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Review Article

Targeting neuroinflammation in the treatment and prevention of Alzheimer's disease

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Summary

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment, language deterioration and visuospatial deficits. The central neuropathological hallmarks of AD are neuronal degeneration, loss of synapses, the formation of neurofibrillary tangles, gliosis and amyloid-beta (Aβ) accumulation. Chronic neuroinflammation is thought to play a role in AD pathology, and numerous studies have indicated that microglia-mediated neuroinflammatory responses promote the neurodegeneration observed in AD. However, vascular inflammation and leukocyte accumulation in the AD brain and in transgenic animals with AD-like pathology suggest a role for new inflammation mechanisms in this disease. Notably, recent animal studies have shown that neutrophils migrate into the AD brain and play an unexpected role in the induction of cognitive deficit and neuropathological changes. Furthermore, blocking LFA-1 integrin, which controls leukocyte–endothelial interactions in AD mice, inhibits both Aβ deposition and tau hyperphosphorylation and reduces memory loss. Thus, the emerging role of peripheral leukocytes in the pathogenesis of AD opens new avenues of investigation and may lead to the identification of new therapeutic approaches. This review summarizes our current understanding of the roles of vascular inflammation and circulating leukocytes in AD, focusing on recently discovered neuroinflammation mechanisms. We also discuss a role for adhesion molecules, chemokines and other inflammation mechanisms that may promote brain damage in AD. Given that all current AD therapies are symptomatic, we highlight existing anti-inflammatory treatments as well as novel approaches that may contribute to AD prevention and therapy.

Key words: Alzheimer's disease – Vascular inflammation – Leukocyte trafficking – Anti-adhesion therapy

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly and is characterized clinically by an amnesic type of memory impairment, deterioration of language and visuospatial deficits (1). The disorder involves progressive functional and behavioral disturbances including the impairment of daily activities, mood changes, apathy, psychosis and agitation (1). The neuropathological hallmarks of the disease include the loss of neurons and synapses, beta-amyloid (Aβ) accumulation in parenchymal senile plaques and in the vascular vessel bed, and the aggregation of the hyperphosphorylated microtubule-associated protein tau into neurofibrillary tangles (NFTs) (2). During early disease phases the pathological process is characterized by abnormal protein processing, leading to the aggregation and accumulation of Aβ peptides, aberrant activation of the brain's innate immune system cells, and neurotoxicity (3-5). However, clinical trials with anti-Aβ drugs have not shown efficacy (6). The current treatment of AD is symptomatic and includes neuroprotective strategies, cholinesterase inhibitors, psychopharmacologic agents to reduce behavioral disturbances, and health maintenance activities (1). These existing therapies do not interfere with the course of the disease and novel drugs are needed to slow the progression of AD (7).

Data from both basic and clinical research suggest that inflammatory mechanisms may contribute to AD pathology (8-10). Indeed, it is widely accepted that innate immune mechanisms in the central nervous system (CNS), particularly microglia, underlie the neuropathological changes in AD (5). Immunosurveillance for the presence of pathogens and cellular debris is carried out by microglia using their highly motile branching processes; these cells also provide trophic factors that support tissue maintenance (5, 11). The plasticity of microglial cells highlights the transition between several activation states evident during the progression of AD pathology (12, 13). Indeed, microglia may initially play a protective role by contributing to tissue repair, Aβ clearance and anti-inflammatory activity (14, 15). However, prolonged intracerebral Aβ production and the presence of inflammatory stimuli lead to the chronic activation of microglia during later stages of the disease (5, 13, 16-19). This is characterized by less effective Aβ clearance and the augmented secretion of pro-inflammatory cytokines, chemokines, reactive oxygen species (ROS) and other neurotoxic products, leading to the accumulation of Aβ and thereby amplifying the general inflammatory environment that promotes neuronal and synaptic damage (13, 16-19).

Although inflammation appears to arise and develop within the CNS, peripheral inflammatory factors also play a role in the onset and progression of AD (20, 21). Indeed, microarray data from blood samples collected from patients with mild cognitive impairment (MCI) and AD suggest that a peripheral immune response is a very early feature of the disease (20). These changes are consistent with the activation of circulating leukocytes and their infiltration into the brain across the vascular wall (10, 22-29). Among the blood-derived leukocyte subpopulations, monocytes migrate into the brain in animal models of AD and their presence is associated with Aβ clearance (30, 31). T cells are also present in the brains of AD patients, but their role appears to depend on the T cell subset and the disease phase (10, 32-35). Vascular inflammation and the trafficking of immune system cells have recently been implicated in the pathogenesis of AD, based on studies showing that neutrophils invade the brain and contribute to the induction of cognitive dysfunction and the neuropathological hallmarks of AD (36, 37). Thus, the inhibition of neutrophil trafficking may offer a new therapeutic approach for the treatment of this devastating neurodegenerative disease.

Epidemiological studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk of AD, supporting a role for inflammatory mechanisms in AD pathogenesis (38, 39). However, NSAIDs have not shown efficacy in clinical trials involving AD patients, suggesting that more specific inflammatory mechanisms must be identified to interfere with disease progression (39-41). In this review we describe vascular inflammation in the context of AD and the potential role of leukocyte populations in AD pathology. We discuss novel strategies targeting selectins, integrins, chemokines and other leukocyte-dependent inflammation mechanisms in AD, and suggest anti-inflammatory therapies that have already been tested in humans and that could be translated rapidly into the clinic for the prevention and treatment of AD.

Blood–Brain Barrier Dysfunction in AD

The blood–brain barrier (BBB) is a highly specialized endothelial cell membrane that coats cerebral microvessels and regulates the entry of plasma components and blood cells into the CNS (10, 42-45). The intercellular junctions between endothelial cells, which include tight junctions, control the diffusion of proteins as well as leukocyte trafficking from the blood to the CNS (10). The BBB functions within the neurovascular unit (NVU), which also includes pericytes, vascular smooth muscle cells from the vessel wall, glial cells and neurons (10, 44, 46). The NVU structure controls BBB permeability and cerebral blood flow, and maintains the chemical composition of the brain interstitial fluid, which is required to support functional neuronal circuits (45). For example, pericytes regulate the structural stability of microvessels and blood flow through brain capillaries by controlling cellular contraction/relaxation (10, 43, 45, 46). These cells also control vesicle trafficking in the CNS, clear toxic cellular byproducts from the CNS, and regulate leukocyte trafficking in the brain (10, 42-46). Astrocytes and microglia surround the parenchymal basal membrane of brain microvessels, with astrocyte endfeet supporting endothelial functions and providing the cellular link to neuronal cells (10).

The loss of BBB integrity may be involved in the initiation and progression of AD (45, 47-53). Recent data from MCI patients has shown that that BBB disruption is associated with cognitive impairment (54). The vascular deposition of Aβ in intracerebral and leptomeningeal vessels, also known as cerebral amyloid angiopathy, is associated with the degeneration of smooth muscle cells, pericytes, endothelial cells and ultimately the breakdown of the BBB (10, 49, 55, 56). Furthermore, in vivo studies have shown that the intravenous infusion of $A\beta_{1\text{-}40}$ produces extensive vascular disruption with endothelial and smooth muscle damage, whereas the direct injection of $A\beta_{1\text{-}42}$ into the rat hippocampus triggers microgliosis and exacerbates leakage from the BBB (22, 57). Similarly, tau was able to trigger a significant loss of transendothelial electrical resistance—thus increasing endothelial permeability—in a rat BBB model, suggesting tau may promote BBB damage during AD (58). These data were confirmed in a transgenic tauopathy mouse model showing a correlation between BBB dysfunction and the appearance of perivascular tau around major hippocampal blood vessels (59). Collectively, these data suggest that Aβ and tau play a role in the loss of BBB integrity, potentially

exacerbating the vascular inflammatory response and neurodegenerative process.

