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Clearance systems in the brain—implications for Alzheimer disease

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Abstract

Accumulation of toxic protein aggregates—amyloid-β (Aβ) plaques and hyperphosphorylated tau tangles—is the pathological hallmark of Alzheimer disease (AD). Aβ accumulation has been hypothesized to result from an imbalance between Aβ production and clearance; indeed, Aβ clearance seems to be impaired in both early and late forms of AD. To develop efficient strategies to slow down or halt AD, it is critical to understand how Aβ is cleared from the brain. Extracellular Aβ deposits can be removed from the brain by various clearance systems, most importantly, transport across the blood–brain barrier. Findings from the past few years suggest that astroglial-mediated interstitial fluid (ISF) bulk flow, known as the glymphatic system, might contribute to a larger portion of extracellular Aβ (eAβ) clearance than previously thought. The meningeal lymphatic vessels, discovered in 2015, might provide another clearance route. Because these clearance systems act together to drive eAβ from the brain, any alteration to their function could contribute to AD. An understanding of Aβ clearance might provide strategies to reduce excess Aβ deposits and delay, or even prevent, disease onset. In this Review, we describe the clearance systems of the brain as they relate to proteins implicated in AD pathology, with the main focus on Aβ.

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Competing interests

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Author contributions

M.J.d.L., J.M.T.-C., R.O.C., R.S.O., T.B., H.R., C.N., B.V.Z., K.B., H.Z. and T.W. researched data for article. M.J.d.L., J.M.T.-C., R.O.C., B.V.Z., H.Z. and T.W. wrote the article. M.J.d.L. and J.M.T.C. provided substantial contributions to discussion of the content. All authors participated in reviewing and editing of the manuscript before submission.

Introduction

Alzheimer disease (AD) is the most common type of dementia and comprises early-onset AD (EOAD) and sporadic or late-onset AD (LOAD).[1–3] EOAD affects a minority of AD patients, whereas LOAD afflicts over 95% of patients with AD.[4–6] Both EOAD and LOAD are characterized by excessive accumulation of toxic forms of amyloid-β (Aβ), which has been hypothesized to result from an imbalance between its production and clearance.[7–9] Emerging evidence suggests that Aβ clearance is impaired in both early-onset and late-onset forms of AD.[10,11] Specifically, carriers of EOAD-associated presenilin mutations show both increased Aβ production[10,12] and decreased Aβ clearance,[10] whereas individuals with LOAD exhibit decreased Aβ clearance only.[11]

Failure of Aβ clearance is increasingly recognized in the pathogenesis of AD. It is critical to understand how Aβ is cleared from the brain, and to find new ways of investigating this process in carefully phenotyped patients and healthy controls. Because Aβ deposition can be increased in presymptomatic individuals years or even decades before the hallmark symptoms of AD manifest,[20] an understanding of Aβ clearance might eventually provide strategies to reduce excess Aβ deposits and delay, or even prevent, disease onset.

Soluble Aβ can be removed from the brain by various clearance systems, including enzymatic degradation and cellular uptake, transport across the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB), interstitial fluid (ISF) bulk flow, and cerebrospinal fluid (CSF) absorption into the circulatory and lymphatic systems.

In the early 2000s, mouse studies demonstrated that the majority (75%) of extracellular Aβ (eAβ) is cleared by the BBB, with only a minority (10%) being cleared by ISF bulk flow.[14,15] However, two-photon imaging studies from the past few years have suggested that ISF bulk flow—facilitated by astroglial aquaporin-4 (AQP4) channels and named the glymphatic (glial + lymphatic) system—contributes to a larger portion of eAβ clearance than previously thought.[16,17] Furthermore, the discovery of meningeal lymphatic vessels suggests yet another potential clearance route.[18] Although the relative contributions of each of these systems to overall clearance are unknown, they act together to drive eAβ from the brain, meaning that alterations in any given system can contribute to the altered pathophysiology and accumulation of lesions in AD.

