possibly reflecting their diverse responses and movement toward injury or "toxic" stimuli.

Activated parenchymal and perivascular microglial cell populations express variable levels of extracellular markers of myeloid lineage in which some resemble tissue macrophages attesting to microglial heterogeneity.[9] It remains unclear whether hematopoietic-derived progenitors contribute to the expansion of adult parenchymal microglia-like cells when homeostasis is disrupted, such as during brain injury, or whether microglia can maintain and transform their function independently of hematopoietic-derived progenitors throughout adult life in the absence of temporary disruption of the blood-brain barrier (BBB). The experimental manipulation of lethally irradiated and bone marrow transplanted mice have provided contradicting views regarding the recruitment of circulating bone marrow cells from the bloodstream in parabiotic chimeras. While some studies show that donor-derived cells can replace considerable population of microglia in the recipients over time,[10-13] others have failed to find evidence for recruitment of mobilized bone marrow cells using models of acute and chronic microglia activation and neurodegeneration with intact BBB.[14] A recent study that avoided concerns associated with irradiation-bone marrow

> **Advanced age is the greatest risk factor in AD and is associated with replicative senescence (loss of mitotic ability after repeated rounds of replication) in myeloid cells**

transplantation models found that the expansion of resident microglia was mainly responsible for microgliosis proximal to the ischemic area in a photothrombotic stroke model.[15] Under the pathological disruption of BBB, some of the circulating monocytes were able to infiltrate CNS, but did not proliferate and their numbers declined over time due to active apoptotic processes.[15,16] Unlike macrophages, microglia have been shown to respond to damage by rapidly entering the cell cycle program and undergoing extensive local expansion.[17] Additionally, experimental pharmacological and genetic ablation of microglia has confirmed that microglial repopulation occurs rapidly[18] suggesting that local proliferation of existing parenchymal progenitors provides a rapid mechanism for mobilizing defense responses, rather than recruitment of systemic bone marrow derived precursors, which is believed to be restricted by the BBB when intact.[19] Therefore, the robust mitotic potential of microglia in advanced age may be among the critical factors for tissue repair and preservation of cognitive function in healthy aging,[19] whereas replicative senescence (please see below) may contribute to pathological aging.

Under the inflammatory conditions of an active immune response, microglia must moderate the potential damage to the nervous system by supporting tissue repair and remodeling, including the maintenance and restoration of synaptic connections. Microglia achieve neuroprotection by clearing debris, suppressing inflammation, restoring BBB integrity[20] and promoting cortical synaptogenesis/neurogenesis by stripping inhibitory synapses.[21] To protect from attacking its own antigen/proteins microglia must also maintain natural self-tolerance. To induce central systemic self-immune tolerance, resident dendritic cells must present self-antigens from circulating dying cells to T cells. It is not clear how microglia maintain self-tolerance of neural cell types. However, examination of the effector macrophages derived from microglia or monocytes in animal models of autoimmune disease has shown that microglia-derived macrophages were protective and oriented toward resolving neuroinflammation, whereas monocyte-derived macrophages were highly phagocytic and proinflammatory[22] suggesting that self-immune tolerance may be an inherent feature of microglia.

Additionally, it has been shown that locally secreted immunosuppressive cytokines (eg, TGFβ, G-CSF, and IL-10) by immune cells may promote immune tolerance to self-associated antigens.[23] It is plausible that self-tolerance mechanisms may contribute to the accumulation of "toxic" signals in neurodegenerative disorders such as AD.

We will discuss whether the engagement of systemic adaptive immune responses can overcome self-tolerance suppression of the brain immune response in order to ameliorate AD neuropathology.[24] We will additionally summarize microglia-related data from postmortem AD studies and examine supporting evidence from AD animal models to answer the following questions: i) What is the temporal sequence of immune activation in AD progression and what is its impact on cognition? ii) Are there discordant, "primed," microglia responses in AD vs successful cognitive aging? iii) Does CNS repair in aging depend on recruitment of the elements of cellular adaptive immune response such as T cells (subtypes of effector T cells), and do they influence multiple microglia activation states revealed by transcriptomics approaches? iv) How effective are the immune-system-based therapeutic approaches currently employed for the treatment of AD?