Aβ-rich brain regions in AD patients frequently show collapsed or extensively degenerated endothelium, and the major mechanism leading to cerebrovascular dysfunction is vascular oxidative stress (60). Indeed, in Tg2576 AD-like mice, $A\beta_{1\text{-}40}$ triggers NADPH oxidase activation, the major source of ROS in blood vessels (61). Moreover, vascular oxidative–nitrosative stress was recently demonstrated in cerebral endothelial cells treated with soluble $A\beta_{1\text{-}40}$, resulting in the loss of BBB integrity and massive endothelial dysfunction (62). Therefore, the generation of ROS by brain endothelial cells during AD may represent a key detrimental mechanism contributing to vascular damage in the brain (63). Aβ deposition in the vasculature also modulates the expression of tight junction proteins and changes the mechanical properties of endothelial membranes, potentially favoring the leakage of plasma molecules and the transmigration of immune system cells (10, 64).

Vascular Inflammation in AD

Previous in vitro studies have shown that brain endothelial cells cultured with Aβ produce inflammatory cytokines, suggesting that Aβ is a pro-inflammatory stimulus that activates vascular cells (65). Moreover, Aβ upregulates endothelial adhesion molecules that may in turn promote the transendothelial migration of leukocytes, further supporting the hypothesis that Aβ acts as a vascular activator (37, 66). Aβ may also bind to pericytes through formyl peptide receptor 1 (FPR1), low density lipoprotein receptor-related protein 1 (LRP1), low-density lipoprotein receptor (LDLR), receptor for advanced glycation end-products (RAGE) and CD36, which are expressed on pericytes in post-mortem AD brains, thus triggering pro-inflammatory signals and potentially promoting leukocyte recruitment (67-71). Aβ is also a NLRP3 inflammasome activator in microglial cells, which are part of the NVU and contribute to the function of the BBB (72, 73).

Activated microglial cells may also induce vascular inflammation by releasing a myriad of pro-inflammatory cytokines and chemotactic factors, thus favoring the recruitment of leukocytes through the BBB into the inflamed brain (13,74-76). For example, microglia release IL-1β, a major pro-inflammatory cytokine that increases the permeability of the BBB and abolishes the ability of astrocytes to maintain BBB integrity (77). Recent studies have shown that IL-1β also promotes neutrophil transmigration in a BBB model, suggesting it may have a role in leukocyte recruitment during AD (78). Microglia may also release the cytokines IL-8 and CXCL1/2, which may in turn attract neutrophils (79). Accordingly, neutrophils were recently found in the AD brain and may be responsible for the induction of cognitive decline and neuropathological changes in AD (37). Therefore, the activation of microglia together with the deposition of Aβ may affect endothelial cell function and may favor leukocyte transendothelial migration into the AD brain.

Under physiological conditions, vascular adhesion molecules are expressed at low levels in pial and choroid plexus venules (80). During brain inflammation, the expression of endothelial adhesion molecules increases rapidly, and their soluble forms may be released into the peripheral circulation, providing biomarkers of vascular inflammation and endothelial dysfunction (10, 81). Endothelial activation is associated with two main classes of adhesion molecules: endothelial selectins and integrin ligands from the immunoglobulin (Ig) superfamily (81).

The selectins are a family of three adhesion molecules: L-selectin, P-selectin and E-selectin. They are involved in the primary capture of leukocytes onto the endothelium in the blood vessels of lymphoid or nonlymphoid organs under physiological and pathophysiological conditions. L-selectin (CD62L) is expressed on most circulating leukocytes and controls homing to secondary lymphoid organs and migration to sites of inflammation (81). E-selectin (CD62E) and P-selectin (CD62P) are constitutively expressed on endothelial and nonendothelial cells or cell fragments, but are also upregulated by the activated endothelium during inflammatory diseases (81).

Recently, we reported that E-selectin and P-selectin are induced in transgenic animal models of AD compared to sex-matched and age-matched wild-type controls. In 5xFAD mice, which overexpress mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V) and London (V717I) familial Alzheimer's disease (FAD) mutations, and human PS1 harboring two FAD mutations, we observed that adhesion molecules were expressed in the early phases of the disease mainly in the vessels of the meninges and cortex, but also in the choroid plexus, hippocampus and amygdala of the mutant mice (37). Similarly, in 3xTg-AD mice, which express the three mutant human proteins PS1 (M146V), βAPP (Swedish) and tau (P301L), we observed the strong expression of endothelial selectins in the hippocampus and cortex during the early phases of the disease when the mice were 6 months old. These vascular adhesion molecules were also expressed during the preclinical and late disease phases, suggesting that vascular inflammation may play a continuous pathological role (37). Our recent data have also shown that $A\beta_{1\text{-}42}$ oligomers induce the expression of endothelial selectins in mouse brain endothelial cells, suggesting a direct role for Aβ in endothelial activation and selectin expression, which may support leukocyte adhesion during AD (37). Also, the treatment of cerebral endothelial cells with oligomeric Aβ induced the expression of P-selectin and enhanced cell stiffness, potentially increasing the likelihood of leukocyte adhesion (64).

Soluble P-selectin and E-selectin have been detected in the plasma of patients with AD, suggesting the presence

of vascular inflammation, although some studies failed to obtain similar results, probably due to the low number of patients investigated (10, 82-86). Interestingly, circulating levels of E-selectin were also higher in subjects with increased risk of diabetes, a condition that is generally associated with cardiovascular disease and AD (87-89). Moreover, E-selectin levels appear to show an inverse correlation with the tau/$A\beta_{1-42}$ ratio in the cerebrospinal fluid (CSF), and E-selectin is more abundant in AD patients without the typical CSF biomarker signature (82, 90-92).

The expression of Ig superfamily integrin ligands such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) is upregulated on endothelial cells in transgenic mouse models of AD and human AD patients (37, 93-95). Recent data from our laboratory have shown higher expression levels of both ICAM-1 and VCAM-1 in 4-month-old 5xFAD and 3xTg-AD mice compared to sex/age-matched wild-type controls. Like the endothelial selectins, these integrin ligands were mainly expressed in the vessels of the meninges, cortex, hippocampus and amygdala in the mutant mice. In addition, a recent study using Arc/SweAβ mice found that VCAM-1 and ICAM-1 levels increased in the brains of 20- to 24-month-old mice compared to wild-type littermates, further supporting a role for integrins and their ligands in AD (34). The expression of ICAM-1 and VCAM-1 was observed in areas with Aβ deposits in both animal AD models and AD patients, suggesting a vascular role for Aβ in AD (34, 37, 96). In support of these results, our recent data show that oligomeric Aβ peptides induce the expression of ICAM-1 and VCAM-1 in brain endothelial cells, demonstrating that Aβ may directly activate endothelial cells (37).

Soluble integrin ligands have been found in AD body fluids, including serum and the CSF, providing further evidence for vascular inflammation in AD (82, 91, 97-101). A key study involving 260 AD patients reported a strong correlation between the presence of soluble forms of ICAM-1, VCAM-1 and PECAM-1 and the occurrence of AD (99). Moreover, soluble ICAM-1 was included among the 18 plasma proteins that can be used to classify blinded samples from AD and control subjects with 90% accuracy, and to identify patients that progress from MCI to AD within 2-6 years (102). However, these results have been challenged more recently by other researchers using a different algorithm, which did not confirm the findings concerning circulating soluble ICAM-1 in AD patients (103). Soluble VCAM-1 was also identified as a plasma biomarker in a study aiming to distinguish between AD patients and cognitively healthy control subjects (101). Elevated levels of soluble VCAM-1 in the plasma of AD patients correlate with the severity of dementia, macro- and microstructural brain changes observed by magnetic resonance imaging, and short-term memory loss (91). Soluble ICAM-1 and VCAM-1 are also serum markers of inflammation and endothelial dysfunction associated with aging, suggesting they may also represent a marker of age-dependent cognitive decline (104).

Together, these data demonstrate that brain endothelial cells are activated in transgenic animals with AD-like disease and in AD patients, suggesting they may mediate leukocyte adhesion and transmigration into the CNS, thus contributing to AD pathogenesis and memory decline.