In this Review, we aim to describe the brain's clearance systems that are related to removal of toxic accumulation of proteins in AD. Here, 'clearance' is defined broadly as the removal of any substance, such as Aβ, from the brain. We focus on Aβ, given its ability to form aggregates within the extracellular space, but also briefly cover tau, which needs to be investigated in parallel with Aβ.

Background

Risk factors for AD

When characterized by autosomal dominant inheritance, EOAD is related to mutations in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) or amyloid precursor protein (*APP*) genes.[2–4] However, epidemiological data suggest that only a minority of EOAD cases demonstrate

autosomal dominant transmission, leaving the genetic association of the majority of EOAD cases unexplained.[2,19]

Various factors have been reported to positively and negatively modulate the risk of LOAD. Specifically, the greatest overall risk factor for LOAD is ageing;[20] for example, in the USA, over 40% of individuals above the age of 85 years have been diagnosed with AD.[21–24] The strongest identified genetic risk factor for LOAD is the apolipoprotein E (APOE) ε4 allele (*APOE**ε4*),[25,26] although genome-wide association studies have linked LOAD to several other genetic variants, such as *TREM2* (triggering receptor expressed on myeloid cells 2),[27] clusterin (*CLU*),[28] and phosphatidylinositol-binding clathrin assembly protein (*PICALM*).[28,29] Known environmental risk factors for LOAD include cardiovascular disease, and factors conferring a risk of cardiovascular disease, such as diabetes mellitus and hypertension. Head trauma, physical and mental inactivity, and sleep impairment are additional risk factors for LOAD.[13,30–35]

Pathological changes

AD is characterized by specific neuropathological and biomarker changes. The gross pathological changes consist of brain atrophy, particularly in the hippocampal formation, temporal lobes and parietotemporal cortices, accompanied by cortical thinning, enlarged ventricles and white matter abnormalities, as evident on MRI.[36,37] Microscopic changes include accumulation of Aβ into parenchymal senile plaques (also known as neuritic plaques) or in the walls of cerebral capillaries and arteries (known as cerebral amyloid angiopathy, or CAA), as well as aggregation of hyperphosphorylated tau into intracellular neurofibrillary tangles (NFTs) and neuropil threads.[36,38] The severity of CAA, the NFT load, and the magnitude of synapse loss—but not the number and extent of amyloid plaques—correlate well with the degree of cognitive decline.[39–44] These AD-related neuropathological changes presumably occur decades before symptom onset, because they are also found in individuals with mild cognitive impairment (MCI), and in people with no cognitive symptoms.[20]

In vivo AD biomarkers

Recent advances now enable several AD-related brain changes to be detected *in vivo*: ^{18}F-FDG-PET detects decreases in glucose metabolism,[45,46] and MRI detects brain atrophy, as well as diffusion and perfusion abnormalities, which are most prominent in the vulnerable hippocampal formation and cortical regions.[47–49] The pathological accumulation of Aβ and tau proteins in the brain can be inferred by analysing their levels in the CSF, with longitudinal changes having been described and modelled.[50–53] Specifically, Aβ accumulation into extracellular plaques is marked by decreased CSF levels of $A\beta_{1–42}$, and tau accumulation into NFTs is marked by increased CSF levels of total tau and hyperphosphorylated tau.[51,54] In addition, PET can be used to assess Aβ brain accumulation directly,[50] and PET for tau is currently under investigation.[52,55]

Clearance systems

The removal of soluble waste from the brain occurs via various overlapping clearance systems, which can be classified according to the compartment from which the waste is directly cleared, and the compartment into which the waste is directly cleared. Protein waste can be cleared from the intracellular compartment, or from the extracellular compartment, which comprises the ISF that surrounds neurons and the CSF that surrounds the brain. These proteins can then be removed by enzymes or cellular uptake, exported into the blood or lymph, or recirculated in the CSF (Table 1). The relative contributions of each of the various clearance systems are currently unknown; the prevailing view is that the BBB clearance predominates, though recent studies involving perivascular CSF circulation challenge this view, making clearance systems of the brain an important area for future research.