Brain inflammatory response – relevant factor in Alzheimer disease and successful cognitive aging: evidence from neuropathology and molecular studies

Alzheimer disease and its associated dementia is the most common type of neurodegenerative disorder, with age and aging representing the greatest risk factors. The neuropathological hallmarks of AD include neuronal loss, abnormal neuronal cytoskeletal changes, known as neurofibrillary tangles (NFT)[25-27] comprised of hyperphosphorylated tau protein, and extracellular protein deposits called neuritic amyloid β protein (Aβ) plaques (NP).[28,29] In addition to the NFTs and NPs, evidence from multiple domains support the hypothesis that inflammatory-immune mechanisms contribute to AD neuropathology.[30] Elevated levels of the inducers of acute phase response, such as inflammatory cytokines and acute phase reactants[31,32] are identified in CSF,[33] plasma and in amyloid-laden plaques[34]; the presence of reactive microglial cell clusters around senile plaques[35-38] and complement components of the membrane attack complex in vicinity of dystrophic neurites and NFTs[39-41] are well-documented in postmortem studies of individuals

with AD. Additionally, changes in gene expression of inflammatory signaling pathways and regulatory gene networks[42,43] and immune markers[44] have been consistently found in brains of individuals with AD.

Given the evidence of genetic linkage of several immune specific genes (*TREM2*,[45] *CR1*,[46] and *CD33*[47,48]), the immune-inflammatory processes may be pivotal drivers of AD pathogenesis suggestive of a feed-forward self-amplifying cycle model of AD.[49] This model proposes that impaired clearance of amyloid deposits and apoptotic cellular material as a result of persistent microglia dysfunction would drive toxic inflammatory responses and complement activation causing extensive synaptic loss/neuronal damage[50] further releasing more apoptotic material through the self-amplifying toxic neuroinflammatory cycles.

Despite their potential lack of construct validity, studies in AD transgenic animals demonstrated that reactive proinflammatory microglia are sufficient to stimulate neuronal degeneration and targeting the reactive microglia alone can relieve cognitive decline in these mouse models. For instance, reactive microglial cells were sufficient to promote tau hyperphosphorylation in mice expressing only human tau isoforms, while inhibition of antagonist-mediated IL-1 receptor signaling reduced microglia-induced tau pathology.[51] Elimination of chronically activated microglia in AD mice (5xFAD) prevented neuronal and synaptic loss and improved memory function in the absence of any change in their Aβ levels or plaque load.[52] Similar improvement in learning and memory without changes in Aβ levels was achieved in AD mice with sustained pharmacological elimination of adult microglia.[53] In contrast, augmenting microglial phagocytic activity by genetic manipulation reduced amyloid load, but led to non-cell-autonomous neurotoxic effect mediated by drastic synaptic loss even in the absence of Aβ.[54] Overall, these findings revealed that unrestrained robust inflammatory mechanisms are sufficient on their own to impede learning and memory in AD-model mice.

Extrapolating from these admittedly imperfect model systems to AD suggests that elucidation of specific microglial responses to CNS insults may be imperative for understanding, and potentially treating, the contribution of inflammatory processes to neurodegeneration and impairment of cognitive function in AD. Recent imaging studies aiming to assess longitudinal changes in microglial activation and

amyloid deposition in mild cognitive impairment and AD provided some clues regarding the temporal sequences and trajectories of microglia activation in the progression of AD.[55] Initial activation during mild cognitive impairment followed by longitudinal (>14 months) reduction of microglial activation was observed during prodromal AD. A second wave of activation of microglia was observed in individuals with definite AD, suggesting the prevalence of proinflammatory phenotype of microglia in advanced stages of AD.[55] The vulnerability of cholinergic neurons, cholinergic atrophy, and decline of acetylcholine release in AD[56] may be at least in part responsible for proinflammatory phenotype in definite AD as acetylcholine directly modulates immune responses[1] by acting on the α 7 nicotinic acetylcholine receptor and suppressing the proinflammatory responses in microglia[57] and in blood-borne macrophages.[58]