Overview of Leukocyte Trafficking During Inflammation

The cascade of adhesion events leading to leukocyte recruitment at inflammation sites in a range of diseases has mostly been characterized by intravital microscopy (105-107). This multistep process involves a sequence of adhesion and activation events including: i) capture and rolling, followed by slow rolling, mediated by selectins interacting with their ligands from the mucin family and by leukocyte integrins interacting with their endothelial counterligands; ii) activation, during which G protein-coupled receptor (GPCR) signaling leads to the activation of integrins triggered by chemoattractants exposed by endothelial cells; iii) integrin-mediated arrest, triggered by interactions between leukocyte integrins and endothelial counterligands; iv) crawling, mediated by leukocyte integrins and their endothelial counterligands; and v) paracellular or transcellular transmigration, also known as diapedesis (Fig. 1) (106, 108).

Under inflammatory conditions, the activated endothelial cells upregulate the expression of both E-selectin and P-selectin, thus enabling the tethering and rolling of leukocytes on the vessel walls. During rolling, leukocytes also interact with chemokines, which are immobilized on endothelial cells and bind to their specific GPCRs on leukocytes, leading to the subsequent activation of integrins during the adhesion cascade (Fig. 1) (109). Integrins, which consist of α and β chains, are central molecules in the adhesion cascade. Some key integrins in the adhesion cascade belong to the β_2 family, including integrin $\alpha_L\beta_2$, which is also known as lymphocyte function-associated antigen 1 (LFA-1) or CD11a/CD18, and integrin $\alpha_M\beta_2$, which is also known as macrophage-1 antigen (Mac-1), complement receptor 3 (CR3) or CD11b/CD18. Others belong to the β_1 family, such as integrin $\alpha_4\beta_1$, which is also known as very late antigen 4 (VLA-4) or CD49d/CD29. Integrin ligands belong to the Ig superfamily, and the most prominent vascular adhesion molecules controlling leukocyte trafficking during inflammatory responses are ICAM-1 and ICAM-2, which bind β_2 integrins, and VCAM-1, which binds VLA-4 (81).

The infiltration of leukocytes leaving the circulation and migrating into the CNS is more complex than in other organs due to the presence of the endothelial basement membrane and glia limitans in postcapillary venules (10). During extravasation in the CNS, leukocytes need to express glycosidases and proteases in order to degrade the tight junctions and the sheath of extracellular matrix molecules composing the basement membranes (10).

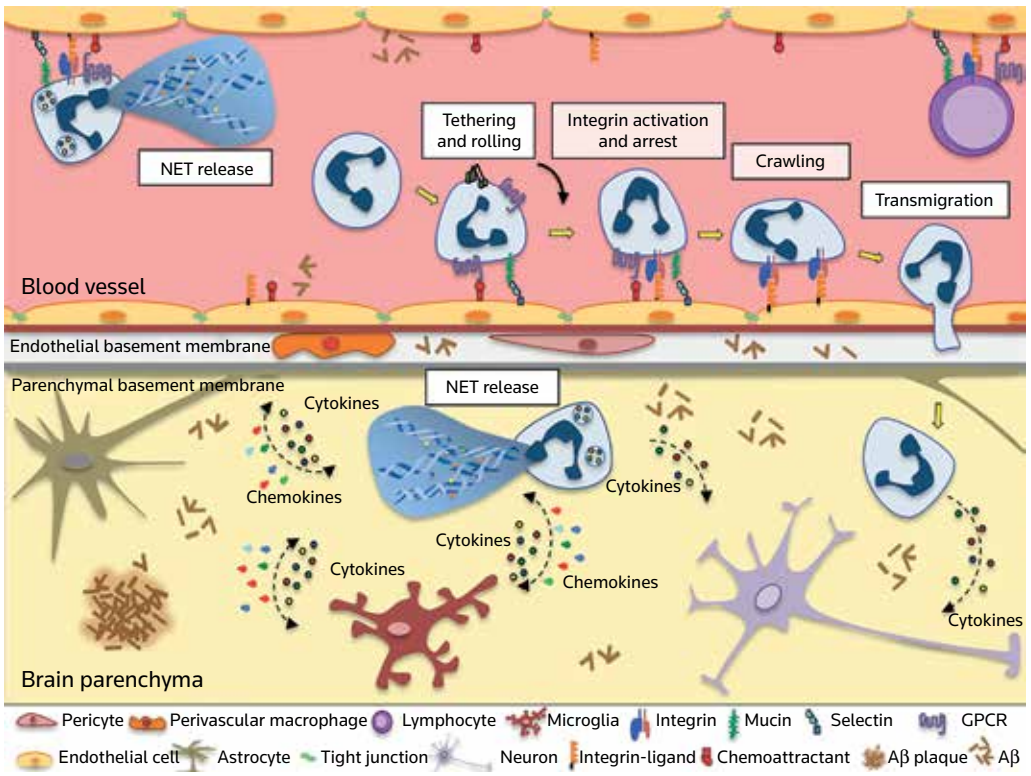

Figure 1. Schematic representation of leukocyte extravasation in the AD brain. This model illustrates the key steps of the leukocyte migration cascade in postcapillary brain venules, using neutrophil extravasation as an example. The activated endothelium in AD brain venules induces the expression of adhesion molecules and chemoattractants, supporting the adhesion of leukocytes that may subsequently transmigrate into the brain parenchyma. The first step of the adhesion cascade is tethering and rolling, followed by a slow rolling phase mediated by endothelial selectins and mucins expressed by leukocytes. Chemoattractants such as chemokines expressed by the endothelial cells bind to leukocyte GPCRs and induce integrin activation and integrin-mediated arrest. Integrins and their endothelial ligands from the IgG superfamily also control leukocyte crawling on the endothelial cells. After crawling, leukocytes transmigrate using a paracellular route as shown here, or transmigrate through the endothelial cell body (not shown). Neutrophils that adhere to the blood vessel walls may acquire a particularly toxic phenotype and release intravascular NETs along with reactive oxygen species (ROS), cytokines, chemokines and enzymes. Leukocytes then infiltrate the brain parenchyma and become harmful to neural cells. They can release intraparenchymal NETs and ROS (in the case of neutrophils), enzymes, cytokines and chemokines, thus amplifying the activation of glial cells and causing neuronal impairment, further contributing to the exacerbation of the neuroinflammatory process in AD.

Under conditions of inflammation in the brain, leukocytes exit the vasculature to infiltrate the CNS via three distinct routes: the first is from the peripheral circulation to the parenchyma through the walls of parenchymal postcapillary venules; the second is from the blood through the meningeal vessel wall to the subarachnoid space; and the third is through epithelial cells of the choroid plexus localized in brain ventricles, which is the route exploited by leukocytes for CNS immunosurveillance (110).

Migration of Circulating Leukocytes in the AD Brain

Although leukocyte trafficking during AD has not been investigated in great detail, several lines of evidence now support the hypothesis that both innate and acquired immune system cells adhere in CNS vessels and migrate into the brains of AD patients and equivalent animal models (Fig. 2) (27, 32, 36, 111, 112).

Recruitment of monocytes in the AD brain

Blood-derived myeloid cells migrate into the brain in transgenic animal models of AD and in AD patients (31, 113, 114). Previous studies using a myeloid-specific ablation system in APP_{Swe}/PS1 mice have shown that the recruitment of myeloid cells near Aβ plaques from the bloodstream promotes Aβ phagocytosis, suggesting these cells have a positive role in the control of AD progression (14). $Aβ_{1-42}$ exerts chemotactic and activating functions on human monocytes, and induces them to undergo in vitro transmigration in a model of the BBB (115). Furthermore, the stimulation

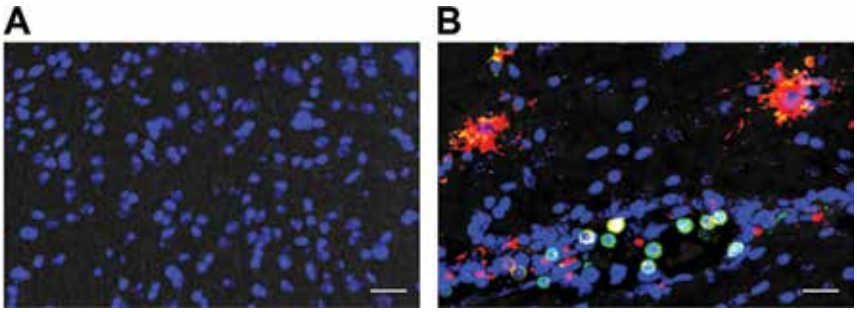

Figure 2. CD45$^+$ and CD18$^+$ cells are recruited in the brains of mice with AD-like disease. Confocal microscopy images show the presence of CD45$^+$ (red) and CD18$^+$ (green) cells colocalized in the cortex of sex/age-matched wild-type control animals (A) and 5xFAD mice (B) at 4 months of age. Nuclei are stained with DAPI (blue). Scale bar = 20 µm. The images are original.

of cultured human peripheral monocytes with $A\beta_{1-42}$ leads to the secretion of pro-inflammatory cytokines and chemokines, which further potentiate the recruitment and transmigration of these cells, thus amplifying the magnitude of inflammation during AD (115). These in vitro studies suggest that Aβ may play a role in monocyte recruitment in the AD brain.

