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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 mechanisms contribute to Alzheimer disease (AD) neuropathology and genetic association of several immune specific genes (REM2, CR1, and CD33) suggests that inflammatory 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 systemically derived immune cells ameliorate AD neuropathology? iv) How effective are the immune-system-based therapeutic approaches currently employed for the treatment of AD?

**Keywords:** Alzheimer disease; gene expression; immune response; inflammation; macrophage; microglia, oldest-old; T cell

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.

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 monocytes that populate the developing brain during mid-embryogenesis, 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. These cell populations include perivascular, subdural, and choroid plexus macrophages which are distinct from parenchymal microglia and are likely derived from hematopoietic precursors during development. Throughout this review, we will discuss brain injury responses of adult microglia, the functions of which may be inherently different from the role(s) of systemically derived immune cells.
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, and effect functional connectivity.\(^6\)

Under homostatic conditions, microglia are ramified cells with multiple branches and processes. Microglia extend processes to contact neurons, microglia (astrocytes and oligodendrocytes), and blood vessels and constantly monitor and remodel microvascular coverage. Ameboid morphology is associated with phagocytosis and proinflammatory functions. Additional differently shaped activated microglia are recognized (e.g., 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.\(^7\) It remains unclear whether hem atopoietic-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 hem atopoietic-derived progenitors throughout adult life in the absence of temporary disruption of the blood-brain barrier (BBB). The experimental manipulation of 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 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.

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

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 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 system self-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 macrophage-derived macrophages were highly phagocytic and proinflammatory 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 microglial cells promote immune tolerance to self-associated antigens. 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 in AD one response in order to ameliorate AD neuropathology. We will additionally sum arize microglia-related data from post-mortem AD studies and examine supporting evidence from AD animal models to answer the following questions: (i) What is the temporal sequence of microglia activation in AD progression and what is its impact on cognition? (ii) Are there discordant, "primed," microglial 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 transcriptomic 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 (NFTs), amyloid plaques (NP), and extracellular protein deposits called neuritic amyloid β protein (Aβ) plaques (NP). In addition to the NFTs and NPs, evidence from multiple studies supports the hypothesis that inflammatory responses contribute to AD neuropathology. Elevated levels of the inducers of acute phase response, such as inflammatory cytokines and acute phase reactants, are identified in CSF, plasma a and in am yloid-laden plaque deposits, the presence of reactive microglial cell clusters around senile plaques, and com protein of the em brane attack complex in vicinity of dystrophic neurites and NFTs are well-documented in post-mortem studies of individuals with AD. Additionally, changes in gene expression of inflammatory signaling pathways and regulatory gene networks and in microglia have been consistently found in brains of individuals with AD.

Given the evidence of genetic linkage of several immune specific genes (TREM2, CR1, CD33), the immune-inflammatory processes may be pivotal drivers of AD pathogenesis suggestive of a feed-forward self-amplifying cycle model of AD. This model proposes that immune-inflammatory mechanisms to CNS insults may be imperative for understanding and potentially treating, the contribution of inflammatory processes to neurodegeneration and targeting the reactive microglia alone may relieve cognitive decline in these individuals.

Despite their potential lack of construct validity, studies in AD transgenic animals demonstrated that reactive inflammatory microglial cells are sufficient to promote tau hyperphosphorylation in AD mice expressing only human tau isoforms, while inhibition of antagonist-mediated IL-1 receptor signaling reduced microglia-induced tau pathology. 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. Similar improvement in learning and memory without changes in Aβ levels was achieved 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. Simiarly, immune cell-mediated inhibition of inflammation led to robust inflammatory microglial cell clusters around senile plaques, and com protein of the em brane attack complex in vicinity of dystrophic neurites and NFTs are well-documented in post-mortem studies of individuals with AD. Additionally, changes in gene expression of inflammatory signaling pathways and regulatory gene networks and in microglia have been consistently found in brains of individuals with AD.