Degradation clearance

Degradation clearance is the enzymatic breakdown of proteins in the brain, and entails both extracellular and intracellular degradation. Extracellular degradation of ISF proteins mainly consists of degradation by proteases expressed and secreted by cells such as astrocytes.[56,57] ISF proteins can also be taken up from the extracellular space to be degraded intracellularly in neurons or glia, including phagocytic microglia and astrocytes.[58–60] Intracellular degradation of proteins occurs via the ubiquitin– proteasome pathway, the autophagy–lysosome pathway, and the endosome–lysosome pathway.[56]

Blood–brain barrier clearance

Interstitial proteins can be cleared into the blood directly at the BBB through specialized transport systems located in the brain endothelium.[61–64] The BBB endothelial cells are connected by tight junctions and have two functionally distinct sides: the luminal side facing the blood circulation, and the abluminal side facing the brain parenchyma.[65] In addition to the BBB, the brain is also protected by the so-called ‘glial barrier’ (also known as the glia limitans) that surrounds the BBB and consists of astroglial endfeet processes that cover the majority of the parenchymal vasculature, with the remaining area consisting of intercellular astrocytic endfeet clefts forming gap junctions.[66] The BBB and glial barrier are part of the neurovascular unit, which comprises various components, including cerebral microvascular endothelium, basement membrane, contractile pericytes (which share the capillary basement membrane with the endothelium), smooth muscle cells (which invest the endothelium of precapillary arterioles), astroglia, and neurons.[67]

Transport at the neurovascular unit across the glial barrier and BBB depends on the solubility, molecular weight and diameter of the protein.[68,69] The relatively large size of the intercellular clefts (20 nm)[70,71] implies that the glial barrier is permeable to nearly all proteins.[66] Given the size of AD-related proteins, monomeric $A\beta_{1–40}$, $A\beta_{1–42}$ and tau, should be able to pass freely through astrocytic endfeet clefts at the glial barrier.[72] However, endothelial tight junctions at the BBB prevent free passage of Aβ and tau into the blood, so they must instead be transported across the endothelium by specialized transporters, which, as descried below, have been identified for Aβ, but not for tau.

Interstitial fluid bulk-flow clearance

ISF proteins can also be cleared directly into the CSF via ISF bulk flow[73] that enters the CSF sink (described below) or the perivascular space (sometimes referred to the paravascular space—we use the term 'perivascular space' to describe the region surrounding the parenchymal vasculature).

Cerebrospinal fluid sink clearance—In parts of the body other than the CNS, lymphatic vessels run in parallel with the circulatory system to clear waste from the ISF in the form of lymph. Lymphatic vessels have recently been described in the meninges surrounding the mouse brain,[18] but the brain parenchyma itself is devoid of such vessels, leading to the long-held assumption that CSF serves as a 'lymph equivalent' to clear waste from the CNS.[74,75] Apart from transport across the endothelium, the removal of ISF from the brain parenchyma was traditionally believed to occur by diffusion[76] or ISF bulk flow into the CSF sink, which comprises the ventricles and subarachnoid space.[77] Given that diffusion is dependent on molecular size, diffusion into the CSF sink has been proposed to be too slow for the highly metabolic and large human brain.[77] As such, ISF bulk flow, which is independent of molecular size, has been proposed as the predominant pathway for movement of large molecules into the CSF sink.[77] ISF bulk flow was initially thought to course through the brain in a diffuse manner, but later evidence (described below) suggests the existence of definite pathways.[78]

Perivascular clearance—Support for an anatomically specific bulk-flow system came from a study of perivascular circulation.[79] Following infusion of horseradish peroxidase into the lateral ventricles or subarachnoid space of anaesthetized cats and dogs, CSF within the subarachnoid space flowed freely through the Virchow–Robin space—a histologically defined space where the subarachnoid space meets the perivascular space. From the Virchow–Robin space, CSF travelled into the periarterial spaces that surround penetrating arteries, moving along specific pathways in the same direction as blood flow.[79] CSF was also shown to move from the perivascular space into the interstitial ISF.[79] This perivascular circulation hypothesis challenged the traditional model of one-way flow of ISF into the CSF sink.[77,80,81]