The mechanism of monocyte recruitment during inflammation is also dependent on CCR2, a GPCR that is constitutively expressed on the pro-inflammatory subset of monocytes but sparse or absent among the patrolling/anti-inflammatory monocytes (116). Interestingly, the main CCR2 ligand (CCL2) is upregulated in mature senile plaques and microvessels in the brains of AD patients as well as in transgenic mouse models of the disease, suggesting a role in the recruitment of monocytes in the CNS (14, 113, 117-120). CCR2 deficiency in the Tg2576 and APP_{Swe}/PS1 mouse models of AD accelerated amyloidosis and memory deficit, suggesting that monocytes play a beneficial role in Aβ clearance (113, 121). These data were confirmed in APP_{Swe}/PS1 mice, which are deficient in the production of CCR2$^+$ monocytes and characterized by cognitive decline, disruption of synaptic activity and a higher Aβ burden (31, 122). The selective removal of patrolling monocytes in APP/PS1 mice has been shown to increase the Aβ load in the cortex and hippocampus, thus revealing the ability of these cells to naturally target and eliminate Aβ within the venous lumen (111). However, there is no general consensus on the role of monocytes in Aβ clearance. Two recent reports in mouse models of cerebral amyloidosis have shown that the replacement of resident microglia with peripherally derived myeloid cells does not modify the Aβ load or ameliorate AD pathology, failing to support a role for circulating monocytes in Aβ clearance (112, 123).

Neutrophil migration in the AD brain

Neutrophils are highly reactive immune system cells with a pivotal role in host defense. They are equipped with a plethora of cytotoxic mechanisms including the production of ROS and the release of enzymes, cytokines and neutrophil extracellular traps (NETs). Inappropriate activation of neutrophils contributes to long-term collateral tissue injury even if large numbers do not accumulate within the inflamed tissue. Notably, several studies have demonstrated that adhesion on the vessel wall, even in the absence of transmigration, is sufficient to induce endothelial damage and the disruption of junctions between endothelial cells (124-126). Therefore, the inhibition of neutrophil migration or of their pro-inflammatory functions has a positive outcome in experimental models of brain inflammation diseases such as stroke and multiple sclerosis (MS) (127-134).

A few studies, including our own, have shown neutrophil migration in the brains of AD patients or transgenic animals with AD-like disease, suggesting a role for these cells in AD (Fig. 2) (36, 37, 135-139). The presence of neutrophils in AD brains was previously underestimated for several reasons, potentially including their short life span, high turnover and phenotypic plasticity (140). Additionally, neutrophils invading the brain may rapidly disappear because they are engulfed by microglial cells, as recently reported in brain ischemia (141). It is difficult to identify cells with polysegmented nuclei (neutrophils) in the context of low-grade tissue inflammation using conventional staining methods, thus requiring either high-resolution light or electron microscopy, or the detection of specific neutrophil markers. The identification of such markers has helped to determine the key role of neutrophils in chronic inflammatory conditions such as atherosclerosis, adipose tissue inflammation, rheumatoid arthritis, systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody-related vasculitis, deep vein thrombosis, chronic obstructive pulmonary disease, cystic fibrosis, inflammatory bowel disease and MS (142-147). During low-grade chronic sterile inflammation, neutrophils release ROS, enzymes, cytokines and NETs, and can thus cause chronic collateral tissue damage even if large numbers of neutrophils do not accumulate within tissues. Indeed, earlier reports as well as our own data show

that large numbers of neutrophils do not necessarily need to accumulate in tissues in order to induce damage: intravascular adhesion per se without transmigration is sufficient to induce endothelial injury and the resulting tissue damage (124-126).

Our recent studies demonstrated that circulating neutrophils migrate into the brain and play a role in the induction of neuropathological changes and memory deficit in 5xFAD and 3xTg-AD mice (37). Interestingly, we found neutrophils adhering to cerebral vessels and invading the parenchyma in higher numbers at the onset of memory deficit in AD mice compared to wild-type controls, suggesting these cells play a key role in AD pathogenesis. Indeed, the migrated neutrophils produced IL-17, a cytokine that has previously been shown to induce BBB damage in vitro (148). In addition, neutrophils produce intravascular and intraparenchymal NETs, which may be harmful to endothelial and neural cells (Fig. 1) (37, 149, 150). Our two-photon laser-scanning microscopy experiments revealed that neutrophils firmly adhere and crawl inside blood vessels with Aβ angiopathy, and migrate into the parenchyma in areas with Aβ deposits, supporting a role for Aβ in the recruitment of neutrophils into the AD brain (37). The capacity of neutrophils to invade the AD brain was supported by a recent imaging study showing $Gr1^+$ cells infiltrating in the brain parenchyma and migrating toward Aβ plaques in 5xFAD mice (36). Importantly, depletion of neutrophils or blocking their migration into the AD brain reduced the severity of AD symptoms in animal models, suggesting that neutrophil-directed therapies may also benefit AD patients.

Adaptive immune cells in AD

$CD4^+$ and $CD8^+$T cells can adhere to the vascular endothelium or migrate into the brain parenchyma in mice with AD-like disease and post-mortem AD subjects, mostly in the hippocampus and cortical regions (27, 34, 151-153). Although these migrated T cells do not appear to co-localize with parenchymal Aβ deposits, as shown for neutrophils, chronic vascular inflammation induced by Aβ may favor the recruitment of these cells into the AD brain (34, 37). T cells were found to be associated with Aβ deposits in leptomeningeal and cortical vessels, further suggesting that Aβ angiopathy rather than intraparenchymal Aβ plaques drive T-cell infiltration into the AD brain (154). In support of these findings, recent studies have shown that Aβ causes the release of tumor necrosis factor-α (TNF-α) by microglial cells, promoting the subsequent transendothelial migration of T cells in an in vitro BBB model (155). Activated T cells that have migrated into the AD brain may trigger an inflammatory response that may in turn activate resident glial cells and recruit other inflammatory cells with detrimental effects on the CNS.

The role of adaptive immunity in AD has recently been explored in lymphocyte-depleted transgenic models of AD. Rag-5xFAD mice lacking T, B and natural killer (NK) cells have a greater Aβ burden and more severe gliosis and neuroinflammation (35). However, another study using APP/PS1 transgenic mice produced contradictory results, i.e., the Rag-PSAPP mice showed evidence of increased Aβ clearance—probably mediated by microglial cells—, suggesting that adaptive immunity has a negative role during AD (33). The only link between these two studies is that adaptive peripheral cell populations were found to act in concert with microglial cells. Accordingly, more recent studies have demonstrated that peripherally derived regulatory T (T_{reg}) cells modulate the inflammatory properties of monocytes and microglia in AD mice (32, 156-158). Notably, the systemic inhibition of T_{reg} cell functions for a very short period of time was sufficient to significantly reduce Aβ accumulation in 5xFAD mice during the later stages of AD, which is characterized by a robust cerebral Aβ plaque load (32). In apparent contradiction of these results, two recent studies in APP/PS1 and 3xTg-AD mice have shown that T_{reg} cells play a protective role during the early stages of the disease, i.e., at the onset of Aβ deposition and microglial activation, suggesting that they may regulate the activation state of the immune system cells (157, 158). Splenocytes from both young and old 3xTg-AD mice also showed a consistently lower level of Foxp3 expression compared to age-matched controls, suggesting that the limitation of regulatory activity may enhance systemic inflammation (139). Similarly, $CD4^+CD25^{high}$ T_{reg} cells were less abundant in AD patients with mild symptoms, indicating a dysregulation of peripheral T-cell subsets (159). These studies therefore suggest that T_{reg} cells may have opposing functions depending on the phase of disease, such that enhancing or inhibiting T_{reg} cell activity in the correct context could be used to therapeutic effect in AD.