Acquired anti-inflammatory protective phenotype during prodromal AD may be tied to self-tolerance mechanisms,[55,59] or it may exemplify chronic para-inflammatory response (low-grade inflammation) switched on due to cellular malfunction as a result of genetic variance and gene-by-exposome interactions such as age-associated low level of physical activity, high-calorie diet, and environmental toxin exposure, rather than tissue injury/infection.[60] Divergent immune responses along with age-related immune senescence may suppress the anti-inflammatory protective phenotype of microglia during the prodromal phase of AD and enable progression to frank dementia, synaptic loss, neurodegeneration, and AD. Inarguably, microglia activation will demand a high rate of metabolic activity necessary for synthesis and production of immune-modulating factors, such as cytokines and other cellular proteins associated with microglial hypertrophy immunophenotype. The well-documented reduction of glucose metabolic rate and expression of energy metabolism genes[61] together with cerebrovascular hypoperfusion[62] recognized as early events in AD progression, may also contribute to microglia dysfunction and the inhibition of anti-inflammatory protective phenotypes.

Advanced age is the greatest risk factor in AD and is associated with replicative senescence (loss of mitotic ability after repeated rounds of replication) in myeloid cells. Morphological assessment of microglia in the brains of elderly humans has provided evidence of structural deterioration of microglia[63,64] and age-associated reduced expression of genes related to motility, adhesion, and chromatin organization.[65] In addition, in vitro studies have shown that microglia are subject to replicative senescence in aging.[66] Together these observations raise the possibility that advanced age-associated factors adversely affect the viability and self-renewal capacity of microglia, resulting in immune senescence/or dysfunctional immune-related cells.[19,67] In this regard, investigation of immune responses in persons surviving beyond the 9th decade of life who remain cognitively intact may shed light on the contribution of microglia to successful cognitive aging with scarce presence of AD neuropathology.[68-70] Investigation of systemic immune responses in centenarians suggests that immune function is not compromised by extreme age, but rather undergoes remodeling processes in which innate immunity is preserved, while adaptive immunity manifests profound modifications[71] suggestive of elevated levels of regulatory T cells and their immunosuppressive activities.[24] Moreover, assessment of the microglial transcriptome in healthy adult mice showed that during physiological aging the microglial transcriptome undergoes disproportionate downregulation of genes involved in sensing endogenous ligands and upregulation of genes associated with alternative neuroprotective microglial priming states[72] suggesting that aging is associated with the microglia transcriptome signature shifting toward neuroprotection by downregulating debris-sensing receptor signaling. Thus, it can be argued, and studied further, that neuroprotection processes assisted by activated microglia are likely central to the cognitive resilience.[7] The presence of ubiquitin-immunoreactive dystrophic neurites in the neocortex of nondemented oldest-old (90+ years old) human brains and granular degeneration of myelin in white matter in the absence of amyloid deposition and neocortical neurofibrillary degeneration[73] support activation of immune responses. However, extrapolation from mice to humans must be approached with caution. A recent purified microglia study in humans found little overlap between genes differentially expressed during aging in humans and those of the mouse brain suggesting that that the microglia of physiologically aged mice do not necessary recapitulate the effect of aging on human microglia.[65]