Mouse model[82] and human[83] studies involving fluorescent soluble tracers and confocal microscopy have demonstrated that following intracerebral injection, ISF solutes diffuse and enter perivascular drainage pathways along the basement membrane of capillary and arterial walls separating smooth muscle cells,[84] then move towards the leptomeningeal arteries at the surface of the brain and, ultimately, to cervical lymph nodes (Figure 1).[84] This pathway was named the perivascular drainage pathway, and was deemed to be the lymphatic drainage of the brain.[44]

The perivascular circulation hypothesis[79] was recently confirmed and expanded on by a study in mice.[16] Following tracer injection into the CSF at the cisterna magna, two-photon microscopy was used to visualize in real time the flux of CSF in living mice through a closed cranial window.[16] As already hypothesized decades ago,[74] CSF was found to act like lymph: it flushed out interstitial substances in a process facilitated by glial cells, prompting

the authors to name it the glymphatic system (Figure 1).[16] This evidence corroborated prior findings that CSF, driven by arterial pulsation, flows into the periarterial space, following the course of the arterial vascular smooth muscle basement membrane to reach the basal lamina of the brain capillary bed, and entering the interstitium at all levels of this perivascular route.[79] The work also confirmed that ISF moves by bulk flow.[77] Moreover, the study extended these findings to include a role for astroglial AQP4 channels. These channels were found to mediate CSF transport from the periarterial space across the pial–glial membrane into the interstitium, where it mixes with ISF. The pia has been shown to be relatively permeable:[16,85] tracers injected into the subarachnoid space rapidly enter the perivascular space and brain parenchyma.[16] CSF–ISF movement from the interstitium into the perivenous space of deep draining veins runs ventromedially towards ventricular and deep white matter structures.[85] Hydrostatic pressure of periarterial bulk flow has been speculated to drive CSF water through the AQP4 channels, which is followed by astrocytic passage of molecules both through clefts and across astrocytes to maintain osmotic balance, although the mechanism has not been fully elucidated.[16] Any remaining CSF components course along the capillary basal lamina.[87,79]

It is unclear whether the perivascular drainage pathway and the glymphatic pathway are in fact distinct pathways, or whether they simply reflect transport along the same pathway captured under differing physiological or experimental conditions. In another study using two-photon imaging, fluorescent tracers were injected directly into the mouse brain via an open skull. Periarterial tracer accumulation was observed, although the direction of the flow was not discerned.[88] Opening of the skull is suggested to be a confounding factor in many experiments involving circulation of CSF, because skull removal can lead to inflammation and mechanical injuries to the cortical surface, or disturb local blood perfusion, BBB permeability and brain homeostasis.[89–92] Regardless, as recently suggested, the perivascular drainage and glymphatic pathways are not mutually exclusive: both could be active depending on the conditions, and the pathway in use could even be different between vessels or within the same vessel at different times (Figure 1).[86]

Cerebrospinal fluid absorption clearance

Following clearance from the ISF into the CSF, proteins must be cleared from the brain. Circulating CSF can be absorbed directly into the circulatory or lymphatic systems.

Circulatory clearance—Although other CSF production sites have also been suggested,[93–95] the majority of CSF seems to be produced at the BCSFB by the choroid plexus, a vascular unit of capillaries comprised of fenestrated endothelium and covered by choroid plexus epithelium (modified epen-dymal cells with tight junctions), located in the ventricles. The BCSFB serves not only as a CSF production site, but also as a ventricular CSF solute clearance site.[96,97] According to the traditional view, following CSF production by the choroid plexus, CSF circulates within the subarachnoid space, from where it is primarily cleared from the brain at arachnoid villi (also known as arachnoid granulations)—one-way valve structures leading to the dural venous sinuses.[98]