A role for T cells in AD is also suggested by the number of $CD4^+$ and $CD8^+$ T cells in the peripheral circulation of AD patients compared to patients with other forms of dementia or age-matched controls (26, 27, 160-162). A significant reduction in the number of circulating $CD4^+$ T cells was reported in AD patients with severe symptoms compared to those with mild to moderate symptoms and to controls, apparently reflecting an increased sensitivity to apoptosis (26, 161, 163). However, other reports showed either no difference in the number of $CD4^+$ T cells or even an increase in AD patients compared to healthy controls (164-167). These discrepancies may be due to the low number of AD patients enrolled, the diagnostic criteria used to select AD subjects, and the cell-surface antigens used to identify lymphocyte subpopulations.

Although there are fewer studies focusing on circulating $CD8^+$ T cells in AD patients, the results are generally more consistent. The number of $CD8^+$ T cells in AD patients is lower than in elderly control subjects (165, 166, 168). Notably, these circulating $CD8^+$ T cells in AD subjects were

found to express the activation marker CD38, which is associated with migration into peripheral tissues and cytotoxic effector functions (169). These results are in agreement with a recent report showing higher numbers of activated $CD8^+$ T cells in the peripheral blood and the CSF of MCI and mild AD patients, correlating with clinical AD markers (162).

Collectively, these results show that adaptive immune system cells invade the AD brain, and the enhanced activation of circulating T cells—especially during early disease stages—may favor their migration into the CNS, thus promoting subsequent brain damage. Future studies aiming to better understand the role of lymphocyte subsets in AD will help to determine whether targeting pro-inflammatory functions in activated specific T-cell subpopulations may have a beneficial effect in AD.

Targeting Selectins and Their Ligands in AD

The selectins discussed above are type I transmembrane glycoproteins that bind sialylated carbohydrate structures in a Ca^{2+}-dependent manner. Selectin ligands belong to the mucin family and must carry the correct glycans, which requires the activity of several distinct glycosyltransferases, including α1,3-fucosyltransferases (FucT), the *O*-linked branching enzyme core-2-β1,6-glucosaminyltransferase-I (C2GlcNAcT-I), β1,4-galactosyltransferase-I (β1,4GalT-I), tyrosine sulfotransferases, sialyltransferases and sulfotransferases (106, 170, 171). P-selectin glycoprotein ligand-1 (PSGL-1) is a mucin-type glycoprotein that is considered the main ligand for P-selectin, although it can also bind the other two selectins in vivo (172-174). PSGL-1 is expressed on the surface of myeloid cells, activated lymphocytes, monocyte-derived microparticles and inflamed endothelial cells (81). Our studies have shown that glycoprotein T-cell immunoglobulin and mucin domain 1 (TIM-1) is also a major receptor for P-selectin, mediating Th1 and Th17 cell trafficking during inflammatory diseases including brain autoimmune diseases (175, 176). CD44 is an additional ligand for E-selectin that controls rolling interactions in neutrophils and activated T cells by binding to endothelial hyaluronic acid (177, 178). Other ligands have been identified for endothelial P-selectin, such as glycoprotein (GP)Ibα and CD24, and for E-selectin, such as E-selectin ligand (ESL)-1 and CD43, suggesting that selectins are promiscuous in their binding capacity (179).

Previous studies have found higher levels of soluble E-selectin and P-selectin in plasma samples from AD patients compared to control subjects, suggesting these molecules are expressed on the brain endothelium during AD (10, 82, 83). Soluble E-selectin and P-selectin can bind their ligands on circulating leukocytes, thus enhancing their adhesion capacity (180-182). For example, soluble P-selectin from plasma may ligate and crosslink PSGL-1 or TIM-1 on the leukocyte surface, triggering intracellular signaling pathways in leukocytes that promote adhesion and activate pro-inflammatory functions, thus favoring chronic inflammation in AD (175, 176, 182).

Selectin-mediated leukocyte recruitment in inflamed tissues has been reported for most organs including the brain, suggesting that selectins and their ligands may offer therapeutic targets for the treatment of inflammatory diseases (105, 179). Anti-selectin therapies have been tested in humans, but the outcome depends on which disease is targeted and the therapeutic approach used to inhibit selectin functions (183, 184). Initial studies have shown that blocking individual selectins using monoclonal antibodies does not improve the outcome of disease in humans, despite encouraging results in several animal models (179). The treatment of psoriasis patients with an E-selectin-specific antibody (CDP850) did not ameliorate the disease, and a humanized monoclonal L-selectin-specific antibody (aselizumab) had no effect on patients with multiple organ failure after trauma (185, 186). However, intravenous treatment with a humanized P-selectin-specific antibody (SelG1) was recently shown to reduce or prevent the occurrence of sickle cell-related pain crises in a placebo-controlled, double-blind study, suggesting that blocking P-selectin may offer a more effective approach in humans (SUSTAIN study) (187).

To overcome functional redundancy among the selectins, pan-selectin antagonists may offer a more efficient therapeutic approach for selectin-mediated inflammatory diseases. A recombinant human PSGL-1 IgG fusion protein (YSPSL), which binds to all selectins, improved early liver allograft function after liver transplantation, but had no effect in patients with myocardial infarction or kidney transplantation (188-190). However, the results achieved with chemically synthesized pan-selectin antagonists were more promising, and several compounds are being developed as anti-selectin therapies. Initial studies have shown that cylexin (CY-1503), an analogue of the sialyl Lewisx antigen, can reduce postoperative reperfusion-induced lung injury compared to a placebo (191). Bimosiamose, another pan-selectin antagonist, reduced the allergen-induced late allergy response in mildly asthmatic patients, ameliorated ozone-induced airway inflammation, and reduced airway inflammation in patients with chronic obstructive pulmonary disease (192-194). Furthermore, the subcutaneous intralesional administration of bimosiamose in patients with psoriasis reduced epidermal thickness and lymphocyte infiltration in the skin (195). Whereas bimosiamose was administered locally, recent studies with the pan-selectin antagonist GMI-1070 (rivipansel sodium) have shown that the systemic administration of selectin antagonists can also offer an effective therapeutic strategy. A randomized phase II study revealed that GMI-1070 substantially reduces the time to resolution of vaso-occlusive events and also reduces opioid use time in patients with sickle cell disease (196). Together, these studies support the

therapeutic potential of pan-selectin antagonists for the treatment of human inflammatory diseases, including AD.

Targeting Integrins in AD

Integrins are heterodimeric cell-surface proteins that play a major role in the migration of cells across the vascular wall and their trafficking inside inflamed tissues. In order to mediate leukocyte arrest on the endothelial wall, integrins require an activation process induced by vascular chemoattractants that bind GPCRs on the leukocyte surface (Fig. 1). This involves the activation of intracellular pathways known as "inside-out signaling," which enhances ligand binding affinity and clustering on the plasma membrane (106, 109, 197).