Extrapolating to the CNS microglia and successful cognitive aging, one may hypothesize that robust immune response involving ubiquitination may promote intact cognitive function in extreme age and protect against accumulation of toxic molecules and Aβ deposition. Evaluation of transcriptional profiles from human postmortem brains
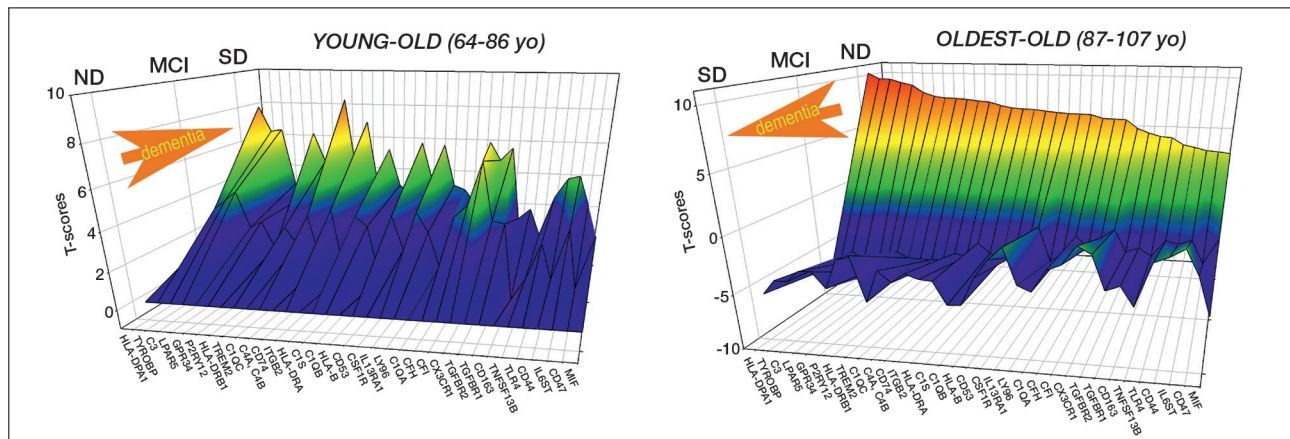
Figure 1. Mesh plot of dramatically upregulated immune response genes in nondemented oldest old (right panel) compared with non-demented young-old (left panel) in different stages of dementia in two aging groups. T scores (Y axis) for each individual gene symbol (X axis) were plotted. T scores - standardized measure of change (extension of fold change algorithm) were derived from contrast analysis.[74] Please note that direction of dementia severity (Z axis) is reversed in the lower panel in order to visualize declining immune response in demented oldest-old. ND, nondemented; MCI, mild cognitive impairment; SD, severe dementia. Figure shows meek increase of inflammatory markers in young-old individuals with dementia severity compare to robust upregulation in the nondemented oldest-old, which is suppressed in the earliest stage of dementia in oldest-old.

show support for contribution of immune activation to the cognitive resilience in aging. Direct comparison of neocortex transcriptomes from young-old (64 to 86 years old) and oldest-old (87 to 103 years old) cognitively normal and demented individuals suggest that inability to scale up robust immune activation that typify oldest-old individuals who evaded dementia (*Figure 1*) may be directly associated with cognitive decline in the oldest-old.[1] Additionally, examination of transcriptional changes in cognitively normal oldest-old compared with younger individuals found evidence for the upregulation of genes involved in sensing extracellular signals released during tissue injury, including *LPAR5, GPR34, TREM2, CSF1R* and *P2RY12* (*Figure 1*). Upregulation of genes involved in host defense and phagocytosis including antigen presentation, Fc receptors, and complement cascade support engagement of inflammatory response and phagocytosis in cognitively normal elderly. Given that microglia and complement cascade mechanisms are intimately involved in synaptic pruning and neuronal loss,[50] the next generation of studies of the role(s) of immune/inflammation and microglia in successful aging must clarify the mechanisms that simultaneously avert synaptic loss while allowing for amplified expression of phagocytosis and host defense genes, including engagement of the complement cascade. The development of new methodologies for isolating and studying different subclasses of microglia may aid in this effort.[75]

One working hypothesis may be that successful aging is associated with an enhanced immune-related signature resulting from priming of microglia, similar to the mechanisms known to occur in peripheral macrophages.[76] The "primed" microglia would respond to triggering stimuli more rapidly and to a greater degree than would be expected from non-primed microglia presumably by modulating accessibility of microglia-specific transcriptional enhancers and promoters (work in transgenic mice suggests that senescent-like dysfunctional neurons are sufficient to induce progressive priming responses in microglia[77]). This hypothesis stands in contrast to, and is underscored by, the fact that there is general consensus that acute and chronic systemic inflammation is detrimental to brain function,[78,79] impedes adult neurogenesis,[80] and is associated with cognitive decline in AD.[76] This "paradox" may be more apparent than real, however, and may be a result of a too-simplistic or too-binary a model of immune/inflammation and microglial function. As we have discussed above, microglia assume diverse phenotypes and can promote both harmful and advantageous/neuroprotective outcomes. Findings in animal models show that upon receiving triggering stimuli, microglia in their classical primed state will release excessive concentration of inflammatory cytokines (IL-1B, IFNG, TNFA etc) associated with neurotoxicity and neurodegeneration, while in the alternative primed state microglia release anti-inflammatory immunomodulators associated

