COMMENT

APOE4-mediated Alzheimer disease and "Vascular"—"Meningeal Lymphatic" components: towards a novel therapeutic era?

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A three-dimensional graphic design representation of the potential role of meningeal vessels in Alzheimer disease. Although there are major differences between APOE4(+) and APOE4(−) Alzheimer disease cases (described in detail in the Comment article by Mentis and colleagues), the figure depicts the clearance of macromolecules and other solutes from meningeal lymphatic vessels. Cover image: Ella Maru Studio.

Molecular Psychiatry (2021) 26:5472–5474; https://doi.org/10.1038/s41380-021-01242-7

Alzheimer disease (AD) is the most common form of chronic neurodegeneration, and it is a significant global health problem [1]. One of the major hallmarks of AD is the formation of amyloid-beta (Aβ) plaques and tau aggregates within the diseased brain, both of which are pathognomonic (from a diagnostic lens) and are primary drivers of AD pathogenesis. Aβ removal from the brain occurs via various processes, including phagocytosis by glial cells, proteolytic degradation by mononuclear and vascular smooth muscle cells, and through the recently described lymphatic system [2]. The rapid aggregation of certain Aβ peptides (i.e., Aβ 1-40 and Aβ 1-42) may lead to major vascular blockage and cellular injury, resulting in impeded fluid drainage from the brain and neuronal dysfunction [3].

In contrast to the prevailing dogma of Aβ as the cause of AD pathogenesis, it is becoming increasingly accepted that pathophysiological processes driving the formation of amyloid plaques and vascular blockage potentially overlap. On the one hand, microglia activation results in the production and release of inflammatory cytokines, such as IL-1β and TNF-alpha, leading to neuronal damage [4]. These proinflammatory cytokines accumulate in those region brain regions, where tau is also present ultimately leading to the generation of reactive nitrogen species and neuronal cell injury [5]. On the other hand, neuronal damage is also mediated by Aβ binding to signaling receptors to activate vascular endothelial cells (VECs) [6], diminishing the expression of ATP-binding cassette (ABC) transporters across the endothelial membrane. In AD, Aβ plaque-mediated downregulation of ABC transporters may contribute to both Aβ clearance deficiencies and blood-brain barrier (BBB) dysfunction [7]. Neuronal homeostasis is maintained by the BBB, which regulates electrolyte balance, controlling the flow of metabolites and xenobiotics between the bloodstream and the brain [8]. Also, Aβ1-42 oligomers interact with the calcium-sensing receptors that are expressed on astrocytes, microglia, and oligodendrocytes; this interaction increases the formation and release of nitric oxide, vascular endothelial growth factors (VEGF), and proinflammatory cytokines, all of which collectively contribute to a neurotoxic environment [9]. The Aβ1-42 oligomer was also observed to increase oxidative damage and promote tau protein hyperphosphorylation, resulting in both synaptic and mitochondrial malfunction.

It is worth asking whether (or not) the notion of non-Aβ underpinnings of AD is completely novel. More than two decades ago, a *vascular hypothesis* of AD was proposed based on findings from cerebral perfusion and metabolic impairment studies in patients with AD [10]. In several investigations, blood vessels and capillaries appeared distorted mainly in the hippocampal and temporoparietal junctions. These vessels were twisted, curved, spiral, flexuous, knob-like bundles, and indicated the deterioration of smooth muscle in the vascular walls. Moreover, parenchymal tissue injury in AD patients may have affected the vasculature in other ways, resulting in elongated vessel and the formation of wicker-like networks. Aberrant vein structures could raise the peripheral resistance, disrupt the hemodynamic physiology of the vascular system and, hence, alter the rate of blood flow [11]. Of note, such string-like, non-functional capillary remnants, which consisted of cellular tissue lacking endothelial cells, were found elevated in the gray matter of brains from AD patients but not those of patients with Aβ present without dementia [12]. In the white matter, a two-fold rise in string-like vessel density and length was observed compared to that of patients with Aβ but not afflicted with dementia [12]. Vascular defects can lead to amyloid plaque accumulation, neurotoxicity, glial cell activation, metabolic dysfunction, and defects in the clearance of Aβ and other toxic metabolites [9]. Nonetheless, definitive evidence for the string-like

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Received: 5 July 2021 Revised: 8 July 2021 Accepted: 15 July 2021
Published online: 10 August 2021

vessel enlargement in the brain of patients with AD remains to be confirmed [7].

Another evidence supporting the notion that the malfunctioning vascular component is not a passive bystander of AD pathogenesis comes from vascular dementia (VD) [9]. While less frequent than AD, VD is linked to several cerebrovascular abnormalities that are specified by the types of arteries afflicted and the anatomical location of the brain damage. Because VD becomes more common as age increases (as with non-familiar AD), it is oft-perceived as a very common co-morbidity in AD [13]. However, severe vascular dysfunction as the underlying etiology of AD has not received as much focus as the Aβ hypothesis. Added evidence indicating a neurovascular aspect in AD stems from cerebral amyloid angiopathy (CAA), which morphologically harbors both vascular and neurodegenerative alterations in afflicted brain areas.