Our recent results demonstrate that LFA-1 integrin controls the intravascular adhesion, and also the intraparenchymal migration, of neutrophils in transgenic mice with AD-like disease (37). Moreover, we have shown that soluble oligomeric Aβ triggers the integrin-dependent adhesion of neutrophils on fibrinogen and ICAM-1, which are both β_2 integrin ligands. Oligomeric Aβ also triggered the transition of the integrin LFA-1 to the intermediate- and high-affinity states in human neutrophils, suggesting that Aβ induces inside-out signaling leading to integrin activation and cell adhesion. Endothelial ICAM-1 engagement by neutrophil LFA-1 may activate Src tyrosine kinases, calcium signaling, protein kinase C and small GTPases, leading to cytoskeletal changes in brain endothelial cells and increased vascular permeability, as has already been observed in the AD brain (45, 198, 199). High-affinity LFA-1 may also provide stop signals to arrested neutrophils within the parenchyma, potentially explaining the propensity of neutrophils to arrest in areas with a high Aβ burden (37). Notably, 3xTg-AD mice treated with antibodies blocking LFA-1 showed evidence of better memory restoration in behavior tests compared to mice treated with a control antibody. Accordingly, 3xTg-ADx-*Itgal*$^{-/-}$ mice, which lack LFA-1 integrin, showed improved cognition and less severe neuropathological changes compared to wild-type littermates, suggesting that LFA-1 represents a key molecular mechanism of pathogenesis (37). These data together support the hypothesis that targeting LFA-1 may represent an attractive therapeutic approach in AD patients.

The expression of VCAM-1 in the blood vessels of transgenic models of AD suggests that it may interact with VLA-4 expressed by several leukocyte subpopulations. VLA-4 is expressed by activated lymphocytes, and blocking its activity has a therapeutic effect in numerous experimental models of inflammatory diseases, including experimental autoimmune encephalomyelitis (EAE), the animal model of MS (105). Although the role of T cells in AD pathogenesis is unclear, some activated T-cell subsets may promote disease progression (10, 34, 153). VLA-4 is also expressed by approximately 3% of circulating neutrophils in both humans and mice, providing an alternative pathway for neutrophil migration to sites of inflammation (200, 201). Thus, we speculate that the therapeutic targeting of VLA-4 may interfere with leukocyte subpopulations that contribute to AD pathogenesis.

From a therapeutic standpoint, integrins are probably the most important class of cell-adhesion receptors (202). Anti-integrin therapy has been tested in humans with autoimmune diseases, but also in patients with other inflammatory diseases (203, 204). A monoclonal antibody directed against the α_4 chain of VLA-4 (natalizumab) was shown to block leukocyte trafficking at sites of inflammation. Initial clinical studies revealed that natalizumab is effective in patients with Crohn's disease and MS (203, 205). Following these promising results, natalizumab was approved in 2004 by the U.S. Food and Drug Administration and the European Medicines Agency as monotherapy for highly active relapsing–remitting MS. However, the drug was temporary withdrawn due to the rare incidence of a virus-induced neurodegenerative process, progressive multifocal leukoencephalopathy (PML), which affected approximately 1 in 1,000 patients (206-208). A subsequent phase III clinical trial confirmed that natalizumab is the most efficacious treatment of MS, despite the rare incidence of PML as an adverse effect, and it has been reintroduced as a second-line therapy for relapsing–remitting MS (209-211). Other agents have been developed to block α_4 integrin, including small molecules that may be administered orally with lower risk, representing further therapeutic opportunities for inflammatory diseases.

LFA-1 integrin was effectively targeted in psoriasis by the humanized anti-CD11a/CD18 antibody efalizumab (212, 213). However, this drug was withdrawn in 2009 following several reports of PML (occurrence 1:1,000), as previously reported for natalizumab and other immunomodulatory therapies including rituximab and belatacept (214, 215). The generation of new integrin antagonists via structure-based drug design has been proposed as a way to interfere with LFA-1–ICAM-1 interactions (202).

The approval of natalizumab and efalizumab has confirmed that blocking leukocyte trafficking is a valid therapeutic approach in humans. The growing evidence supporting a role for vascular inflammation and leukocyte trafficking during AD suggests that anti-integrin therapies may be useful to prevent or delay the progression of this neurodegenerative disease.

Targeting Chemokines in AD

Chemokines play a crucial role during immune responses by promoting the timely recruitment of specific leukocyte subpopulations to inflammatory sites. These small, soluble proteins exert their chemotactic activity by binding to GPCRs expressed on a wide variety of hematopoietic and

nonhematopoietic cells (216). The redundancy and promiscuity of the chemokine system has been determined predominantly by studying chemokine actions on specific immune system cells. It is evident that chemokines recruit several leukocyte populations, which in turn express receptors for and respond to different chemokines. Previous studies have shown that chemokines induce β_1, β_2 and β_7 integrin-dependent adhesion, particularly chemokines CCL2, CCL3, CCL4, CXCL10, CXCL9, CCL5, CXCL12, CXCL13, CCL19, CCL21, CCL20, CCL17 and CCL22 (109, 217). The binding of vascular chemokines to GPCRs on the leukocyte surface activates integrins and induces inside-out signaling, leading to leukocyte arrest in brain microvessels and subsequent extravasation under inflammatory conditions (109, 197).

Chemokines and their receptors are upregulated in the brain during acute and chronic CNS inflammatory conditions (81, 216). Both glial cells and neurons synthesize chemokines, which may recruit leukocytes and play a role in the initiation and maintenance of immune responses during neuroinflammatory diseases (218, 219). Accordingly, the astrocyte-derived chemokines CXCL10/IP-10 and CCL2/MCP-1 act as potential chemoattractants for inflammatory cells during EAE (220). Transgenic mice designed to produce CXCL10/IP-10 in their astrocytes spontaneously experience the massive infiltration of neutrophils (and to a lesser extent of T cells) in perivascular, meningeal and ventricular regions of the brain (221). Interestingly, higher levels of CXCL10/IP-10 and its receptor CXCR3 were reported in astrocytes associated with Aβ plaques in the AD brain, suggesting CXCL10/IP-10 may also recruit neutrophils and T cells into the brain during AD (222).

Aβ can directly activate glial cells by inducing proinflammatory gene expression and the release of cytokines and chemokines that potentially recruit peripheral immune system cells through the BBB and toward brain lesions and Aβ plaques (10). Indeed, the genes encoding CXCL8/IL-8 and CCL2/monocyte chemotactic protein-1 (MCP-1) are strongly upregulated in cultured microglia from the adult human brain following their stimulation with Aβ (223). These are potent chemoattractants for neutrophils and mononuclear cells, respectively.

Recent studies have shown that neutrophils are recruited into the AD brain and the CNS in mice with AD-like disease (37). In AD models, these cells contribute to the induction of cognitive deficit, microglial activation, Aβ deposition and tau hyperphosphorylation, suggesting that blocking neutrophil functions may have therapeutic effect in AD patients (37). Neutrophils express CXCR1, CXCR2 and CXCR4 on their surface, and these receptors can bind a large number of chemokines, including CXCL1, CXCL2, CXCL8 and CXCL12 (224). The injection of recombinant CXCL8 (IL-8) and CXCL2, also known as macrophage inflammatory protein 2α (MIP-2α), into the murine hippocampus induces a dramatic increase in the number of neutrophils recruited into the CNS as well as a loss of BBB integrity, suggesting this chemokine has a deleterious effect on the brain (126, 225). Aβ-stimulated glial cells secrete large amounts of CXCL8/IL-8, which potentially recruit neutrophils into the AD brain (223). CXCR2 is also expressed on microglial cells in the entorhinal cortex and hippocampus of AD patients at higher levels than in healthy controls, suggesting this receptor may play a role in the inflammation mediated by microglia during AD (225). Accordingly, CXCL8/IL-8 is the most strongly upregulated chemokine in human microglia stimulated with $A\beta_{1\text{-}42}$ (223). Although in vitro studies have shown that CXCL8 confers neuroprotective effects against Aβ-induced toxicity, the injection of $A\beta_{1\text{-}42}$ into the hippocampus of rats induces a time-dependent increase in CXCR2 and CXCL8/IL-8 expression, resulting in enhanced gliosis and a marked reduction in the number of neurons, indicating these molecules have a neurotoxic role in vivo (225). Moreover, the in vivo administration of a CXCR2 pharmacological antagonist led to a global amelioration of the inflammatory phenotype following the injection of Aβ, including reduced microgliosis and neuroprotection against oxidative damage (225). The role of CXCL8 in AD pathogenesis is also supported by studies showing higher levels of CXCL8 in the CSF of MCI and AD patients, correlating with age and neuropathological changes during disease progression (226). CXCL8-specific neutralizing antibodies have been used successfully in several animal models of inflammatory diseases or to reduce tumor load (227). Therefore, blocking the activity of CXCL8 and/or its receptors may also have beneficial effects against AD.