Extrapolating from these admittedly imperfect models, 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 targeting the reactive microglia alone may relieve cognitive decline in these individuals. Recent imaging studies aiming to assess longitudinal changes in microglial activation and...
amloid deposition in mild cognitive impairment and AD provided some clues regarding the temporal sequences and trajectories of microglia activation in the progression of AD. 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. The vulnerability of cholinergic neurons, cholinergic atrophy, and decline of acetylcholine release in AD may be at least in part responsible for proinflammatory phenotype in definite AD as acetylcholine directly modulates immune responses by acting on the α7 nicotinic acetylcholine receptor and suppressing the proinflammatory response in microglia and in blood-borne macrophages.

Acquired anti-inflammatory protective phenotype during prodromal AD may be tied to self-tolerance mechanisms; or it may exemplify chronic para-inflammatory response (low-grade inflammation) switched on due to cellular senescence as a result of genetic variance and gene-by-exposome interactions such as age-associated low level of physical activity, high-calorie diet, and enviromental toxin exposure, rather than tissue injury/infection. 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 demyelination, synaptic loss, neurodegeneration, and AD. Inarguably, microglial activation will demand upregulation of genes associated with alternative neuroprotective microglial priming states suggesting that aging is associated with the microglial 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. The presence of ubiquitin-immunoreactive neurons in the neocortex of nondemented old-old (90+ years old) human brains and granular degeneration of white matter in white matter atrophy in the absence of amloid deposition and neocortical neurofibrillary degeneration support activation of immune responses. However, extrapolation from microglia to humans must be approached with caution. A recent purified microglia study in human brains found little overlap between genes differentially expressed during aging in humans and those of the mouse brain suggesting that the microglia of physiologically aged mice do not necessarily recapitulate the effect of aging on human microglia.

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 and age-associated reduced expression of genes related to motility, adhesion, and chromatin organization. In addition, in vitro studies have shown that microglia are subject to replicative senescence in aging. 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. 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. 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 immune system is preserved, while adaptive immune system manifests profound modifications suggestive of elevated levels of regulatory T cells and their immune suppressive activities. 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 suggesting that aging is associated with the microglial 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. The presence of ubiquitin-immunoreactive neurons in the neocortex of nondemented old-old (90+ years old) human brains and granular degeneration of white matter in white matter atrophy in the absence of amyloid deposition and neocortical neurofibrillary degeneration support activation of immune responses. However, extrapolation from microglia to humans must be approached with caution. A recent purified microglia study in human brains found little overlap between genes differentially expressed during aging in humans and those of the mouse brain suggesting that the microglia of physiologically aged mice do not necessarily recapitulate the effect of aging on human microglia.
State of the art
Brain immunity – relevant factor in Alzheimer disease - Haroutunian

show support for contribution of immune activation to the cognitive resilience in aging. Direct comparison of neocortical 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. 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, the next generation of studies of the role(s) of 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.

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. The “primed” microglia would respond to triggering stimuli more rapidly and to a greater degree than would be expected from non-primed microglia. 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, impedes adult neurogenesis, and is associated with cognitive decline in AD. This “paradox” may be apparent but real, however, and may be a result of a too-simplistic or too-binary 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-1β, IFNG, TNFA etc) associated with neurotoxicity and neurodegeneration, while in the alternative primed state microglia release anti-inflammatory cytokines in unison with other associated

Figure 1. Mesh plot of dramatically upregulated immune response genes in nondemented oldest old (right panel) compared with nondemented 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. 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 compared to robust upregulation in the nondemented oldest-old, which is suppressed in the earliest stage of dementia in oldest-old.
with protection and neuroplasticity. Transcription study of isolated microglia in rodents suggested that during aging the m icrogial phenotype shifts toward an alternative neuroprotective prim ing state. Survey of protein levels of 30 cytokines in cortical grey m attar of nondem ented oldest-old when com pared with cognitively intact younger individuals showed that only a sm all set of cytokines (G-CSF, IL-6, 8, and 15) were significantly upregulated in brain tissue hom og enates (Brodm ann area 22) from nondem ented oldest-old (refer to ref 74 for unpublished data derived from the sam e dataset). At least two of these factors (IL-6 and IL-15) can be considered to elicit both pro- and anti-inflamm atory responses implying that this sim plistic subdivision of cytokine responses m ay also be inadequate to classify their function\textsuperscript{31} and necessitate further exam ination with respect of neuronal and glial responses in the hum an CNS\textsuperscript{82}