Moving forward, the role of meninges has been revisited and is now considered a crucial immunological gatekeeper guiding immune surveillance within the central nervous system (CNS). The recent studies of *meningeal lymphatic vessels* have shown that CNS immune cells, cerebrospinal fluid (CSF)-contained macromolecules, and toxic byproducts can be carried to the cervical lymph nodes by meningeal lymphatic vessels [14, 15]. These vessels run through the middle dura meningeal vessel and near the base of the skull [14]. They exhibit histological, immunohistochemical, and structural properties similar to the lymphatic vessels, allowing them to transport fluid and macromolecules from the brain interstitial fluid into the lymphatic vessels via the efferent paravascular glial lymphatic (*glymphatic*) system [16].

Previously, we provided, to the best of our knowledge, the first indication that the chief genetic risk factor for AD, *APOE4*, may exercise some of its AD-related effects through meningeal lymphatic vessels [16]. *APOE4* is linked to reduced expression of lymphatic vessel genetic markers and peripheral lymphedema-associated genes. Therefore, APOE4's action could result in the early shrinkage and attenuated function of lymphatic vessels in the meninges, leading to diminished CSF flow [17]. Given these results, the question arises as how the APOE4 protein exerts its effects on the meningeal lymphatic vasculature. Future studies will need to assess whether lymphatic vessels access APOE4. Studies have identified diverse sources of APOE4, from those secreted by local cells, such as astrocytes, microglia, or neurons to those derived from the choroid plexus circulating in the CSF [18]. Recent reports have also demonstrated that APOE isoforms in CSF can be distributed into the brain through the glymphatic pathway in mice [19]. Whether APOE4 in CSF affects meningeal lymphatic vasculature, the supposedly downstream pathway of the glymphatic pathway, waits to be elucidated. Additional studies will need to determine whether meningeal lymphatic endothelial cells (MLECs) are involved in AD pathogenesis by expressing receptors for APOE4. Main receptors for APOE4 in the brain include low-density lipoprotein receptor and LDL receptor-related protein 1 (LRP1), both of which are widely expressed in neurons, astrocytes, and microglia of the brain parenchyma, as well as in endothelial cells and smooth muscle cells at the BBB and cerebral arteries [20]. The study of the presence of APOE4 receptors on MLECs can lead to future studies unraveling the role of MLECs in AD pathogenesis. Last but not least, MLECs and VECs at BBB have many molecular properties in common [21]. Hence, the study of MLECs can be approached in a way analogous to VECs at BBB. At BBB, overexpression of *APOE4* can cause degeneration of pericytes which, in turn, significantly diminishes the function of VECs, resulting in cognitive decline [22]. Of note, however, is the fact that meningeal lymphatic dysfunction can present with various outward symptoms and is linked to several neurological disorders and processes, such as ageing and multiple sclerosis [23]. Given that pericytes in the BBB have been found to secrete APOE4, it might be possible that mural cells next to MLECs also secret APOE4 affecting the meningeal lymphatic structures and functions [24]. Taken together, it is intriguing and crucial to know if MLECs and meningeal lymphatic vasculature are compromised by APOE4 in order to further study their roles in AD pathogenesis.

Notably, other parallel roles of APOE4 in brain physiology and AD pathogenesis should not be neglected. First, there are some indications that APOE4 could function as a transcription factor affecting genes relevant to Alzheimer's disease [25]. Moreover, it can redistribute its downstream protein targets from the nucleus to the cytoplasm, altering the signaling cascade [26]. Also, *APOE4* may be a separate risk factor for ischemic vascular conditions, such as stroke and coronary artery disease in some patients [27]. Moreover, another Ab-independent role of APOE, i.e., one that involves activation of the cyclophilin A and matrix metalloproteinase-9 signaling pathway which, in turn, causes disruption of the BBB, has been recently implicated in AD pathogenesis [28]. Furthermore, the notion that the brain can act as neurosecretory organ mediating immune responses, by which the APOE4-mediated drainage can play a regulatory role as opposed to a passive role, cannot be excluded [29, 30]. Lastly, it would be intriguing to assess in future studies whether APOE4's role on COVID-19 infection is mediated not only through neurotropic actions and behavioral aspects (e.g., people with *APOE4* status AD are more prone to COVID-19 infection) [31, 32] but also through vascular-meningeal lymphatic pathways (following initial observations in the peripheral system [33]).

In conclusion, all of the above results support a clear role of the *vascular* and *meningeal lymphatic* components in AD, especially the major AD category that is linked to *APOE4*. In our precision medicine era, and in light of the failed clinical drug trials that aimed to solely target Aβ, it is high time we appreciate the potential value of therapeutic approaches that treat both the *neurodegenerative* and *vascular* component of AD. Crucially, therapies may entail administering both anti-Aβ passive immunotherapy and VEGF C to treat the neurodegenerative and *vascular-meningeal lymphatic* component of AD, respectively [2].

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ACKNOWLEDGEMENTS

Many thanks are expressed to Prof. Chrousos for previous helpful discussion in this research arena, to Prof. Seymour Reichlin for helpful comments on the notion of brain as a neurosecretory organ, and to Ms. Anna Gkika for her continuous moral support in this study.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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