CCL2 (monocyte chemotactic protein-1/MCP-1) immunoreactivity was observed mainly in reactive microglia associated with Aβ plaques in AD brain tissue, suggesting a role for CCL2/MCP-1 in the inflammatory process during AD (117). The upregulation of CCL2 was also observed in the cerebral microvessels of AD patients and in mice with AD-like disease (14, 113, 118). Moreover, higher levels of CCL2 were detected in the CSF of MCI and AD patients (214). The in vitro stimulation of microglia and astrocytes with Aβ also triggers the release of CCL2 (228-230). CCL2 production by glial cells in vivo may induce monocyte recruitment from the blood to the brain, and their differentiation into macrophages, thus enhancing neuroinflammation and AD pathogenesis (31, 121). Collectively, these results support a role for CCL2 in AD pathogenesis and suggest this chemokine may recruit monocytes and T cells in the brain vasculature during AD. However, previous studies have shown that CCR2 deficiency exacerbates and accelerates memory deficit and enhances amyloidosis, suggesting that blocking CCR2 may have detrimental effects in AD (113, 121). CCR2 antagonists are currently undergoing preclinical and clinical investigation in several diseases, including psoriasis, rheumatoid arthritis,

asthma, obesity, chronic obstructive pulmonary disease, HIV infection, CNS diseases such as MS and cerebral ischemia, and cancer (231-235). Further studies are required to determine whether the therapeutic targeting of CCL2/CCR2 may reduce neuroinflammation and alleviate tissue damage in AD.

T-cell recruitment into the AD brain is also promoted by chemokine CCL3 (macrophage inflammatory protein 1α/MIP-1α), which is widely expressed during CNS inflammation (236, 237). Activated microglia secrete CCL3/MIP-1α during EAE, promoting T-cell migration into the CNS, and we suggest this may also be the case in AD (238). CCR5, the receptor for CCL3, has been detected on microglia and brain endothelial cells during aging and in the AD brain, suggesting it may play a role in brain inflammation (236). A small-molecule CCR5 inhibitor was approved by the FDA for the treatment of HIV infection, and future studies are required to determine whether the therapeutic targeting of CCR5 may also have an effect in AD (239).

Peripheral T cells in AD patients overexpress CXCR2, potentially stimulating their transendothelial migration (240). Previous studies have shown that $CD8^+$ T cells (but not B cells or $CD4^+$ T cells) express CXCR2 and, to a lesser extent, CXCR1; both are receptors for CXCL8 (241). Thus, CXCR2 may control the accumulation of not only neutrophils but also T cells in the AD brain, thus representing a further therapeutic target in this disease.

The chemokine system is a key drug target for the treatment of inflammatory diseases, given that inducible cytokines promote leukocyte recruitment to inflammatory sites (216). Accordingly, the biotechnology and pharmaceutical industry have developed several drugs to modulate chemokine receptor activity (216). However, the results from clinical trials have been disappointing, with some drugs (e.g., small-molecule CCR2 antagonists) showing no significant beneficial effects (232). This may reflect the complexity and redundancy of the chemokine network as well as our limited understanding of AD pathogenesis (216). In addition, chemokines not only mediate leukocyte recruitment during inflammation, but also play a physiological role in the CNS, including neurodevelopment and synaptic transmission (216). An additional challenge is therefore to design therapeutic agents selectively targeting the detrimental effects of chemokines and not their beneficial roles in the normal brain. Thus far, only small-molecule antagonists, peptide-derived inhibitors or humanized monoclonal antibodies against chemokines and their receptors have reached clinical trials (242, 243). However, innovative approaches such as siRNA nanoparticles and drugs targeting key signaling pathways and transcription factors responsible for the regulation of chemokine and chemokine receptor expression or function may lead to new therapeutic approaches based on the chemokine system (244, 245).

Targeting Other Leukocyte Functions in AD

One approach to limit tissue damage caused by leukocyte infiltration in the brain is the inactivation of pro-inflammatory compounds released by activated leukocytes, including cytokines, toxic oxygen metabolites, and protein components of the granules that participate as primary mediators of the inflammatory response. For example, neutrophil elastase (NE), a neutrophil-derived serine protease and major component of neutrophil azurophilic granules, is thought to play a key role during sterile inflammation (246). The release of proteases into the extracellular and pericellular spaces is responsible for the degradation of the extracellular matrix and the cleavage of inflammatory mediators, including chemokines and cytokines, and the activation of specific cell-surface receptors (246). These molecules offer further targets for the development of therapeutic agents. ONO-5046, a potent and specific NE inhibitor, has been shown to reduce cerebral ischemic damage in a middle cerebral artery occlusion rat model and in transient forebrain ischemia in rats (247, 248). This compound does not prevent the accumulation of neutrophils in damaged tissues during sterile inflammation, and we speculate that it could provide a benefit in combination with other anti-inflammatory therapies. Based on recent data showing a role for neutrophils in the induction of memory deficit and neuropathological changes in AD models, NE represents an attractive therapeutic target in AD (37).

The role of pro-inflammatory cytokines at different stages of AD has received special attention, offering the possibility for new therapeutic approaches. The pro-inflammatory cytokine TNF-α has been detected in the CSF of MCI patients at risk of AD, suggesting a propensity toward inflammation in these patients (249). TNF-α was also more abundant in the serum and CSF of AD patients compared to controls, suggesting TNF-α may play a continuous role during the disease (82, 249). The therapeutic targeting of TNF-α ameliorates tissue inflammation and has been successfully used in patients with autoimmune diseases such as psoriasis and rheumatoid arthritis (250). A pilot study in AD patients with mild to severe symptoms revealed sustained clinical improvement 6 months after the administration of etanercept, a potent anti-TNF-α fusion protein (251). However, a recent clinical trial did not confirm this promising finding, and failed to show statistically significant changes in cognition, behavior or global function (252). Therefore, additional research is needed to clarify the potential therapeutic effect of targeting TNF-α in the context of AD.

The therapeutic targeting of IL-1 has been used successfully to treat human inflammatory diseases. Anakinra is a recombinant version of the IL-1 receptor antagonist (IL-1RA) which is effective for the treatment of rheumatoid arthritis and other autoimmune diseases, and is also currently undergoing testing as a treatment for stroke (253, 254). IL-1RA reduces the amount of BBB damage in experimental stroke

models, and is therefore suitable as a clinical development candidate (254). Notably, IL-1β serum levels are higher in MCI and AD patients than controls, suggesting this molecule could be used as a stage marker for ongoing brain neurodegeneration in the continuum between normal aging and AD (255). Moreover, a characteristic *IL-1* gene cluster polymorphism appears to play a pivotal role in AD susceptibility (256, 257). The pharmacological inhibition of IL-1 improved cognition and attenuated tau pathology in 3xTg-AD mice, suggesting that targeting the IL-1 signaling pathway may result in promising therapies for AD (258). However, observations in other animal models of AD have suggested a role for IL-1 in Aβ plaque degradation by increasing microglial activation and phagocytic activity (259). Although the manipulation of IL-1 levels appears to be a promising therapeutic approach in AD, further studies are needed to clarify its role in AD disease pathology.