Lymphatic clearance—As described above, circulating CSF within the perivascular space can be cleared from the brain to cervical lymph nodes.[84,16] Another clearance route for circulating CSF to cervical lymph nodes is along perineural spaces, extensions of the subarachnoid space surrounding nerves.[99,100] Furthermore, the recent development of a method for mounting of whole meninges, such that mouse meninges can be examined intact on a single slide, led to the discovery of meningeal lymphatic vessels, which might provide another clearance route for circulating CSF proteins.[18] These meningeal lymphatic vessels might also provide a more conventional path for immune cells to exit the CNS, and dysfunction of these vessels might have important implications for neurological disorders associated with altered immune responses.[18]

Clearance of amyloid-β

Amyloid- β aggregation

Aβ is produced during neuronal activity[101] from amyloid precursor protein (APP), a membrane protein that acts as a signalling receptor.[101,102] In nonpathological conditions, APP is cleaved by α-secretase, which precludes formation of Aβ, and the resulting carboxy-terminal fragment is then cleaved by γ-secretase.[103] The resulting products do not aggregate.[104]

If APP is first cleaved by β-secretase 1 (also known as BACE1) instead of α-secretase, the subsequent γ-secretase cleavage will result in soluble monomeric Aβ. The most common soluble monomeric isoforms of Aβ are $A\beta_{1–40}$ (<80%), $A\beta_{1–38}$ (<20%) and $A\beta_{1–42}$ (10%).[105] $A\beta_{1–40}$ is prone to be deposited in the vasculature, as seen in CAA.[15,106] $A\beta_{1–38}$ is less likely to aggregate in either the vasculature or the brain than the other isoforms.[107,108] $A\beta_{1–42}$ has two additional amino acids, making it more hydrophobic than $A\beta_{1–40}$;[109] thus, it is capable of forming insoluble aggregates.[110] The tendency of $A\beta_{1–42}$ to form hard-to-clear aggregates is particularly increased when the concentration of $A\beta_{1–42}$ is high, and at a lower pH.[111–114]

The different forms of Aβ are in a dynamic equilibrium, and dense amyloid plaques can slough off soluble monomeric $A\beta_{1–42}$, which can then reform into aggregates.[115] Reflecting the equilibrium, in a longitudinal study of individuals carrying EOAD-linked presenilin mutation, CSF Aβ levels were initially reduced owing to aggregation of $A\beta_{1–42}$ into plaques.[10] Impaired clearance can, thus, result from Aβ aggregation— especially aggregation of $A\beta_{1–42}$—rather than from an intrinsic defect in the clearance system. Nonetheless, Aβ clearance systems can also become dysfunctional, as discussed below (Figure 2 and Table 2).

Degradation clearance

Intracellular Aβ (iAβ) can be degraded by proteasomes via the ubiquitin–proteasome pathway in neurons,[116] lysosomal cathepsin enzymes,[117] proteases (such as insulin-degrading enzyme, a thiol metalloendopeptidase that degrades monomeric Aβ) and insulin.[118] Extracellular Aβ can also be degraded by proteases, such as neprilysin (a membrane-anchored zinc metalloendopeptidase that degrades the Aβ monomers $A\beta_{1–40}$ and $A\beta_{1–42}$, and Aβ oligomers),[119] matrix metalloproteinases 2, 3 and 9,[120] glutamate

carboxypeptidase II,[121] endothelin-converting enzyme,[122] tissue plasminogen activator,[123] plasmin,[120] angiotensin-converting enzyme,[120] and insulin-degrading enzyme.[124] In addition, eAβ can be degraded following glial phagocytosis. Specifically, ISF Aβ can be taken up by microglia and astrocytes, whereas perivascular Aβ can be degraded by vascular smooth muscle cells, perivascular macrophages, and astrocytes (Figure 2).[125]