It is critical to keep in m ind that m icrogia and the other cell-types of the CNS do not function independently. Rather, neurons, microglia, astrocytes, oligodendrocytes, and m icrovascular endothelial cells interact extensively and function in unison. Notably, the m ost upregulated cyto kine in the brain of nondem ented oldest-old is granulocyte-colony stim ulating factor (G-CSF) which has been shown to induce neurogenesis, neuroplasticity to counteract apoptosis, and is known as a factor involved in vasculogenesis.\textsuperscript{83} G-CSF is currently under investigation for the development of treatments of neurological diseases such as acute cerebral ischem ic stroke.\textsuperscript{84} In this respect, participation of brain m icrovascular endothelial cells in inflam m atory responses is highly critical as they can initiate the release of cytokines/chem okines, reactive oxygen species capable of inducing neurotoxicity, expression of antigen presentation m olecules (MHC class II), phagocytosis-related Fc receptors, and m atrix m etallo peptidases. In contrast, M2 (a,b,c) phenotypes are central for inflam m atory resolution, im m unom odulation, angiogenesis, tissue repair/remodeling and associated with release of neurotrophic/pro-survival factors and anti-inflamm atory 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 environm ental stimuli. Novel approaches such as single-cell and cell-type-specific transcriptomics have revolution ized m odern immunology and suggests that classification systems such M1 and M2 phenotypes m ay be inadequate for the advanced understanding of m icrogia diversity in health and disease. Recent advances in next-generation sequencing and single-cell transcriptomics show that m icrogia activation states associated with development, aging and different neuropathologies are varied, display unique m ultiple transcription signatures that are not only distinct from m yeloid cells/m acrophages in peripheral tissue, but also m uch m ore com plex than those im plied by M1 vs M2 dichotom ies/taxonom y. For exam ple, a com prehensive m apping of all im m une cell clusters in AD m ouse brains revealed novel neurodegenerative disease associated m icrogial subtypes localized in proxim ity to neuritic plaque foci som e of which appear to have the potential to restrict/ confine AD neuropathology.\textsuperscript{75} Another intriguing finding

**State of the art**

**Brain immunity – relevant factor in Alzheimer disease - Haroutunian**

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

Activation of m icrogia is frequently categorized by the M1-classical and M2-alternative phenotypes similar to the categorizations attributed to m acrophages.\textsuperscript{86} The M1 phenotype is considered a proinflamm atory atory state characterized by elevated levels of cytokines/chem okines, reactive oxygen species capable of inducing neurotoxicity, expression of antigen presentation m olecules (MHC class II), phagocytosis-related Fc receptors, and m atrix m etallo peptidases. In contrast, M2 (a,b,c) phenotypes are central for inflam m atory resolution, im m unom odulation, angiogenesis, tissue repair/remodeling and associated with release of neurotrophic/pro-survival factors and anti-inflamm atory 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 environm ental stimuli. Novel approaches such as single-cell and cell-type-specific transcriptomics have revolution ized m odern immunology and suggests that classification systems such M1 and M2 phenotypes m ay be inadequate for the advanced understanding of m icrogia diversity in health and disease. Recent advances in next-generation sequencing and single-cell transcriptomics show that m icrogia activation states associated with development, aging and different neuropathologies are varied, display unique m ultiple transcription signatures that are not only distinct from m yeloid cells/m acrophages in peripheral tissue, but also m uch m ore com plex than those im plied by M1 vs M2 dichotom ies/taxonom y. For exam ple, a com prehensive m apping of all im m une cell clusters in AD m ouse brains revealed novel neurodegenerative disease associated m icrogial subtypes localized in proxim ity to neuritic plaque foci som e of which appear to have the potential to restrict/ confine AD neuropathology.\textsuperscript{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.55,98 Recent meta-analysis of m yeloid transcriptional responses from the fusiform gyrus of individuals with AD and controls, and animals models of different pathological states provided additional information about m yeloid 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/m onocyte, which were absent in AD m ouse m odels and a subtype related to the acute response to endotoxin lipopolysaccharide.9 It is not clear whether m ultiple m yeloid cell clusters represent various dynamic phenotypes of activated m icroglia, such as phagocytic, antigen presenting cells (APC), and/or whether they include CNS-infiltrating m onocyte-derived m acrophages and Aβ-reactive T cells. What these m ore recent studies reveal is a remarkable diversity in m icroglial cell phenotypes that requires reassessment of functional distinctions and perhaps even a new taxonomy.