with protection and neuroplasticity. Transcription study of isolated microglia in rodents suggested that during aging the microglial phenotype shifts toward an alternative neuroprotective priming state.[72] Survey of protein levels of 30 cytokines in cortical grey matter of nondemented oldest-old when compared with cognitively intact younger individuals showed that only a small set of cytokines (G-CSF, IL-6, 8, and 15) were significantly upregulated in brain tissue homogenates (Brodmann area 22) from nondemented oldest-old (refer to ref 74 for unpublished data derived from the same dataset). At least two of these factors (IL-6 and IL-15) can be considered to elicit both pro- and anti-inflammatory responses implying that this simplistic subdivision of cytokine responses may also be inadequate to classify their function[81] and necessitate further examination with respect of neuronal and glial responses in the human CNS.[82]

It is critical to keep in mind that microglia and the other cell-types of the CNS do not function independently. Rather, neurons, microglia, astrocytes, oligodendrocytes, and microvascular endothelial cells interact extensively and function in unison. Notably, the most upregulated cytokine in the brain of nondemented oldest-old is granulocyte-colony stimulating factor (G-CSF) which has been shown to induce neurogenesis, neuroplasticity to counteract apoptosis, and is known as a factor involved in vasculogenesis.[83] G-CSF is currently under investigation for the development of treatments of neurological diseases such as acute cerebral ischemic stroke.[84,85] In this respect, participation of brain microvascular endothelial cells in inflammatory responses is highly critical as they can initiate the release of cytokines/chemokines[86] across the BBB engaging constituents of the neurovascular unit, including microglia, and also release factors to the systemic circulation to elicit systemic immune responses.[87] Given the strong evidence for cerebrovascular dysfunction including cerebral hypoperfusion, microvascular degeneration, and cerebral amyloid angiopathy[62,88-91] preceding the appearance of AD neuropathology, it is plausible that angiogenesis and vascular remodeling will be among the homeostatic responses that accompany and promote microglial function, restore cerebral circulation, and enhance Aβ removal. Additionally, changes in the expression of endothelial and microglial specific genes compared with the other neural cell types are the most reliable predictors of biological aging in the human brain.[92] Intriguingly, the distinct gene signature of human endothelial cells treated with VEGF-A, a critical pro-angiogenic factor, shows the upregulation of inherent inflammatory subset of genes[93] attesting to the cross-regulation of angiogenesis, systemic inflammation, and microglial activation[94] during neovascularization and BBB maintenance. Feasibility of cognitive function improvement and reduction of amyloid pathology due to improved circulation and adaptive activation of immune pathways around plaques and the vasculature involving perivascular macrophages and potential BBB modifications has been demonstrated in AD mice.[95]

Microglia plasticity - different states of activation and altered immune cell composition in Alzheimer disease

Activation of microglia is frequently categorized by the M1-classical and M2-alternative phenotypes similar to the categorizations attributed to macrophages.[96] The M1 phenotype is considered a proinflammatory state characterized by elevated levels of cytokines/chemokines, reactive oxygen species capable of inducing neurotoxicity, expression of antigen presentation molecules (MHC class II), phagocytosis-related Fc receptors, and matrix metallopeptidases. In contrast, M2 (a,b,c) phenotypes are central for inflammation resolution, immunomodulation, angiogenesis, tissue repair/remodeling and associated with release of neurotrophic/pro-survival factors and anti-inflammatory cytokines. These phenotypes are not static, however, and exhibit dynamic changes at different ages, stages of evolution of diseases such as AD, and in response to different environmental stimuli. Novel approaches such as single-cell and cell-type-specific transcriptomics have revolutionized modern immunology and suggests that classification systems such M1 and M2 phenotypes may be inadequate for the advanced understanding of microglia diversity in health and disease. Recent advances in next-generation sequencing and single-cell trascriptomics[75,97] show that microglia activation states associated with development, aging and different neuropathologies are varied, display unique multiple transcription signatures that are not only distinct from myeloid cells/macrophages in peripheral tissue, but also much more complex than those implied by M1 vs M2 dichotomies/taxonomy. For example, a comprehensive mapping of all immune cell clusters in AD mouse brains revealed novel neurodegenerative disease associated microglial subtypes localized in proximity to neuritic plaque foci some of which appear to have the potential to restrict/confine AD neuropathology.[75] Another intriguing finding