In addition to TNF-α and IL-1β, the cytokine IL-17A has recently emerged as an additional therapeutic target in inflammatory diseases (260). Previous studies have shown that IL-17 plays a pathogenic role in animal models of MS, rheumatoid arthritis, psoriasis, Crohn's disease and ulcerative colitis (261-267). Notably, treatment with the humanized anti-IL-17 monoclonal antibody ixekizumab, or with the anti-IL-17R antibody brodalumab, improved the clinical symptoms of psoriasis (268, 269). Furthermore, the fully human anti-IL-17A monoclonal antibody secukinumab was efficacious in patients with moderate to severe plaque psoriasis (269), suggesting that targeting IL-17 and/or its receptor is a promising approach for the treatment of diseases linked to Th17 cells and IL-17 signaling. Notably, elevated serum levels of IL-17 were reported in a cohort of Chinese AD patients (270). Furthermore, we have recently confirmed the presence of IL-17$^+$ neutrophils in the brains of AD mice, suggesting that IL-17 may play a role in neutrophil-dependent damage and pathogenesis (37). IL-17 is also a cytotoxic cytokine for neurons, and may alter the BBB and recruit neutrophils (148, 271). IL-17 released by neutrophils may also exacerbate brain microvessel pathology, thus worsening brain perfusion, which has already been shown in AD (272). Another significant property of IL-17 is its ability to induce NETosis, i.e., the formation of NETs, as reported for neutrophils associated with rheumatoid arthritis (273). Therefore, therapies targeting IL-17 signaling may offer a novel strategy for future AD treatment.

In addition to the important role played by the pro-inflammatory milieu found in the AD brain, key anti-inflammatory cytokines have also been shown to modulate the inflammatory responses (274, 275). IL-10$^+$ glial cells were identified surrounding Aβ plaques in the brains of Tg2576 and APP/PS1 mice, suggesting a role for anti-inflammatory mechanisms in AD (275, 276). IL-10 deficiency in APP/PS1 mice led to the potentiation of Aβ phagocytosis, reducing cerebral amyloidosis and mitigating cognitive impairment (275). These data are consistent with recent results showing that IL-10 expression in two APP mouse models increases the Aβ load, reduces microglial Aβ phagocytosis and causes more severe memory deficit (274). Together, these data suggest that IL-10-dependent inflammation suppressor pathways enhance Aβ pathology in animals with AD-like disease (274, 275). The IL-10 receptor alpha chain (IL-10Rα) accumulates to higher levels in the brains of AD patients than controls, suggesting that IL-10 may also promote AD pathogenesis in humans (275). We thus speculate that blocking the IL-10 pathway using monoclonal antibodies or the recently described IL-10 receptor-binding oligonucleotide aptamers may offer a novel and attractive therapeutic strategy for AD (277).

We have recently observed the release of NETs within the cerebral vasculature and brain parenchyma of AD patients and animal models, which suggests that NETs can potentially damage the BBB and neural cells (Fig. 1) (37). The generation of NETs involves a sequence of events including the production of ROS, the translocation of NE and myeloperoxidase (MPO) from granules to the nucleus, the extrusion of decondensed chromatin into the extracellular space, the processing of histones and, finally, cell disruption (278). These events generate tissue damage in many different diseases, including microbial infections, stroke, autoimmune diseases and other chronic inflammatory diseases, suggesting they may also damage the CNS (146).

The inhibition of NET formation is now considered a key strategy to control diseases associated with sterile inflammation (279, 280). A promising approach to target NETs is the inhibition of histone release and activity. Histones not only package DNA in the nucleus but also act as damage-associated molecular patterns (DAMPs) that mediate sterile inflammation and the associated organ injury (279, 281, 282). Histones are highly conserved proteins that combine microbicidal, cytotoxic and prothrombotic functions. Also, histones directly induce endothelial permeability by influencing junctional protein expression and the release of inflammatory mediators (283). This pro-inflammatory activity can be inhibited by activated protein C, a potent endothelial-derived anti-inflammatory and anticoagulant factor that protects against histone-induced mortality in murine sepsis (281, 283).

We have recently suggested that vascular Aβ deposits trigger the activation and adhesion of platelets and neutrophils on endothelial cells, thus promoting NET extrusion and blood clot formation associated with neutrophil activation (Pietronigro et al., manuscript submitted). Extracellular histones are prothrombotic, and the administration of recombinant thrombomodulin, which binds directly to extracellular histones, was shown to prevent lethal thrombosis in mice and was approved for the treatment of disseminated intravascular coagulation in Japan (284, 285). Heparin, another histone ligand widely used as an anticoagulant, prevents

in vitro cytotoxicity and reduces mortality associated with sterile inflammation and sepsis in mouse models, suggesting heparin could be used to treat patients suffering from sepsis (286). Therefore, we speculate that thrombomodulin or heparin (or the non-anticoagulant heparin, to avoid the risk of bleeding) could also be used as therapeutic approaches in AD.

Enzymes of the peptidylarginine deaminase (PAD) family play a fundamental role during NET formation by promoting histone deimination/citrullination (287, 288). The release of citrullinated histone H3, mediated by PAD4, is particularly important during NET extrusion because this is part of the cell death signaling pathway in the immune system (289, 290). We also detected citrullinated histone H3 in the brains of AD patients and mice with AD-like disease, suggesting this molecule may play a neurotoxic role (37). Furthermore, the formyl peptide fMLP induces histone H3 deimination in neutrophils, suggesting that the formyl peptide receptor (FPR) is required on neutrophils during NET formation and histone H3 deimination (289). We have recently shown that oligomeric Aβ triggers neutrophil rapid adhesion and ROS generation, a fundamental step in the extrusion of NETs, by engaging with FPR, suggesting that Aβ may contribute to NET release and the production of deiminated histone H3 (37). The inhibition of PAD is beneficial in animal models of chronic inflammatory diseases such as systemic lupus erythematosus, atherosclerosis and rheumatoid arthritis, and has been proposed as a novel therapeutic approach in these diseases (280, 291, 292). However, the effect of blocking NET formation in animal models of AD is unknown and further studies are required to determine any potential therapeutic benefits in AD.

Conclusions

Leukocyte trafficking is a key event in the pathogenesis of chronic inflammatory diseases, thereby offering a potential target for therapeutic intervention. Tissue-specific interactions between leukocyte subpopulations and the specialized vascular endothelium have been characterized in the last two decades, with different combinations of adhesion molecules and chemoattractants regulating leukocyte trafficking to different organs. The unexpected recent discovery that neutrophils promote AD pathogenesis in mouse models has opened a new area of investigation highlighting the prominent role of circulating immune system cells in AD. Neutrophil depletion or the blocking of integrin LFA-1 reduces the severity of symptoms in animal models of sterile inflammation, suggesting that neutrophil-directed therapy may have beneficial effects in patients with AD.

Several drugs affecting leukocyte trafficking have been successfully translated to the clinic for the treatment of inflammatory diseases. Promising results have been achieved in clinical trials with pan-selectin antagonists and a P-selectin-specific antibody, clearly supporting the therapeutic potential of anti-selectin therapy for the treatment of human inflammatory diseases. Notably, endothelial selectins are expressed during the subclinical and overt disease phases in AD models, suggesting that selectin inhibition is an attractive therapeutic approach to be tested in AD patients.

A humanized antibody against α_4-integrin (natalizumab) has been approved for the treatment of MS and Crohn's disease, and another against integrin-$\alpha_L\beta_2$ (efalizumab) has been approved for the treatment of psoriasis. Our recent data indicate that natalizumab also reduces the frequency and severity of seizures in a patient with MS and epilepsy, suggesting that anti-leukocyte adhesion therapy is a promising therapeutic approach for brain inflammatory diseases (204). However, anti-integrin therapies and other immunosuppressive drugs have previously been shown to induce PML in patients with autoimmune disorders, especially immunosuppressed patients (293). The availability of improved tests to detect patients at risk of PML, and the fact that AD patients are not normally treated with immunosuppressive drugs, suggests that anti-integrin strategies may cause less collateral damage and may therefore have beneficial effects in AD patients (293).

Recently discovered mechanisms in the chemokine system, such as the recycling of cell-surface receptors, specific signaling molecules, and the presentation of chemokines on glycosaminoglycans, also provide opportunities for novel therapeutic interventions (243, 294). Chemokines promote the neuroinflammatory processes in AD, thus making them potential therapeutic targets. The inhibition of other leukocyte functions, such as the recently described formation of NETs, could also offer a novel therapeutic approach to limit the extensive damage caused during AD (295).

Current AD therapies achieve only short-term memory improvement and do not prevent the overall course of cognitive decline. Therefore, we propose that targeting the mechanisms of leukocyte-dependent inflammation could inhibit AD pathogenesis and protect AD patients from ongoing cerebral injury during the progression of the disease.

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