While the functions of m icroglia as APC for adaptive m une 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 epilepsy99 and encephalopathies.100 These findings are consistent with data acquired in adult transgenic m ice showing prominent appearance of DCs in CNS regions exhibiting plasticity and adult neurogenesis with the m orphologic characteristics of immune/m icroglial cells.101 However, these CD11c-positive DC cells express low levels of MHC class II genes suggesting that their function as APC m ay be flawed,102 or that within the CNS their functions m ay be different or possess features in addition to classical antigen presentation. It is possible that these DC-like cells within the CNS m ay not infiltrate from the periphery. It has been suggested that adult m icroglial progenitors m ay differentiate into m yeloid m utate DCs in the presence of granulocytic-m acrophage colony-stimulating factor and express extracellular m arkers distinct from the rest of m icroglia.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 m ice induced the appearance of a DC-like CD11c m icroglia phenotype which was associated with increased neurogenesis and m proved spatial learning and memory, suggesting that DC-like m onocytes m ay 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 T cell, T lymphocytes are part of the adaptive immune system) in the brain parenchyma of the elderly101 and individuals with AD.104-106 More comprehensive tissue imaging has 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 m emory T cells rather than naive T cells based on the cell-surface marker staining.107 The absence of the IL-2 receptor subunit (CD25), proliferation m arkers, 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 inflammation in AD, 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.
For example, microbiome depletion and antibiotic treatment for the immunosuppressive programmed death receptor 1 in AD have reported strong increases of the subset of Tregs negative for the memory phenotype in individuals with mild AD. Some studies have also found decreased frequency of potential circulating CSF T cells with a naive phenotype in individuals with mild AD.114 Neonatal vaccination experiments with CNS antigens showed enhanced recovery after axonal injury115 and subsequently boosting levels of CNS-specific circulating T cells facilitated the recruitment of onocyte-derived macrophages from the periphery to the CNS sites of injury where they differentiate locally into resolving macrophages.116 The blood-CSF barrier of the choroid plexus is a likely site for a selective site of migration of CNS infiltration and is characterized by a distinct population of effector-memory T lymphocytes expressing T-cell receptors specific for CNS antigens.117 The cellular composition of CSF is different from that of peripheral blood and constitutively predisposed toward 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.118 Studies in human and mouse models have shown that age-induced dysfunction of the choroid plexus and cognitive decline are associated with elevated expression of IFN-I responses interfering with IFN-γ type II regulation of leukocytes homing, rolling, and migration, which may act as a permissive mechanism allowing leukocyte infiltration through the choroid plexus.119 Blocking of brain IFN-I signaling improved neurogenesis and partially restored cognitive function in aging mice.120 Com plex marker classification of diverse Tregs subpopulations is responsible, at least in part, for the inflammatory and anti-inflammatory functions of the T cell response in AD.121 Some studies have reported strong increases of the subset of Tregs negative for the immunosuppressive program m ed death receptor T cells in individuals with m ilder cognitive impairment.122 In contrast, others have found decreased frequency of potential circulating Treg cells with a naive phenotype in individuals with milder 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 m icroglia and need special attention. Ex vivo and in vitro experiments show profound environmentally dependent alteration in human m icroglia transcriptomes and epigenetic landscapes from surgically resected tissue.115 Recent data also suggest that adult m icroglia go through transcriptional and epigenetically distinct differentiation stages,116,117 which can diverge as a function of sex. For example, m icrobiome depletion and antibiotic treatment m ent in m icroglia has sexually dimorphic effects on m icroglia highlighting the importance of the interplay between sex-dependent m icroglia features and environmental factors.