of that analysis was the identification of *TREM2*-dependent activation of gene networks specific to the novel disease-associated microglial subtype, which is consistent with genetic evidence of *TREM2* polymorphism and dysregulation associated with increased risk of AD.[45,98] Recent meta-analysis of myeloid transcriptional responses from the fusiform gyrus of individuals with AD and controls, and animal models of different pathological states provided additional information about microglial population-wide transcriptional changes indicating upregulation of not only the TREM2-dependent core neurodegeneration-related subtype, but also additional cell-type clusters including phenotypes related to neutrophil/monocyte, which were absent in AD mouse models and a subtype related to the acute response to endotoxin lipopolysaccharide.[97] It is not clear whether multiple microglial cell clusters represent various dynamic phenotypes of activated microglia, such as phagocytic, antigen presenting cells (APC), and/or whether they include CNS-infiltrating monocyte-derived macrophages and Aβ-reactive T cells. What these more recent studies reveal is a remarkable diversity in microglial cell phenotypes that requires reassessment of functional distinctions and perhaps even a new taxonomy.

While the functions of microglia as APC for adaptive immune responses has not been characterized in AD, the appearance of perivascular and intraparenchymal dendritic cells (DC) - classical APCs - has been demonstrated in brains of patients suffering from epilepsy[99] and encephalopathies.[100] These findings are consistent with data acquired in adult transgenic mice showing prominent appearance of DCs in CNS regions exhibiting plasticity and adult neurogenesis with the morphologic characteristic of immune/microglia cells.[101] However, these CD11c-positive DC cells express low levels of MHC class II genes suggesting that their function as APC may be flawed,[102] or that within the CNS their functions may be different or possess features in addition to classical antigen presentation. It is possible that these DC-like cells within the CNS may not infiltrate from the periphery. It has been suggested that adult microglial progenitors may differentiate into immature DCs in the presence of granulocyte-macrophage colony-stimulating factor and express extracellular markers distinct from the rest of microglia.[9,100] These DC-like cells can mature fully into DC upon CD40 ligation.[100] Consistent with this hypothesis, T cell-based vaccination in AD mice induced the appearance of a DC-like CD11c+

microglia phenotype which was associated with increased neurogenesis and improved spatial learning and memory[103] suggesting that DC-like monocytes may be of benefit to the brain's resistance to AD by aiding T helper cells to induce T cell activation as APC.

Limited work in postmortem human brains also suggests elevated appearance of T cells (T cell receptor expressing mature T lymphocytes are part of the adaptive immune system) in the brain parenchyma of the elderly[104] and individuals with AD.[104-106] More comprehensive tissue immunostaining using multiple T cell markers have confirmed increased frequencies of T cells in the hippocampus, the entorhinal cortex and associated brain regions of individuals with AD compared with other types of dementia and controls.[107] These observations have supported the view that brain parenchymal T cells are likely memory T cells rather than naïve T cells based on the cell-surface markers staining.[107] The absence of the IL-2 receptor subunit (CD25), proliferation markers, and CD11b staining in CD8+ cells argues against clonal expansion of T cells in AD brains, and their complete differentiation into effector cells.[107] Taken together, these findings suggest ineffective activation of T lymphocytes or a brain-specific T lymphocyte phenotype in AD brain.

A subtype of T cells, regulatory/suppressor T cells (Tregs), is another component of the immune system, critical for the modulation of inflammatory responses, and usually difficult to distinguish from effector T cells. Modulation by Tregs of overall systemic inflammatory status may also translate into differential activation and functional differentiation of parenchymal microglia. The mechanisms involved in this interaction and the infiltration of circulating immune cells into the CNS is a controversial topic and a question that needs to be addressed with high priority. It has been hypothesized that elevated activity of Tregs, key immunosuppressors, and protectors of systemic immune tolerance, is permissive of cerebral plaque pathology and cognitive decline in AD, and may contribute to the limited efficacy of anti-inflammatory treatment trials of AD.[24,108] Under neurodegenerative condition CNS recruitment of circulating immunoregulatory cells, such as T cells, may be critical for moderating microglia-mediated neuroinflammatory response. In support of this hypothesis, temporary reduction of Tregs removed systemic immunosuppression and boosted accumulation of inflammation-resolving cells in the brain parenchyma, which was

associated with reduced brain protein levels of soluble β amyloid and reversed cognitive decline in transgenic AD mice.[24,108] Similarly, early vaccination experiments with CNS antigens showed enhanced recovery after axonal injury[109] and subsequently boosting levels of CNS-specific circulating T cells facilitated the recruitment of monocyte-derived macrophages from the periphery to the CNS sites of injury where they differentiate locally into resolving macrophages.[110] The blood-CSF barrier of the choroid plexus is a likely candidate for a selective site of monocyte CNS infiltration and is characterized by distinct population of effector-memory T lymphocytes expressing T-cell receptors specific for CNS antigens.[111] The cellular composition of CSF is different from that of peripheral blood and constitutively predisposed towards a tissue injury healing, pro-resolving, milieu characterized by elevated levels of anti-inflammatory cytokines, untraceable levels of pro-inflammatory factors and inhibition of development of cytotoxic T lymphocytes.[111] Studies in human and mice have shown that age-induced dysfunction of the choroid plexus and cognitive decline are associated with elevated expression of type I interferon (IFN) responses interfering with IFN- type II regulation of leukocytes homing, rolling, and migration, which may act as permissive mechanism allowing leukocyte infiltration through the choroid plexus.[112] Blocking of brain IFN-I signaling improved neurogenesis and partially restored cognitive function in aging mice.[112] Complex marker classification of diverse Tregs subpopulations is responsible, at least in part, for limited and conflicting information about systemic composition of Tregs subpopulations in AD. Some studies have reported strong increases of the subset of Tregs negative for the immunosuppressive programmed death receptor 1 in individuals with mild cognitive impairment.[113] In contrast, others have found decreased frequency of potential circulating Treg cells with a naive phenotype in individuals with mild AD.[114] Thus, a more thorough understanding of cells with Treg phenotypes in aging and disease is required for disentangling these diverse findings.

The exposome, its individual constituents, and sex are additional factors influencing adult microglia and need special attention. Ex vivo and in vitro experiments show profound environmentally dependent alteration in human microglia transcriptomes and epigenetic landscapes from surgically resected tissue.[115] Recent data also suggest that adult microglia go through transcriptional and epigenetically distinct differentiation stages,[116,117] which can diverge as a function of sex. For example, microbiome depletion and antibiotic treatment in mice has sexually dimorphic effects on microglia[116] highlighting the importance of the interplay between sex-dependent microglia features and environmental factors.

The studies and observations discussed above all point to the centrality of microglia to AD, cognitive compromise, and successful aging. But they also highlight the immense complexity of microglia, their phenotypic diversity and the myriad of responses that can and are evoked depending on factors such as age, sex, environment, and their CNS milieu, all of which are rife with their own complexities. It seems unlikely that we will be able to make significant gains in AD therapeutics and promotion of successful cognitive ageing without a more detailed understanding of the mechanisms that govern the myriad roles and responses of microglia and the ways in which they influence and modulate the functions of the other cells of the CNS. As daunting a task as this may seem, recent advances in cell-type specific omics provides the light at the end of the tunnel.

Challenging immune responsiveness in central nervous system – therapeutic approaches to Alzheimer disease

Current immunotherapeutic approaches to AD aim to reduce amyloid burden and reduce or slow the rate of cognitive decline by increasing amyloid β clearance and microglial phagocytic activity, while dampening pro-inflammatory response and retaining microglial phagocytic activity. Suppression of inflammation alone through non-steroidal anti-inflammatory drug therapy has been disappointing, or had adverse effects in advanced AD. With hindsight, this is not surprising given that, as described above, microglial activation phenotypes are contextually different and evolve at different stages of AD progression (reviewed in ref 118 and ref 119). The anti-inflammatory steroid, prednisone, was explored in a randomized, multicenter trial[120] with no positive outcome and with some notable adverse reactions. Minocycline-antibiotic immunomodulation, neuroprotective in neurodegenerative models and human chronic neurological disorders, showed reduced production of proinflammatory cytokines, while showing contradicting results for amyloid clearance.[121-123] Recent clinical trial in patients with moderate-severe traumatic brain injury showed detrimental effect of chronic phase minocycline treatment - increasing neurodegeneration, while decreasing inflammation.[124]