The studies and observations discussed above all point to the centrality of m icroglia to AD, cognitive compromise, and successful aging. But they also highlight the immense complexity of m icroglia, 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 therapies and promote of successful cognitive ageing without a more detailed understanding of the mechanisms that govern the roles and responses of m icroglia in 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 m icroglial phagocytic activity, while dampening pro-inflammatory m icroglial response and retaining m icroglial phagocytic activity. Suppression of inflammatory mediators 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, m icroglial activation phenotypes are contextually different and evolve at different stages of AD progression (reviewed in ref 118 and ref 119). The anti-inflammatory m icroglial steroid, prednisone, was explored in a randomized, multicenter trial with no positive outcome and with some adverse reactions. Minocycline-antibiotic immunomodulation, neuroprotective in neurodegenerative m odel s and human chronic neurological disorders, showed reduced production of pro-inflammatory m icroglia cytokines, while showing contradicting results for amyloid clearance.121-123 Recent clinical trial in patients with moderate-severe traumatic brain injury showed detrimental effects of chronic phase minocycline treatment while decreasing inflammation at injury sites.124

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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 microglia has sexually dimorphic effects on microglia highlighting the importance of the interplay between sex-dependent microglia features and environmental factors.
One line of defense against toxic amyloid subspecies could be natural neutralizing antibodies, which can be expanded upon immunization with Aβ peptides (active vaccination). The first active vaccine (AN1792, consisting of pre-aggregate Aβ Elan Pharamaceutical) was termed inactivated because it induced autoimmune encephalitis in human. Postmortem tissue examination showed T cell infiltration and inflammation around leptomeningeal blood vessels, near vascular amyloid and infiltration of macrophages in white matter. Despite these negative consequences, immunized patients also showed improvements in amyloid clearance and reduced measures of plaque-associated neuritic dystrophy in the hippocampus, which were associated with increased expression of microglial markers reminiscent of the phagocytic phenotype.

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. The cognitive benefits of the initial clinical studies with bapineuzumab ab 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. Initial evaluation from two pooled phase III studies suggest a positive trend toward slowing of cognitive decline in the mild AD subgroup. 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 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 amloid beta-reactive T cells in healthy elderly and individuals with AD, suggesting that either these cells are either positively selected, or that they have escaped central and peripheral tolerance. 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 hippocampal and the cerebral cortex in provoking neurogenesis and alleviating amyloid burden. Interestingly, T cell function and migration within the brain parenchyma was dependent on IFN γ signaling in neural tissue, which is consistent with IFN γ as a permissive mechanism allowing immune cells infiltration through the choroid plexus. T cells migration was associated with upregulation of MHC class II molecules in choroid plexus revealing that MHC-T cell receptor interactions may be 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 perivascular antigen presenting dendritic cells remains elusive in human, animal studies favor the recruitment of blood-derived monocytes and their differentiation into dendritic cells.

Hyperphosphorylation of tau at specific sites transform 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. Preclinical studies suggest that active immunization may be effective against misfolded tau in AD animal models. Very few preclinical studies of passive immunization against hyperphosphorylated tau protein are currently available. Examination of two Ig isotype antibodies specific to human tau pathological phosphorylation site pS404 showed that IgG2a/kappa, but not IgG1/kappa antibody, reduced hyperphosphorylation of tau and NFT burden in two independent mouse models of tau pathology.

Despite the meager effects of current clinical trials of Aβ inmunization 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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