One line of defense against toxic amyloid subspecies could be natural neutralizing antibodies,[125] which can be expanded upon immunization with Aβ peptides (active vaccination). The first active vaccine (AN1792, consisting of pre-aggregate Aβ Elan Pharmaceutical)[126] was terminated because it induced autoimmune encephalitis in humans. Postmortem tissue examination showed T cell infiltration and inflammation around leptomeningeal blood vessels, near vascular amyloid and infiltration of macrophages in white matter.[127] Despite these negative consequences, immunized patients also showed improvements in amyloid clearance and reduced measures of plaque-associated neuritic dystrophy in the hippocampus,[128] which were associated with increased expression of microglial markers reminiscent of the phagocytic phenotype.[129]

Another approach has focused on passive vaccination by administering antibodies against Aβ. Several anti-Aβ monoclonal antibodies (eg, bapineuzumab, solanezumab and mAb158) have been developed.[130,131] The cognitive benefits of the initial clinical studies with bapineuzumab are still unclear and concerns on the safety of these antibodies have been raised. Solanezumab, a humanized monoclonal antibody directed against the mid-region of the Aβ peptide, was shown to neutralize soluble Aβ.[130] Initial evaluation from two pooled phase III studies suggest a positive trend toward slowing of cognitive decline in the mild AD subgroup.[131] A monoclonal antibody, mAb158 has high selectivity for soluble Aβ protofibrils, which are toxic to neurons. A humanized version of mAb158, BAN2401, has now entered a clinical phase IIb trial for Aβ immunotherapy in early AD[132] with some, but limited positive outcomes. Despite these encouraging trends, none of the trials to date have led to meaningful and substantial clinically significant outcomes.

Experimental approaches toward utilizing cellular mechanisms of adaptive immune responses against Aβ are supported by detection of elevated levels of amyloid beta-reactive T cells in healthy elderly and individuals with AD[104] suggesting that either these cells are either positively selected, or that they have escaped central and peripheral tolerance.[102] A recent animal study provided *convincing evidence* that activated CD4 positive T cells polarized toward the Th1 phenotype (but not Th2 or Th17 subsets) and injected into the lateral ventricles can effectively migrate and target amyloid plaques in the parenchyma of hippocampus and the cerebral cortex improving neurogenesis and alleviating amyloid burden.[133] Interestingly, T cell function and migration within the brain parenchyma was dependent on IFN gamma signaling in neural tissue, which is consistent with IFN gamma regulation of adhesion and migration, which may act as a permissive mechanism allowing immune cells infiltration through the choroid plexus.[112,134,135] T cells migration was associated with upregulation of MHC class II molecules in choroid plexus revealing that MHC-T cell receptor interactions maybe a prerequisite for T cell transmigration to the CNS and agrees with hypotheses that suggest that Aβ induces adaptive immune response in the periphery. While the exact source and functional characteristics of CNS peri-vascular antigen presenting dendritic cells remains elusive in humans, animal studies favor the recruitment of blood-derived monocytes and their differentiation into dendritic cells.[99,136]

Hyperphosphorylation of tau at specific sites transforms normal tau into misfolded tau within paired helical filaments and leads to the canonical NFTs of AD. When secreted by affected neurons, misfolded tau may propagate pathology by inducing tau aggregation in neighboring neurons.[137] Preclinical studies suggest that active immunization may be effective against misfolded tau in AD animal models.[138] Very few preclinical studies of passive immunization against hyperphosphorylated tau protein are currently available.[139,140] Examination of two Ig isotype antibodies specific to human tau pathological phosphorylation site pS404 showed that IgG2a/kappa, but not a IgG1/kappa antibody, reduced hyperphosphorylation of tau and NFT burden in two independent mouse models of tau pathology.[140]

Despite the meager effects of current clinical trials of Aβ immunization and anti-inflammatory treatments, the prospect for effective immune system-based approach for treatment of AD will be enhanced as we expand our knowledge of microglia-mediated immune responses, their phenotypic and functional diversity, and their role(s) in modulating cognition, successful aging, and cognitive decline during AD progression. ■

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