Degradation clearance of Aβ is affected by four main factors: enzyme expression and activity, ligand affinity and competition, activation of cellular uptake, and initiation of intracellular degradation pathways (Table 1), all of which become impaired with ageing and in AD. First, expression of neprilysin is decreased in AD,[126] especially in regions with high Aβ loads such as the hippocampus and temporal gyrus.[127] Although overall matrix metalloproteinase 2 expression is increased in AD,[58] its activity is reduced in astrocytes that surround Aβ plaques.[128] Second, both Aβ and insulin are ligands that compete for degradation by insulin-degrading enzyme; thus, hyper-insulinaemia can reduce clearance of Aβ, which might partly explain the link between type 2 diabetes mellitus and AD.[13] Third, plaques activate the immune effectors of the CNS—microglia and astrocytes[129]—inducing both phagocytosis of Aβ, which facilitates clearance from the extracellular space, and production of neurotoxic inflammatory cytokines.[130] Aβ that has undergone cellular uptake can then be degraded, for example via the autophagy–lysosome pathway[131] or be released back into the extracellular space,[130] as found in the brains of patients with AD.[132,133] Last, in AD, Aβ degradation via the endosome–lysosome pathway is increased relative to lysosomal degradation:[134] endocytic activity is elevated, resulting in accumulation of autophagic vacuoles, presence of lysosomal cathepsin enzymes in Aβ plaques, and abnormally enlarged endosomes containing Aβ, leading to generalized proteasome dysfunction.[134,135]

Blood-brain barrier clearance

Mechanisms of amyloid-β influx and efflux—Aβ is transported from the interstitial space across the BBB and into blood, and vice versa (Figure 3).[123] Specifically, local soluble Aβ is transferred from the interstitium to the brain by LDL receptor (LDLR) family members such as LRP1, and ATP-binding cassette transporters (ABC transporters).[14,136] Some evidence suggests that LRP1 is the main transporter for Aβ efflux at the BBB, whereas other studies have demonstrated its role to be quite minor.[137–139]

The main ABC transporter responsible for Aβ efflux is ABCB1 (also known as P-glycoprotein 1 or MDR1), which directly exports Aβ into the circulation. ABCA1, which is located on the abluminal side of the brain endothelium,[140] does not directly bind and extrude Aβ,[141] but mediates Aβ clearance in an ApoE-dependent manner.[142] The precise mechanism by which abluminal ABCA1 mediates Aβ clearance is unknown, although this transporter has been proposed to induce ApoE lipidation, which facilitates ApoE-Aβ interaction in the perivascular space, making Aβ more accessible to transport by LRP1 or ABCB1.[143] Clearance of Aβ through the BBB is also mediated by α2-macroglobulin (α2M),[14] and LDLR-related protein 2 (LRP2, also known as megalin) when LRP2 forms a complex with clusterin (also known as ApoJ).[14,136] In addition, insulin-degrading enzyme has been

proposed to have a role in Aβ clearance through the BBB, which might explain why BBB clearance is sensitive to insulin.[144]

Free Aβ can be transported from the circulation into the interstitium via RAGE (advanced glycosylation end product-specific receptor).[136,145] Soluble transporters (also known as sequestering agents)—such as the soluble form of RAGE (sRAGE),[14] anti-Aβ IgG,[14] serum amyloid P component (SAP),[14] and the soluble form of LRP (sLRP), which binds 70–90% of plasma Aβ—bind to soluble Aβ and inhibit its binding to RAGE, thereby preventing Aβ from entering the interstitium.[146]

Factors impairing amyloid-β clearance in AD—Clearance of Aβ through the BBB is affected by transporter expression and activity, ligand affinity and competition, and vascular integrity (Table 1). In AD, these factors are impaired in a number of ways. First, expression of the blood efflux transporters LRP1[123] and ABCB1[147] is decreased, whereas expression of the blood influx transporter RAGE is upregulated.[123]

Second, oxidative changes in AD are linked to changes in sLRP that reduce its affinity for Aβ, potentially facilitating Aβ influx into the interstitium by RAGE.[123] Inflammation, a common feature of AD, can affect ligand affinity by making the pH more acidic, which promotes hyperphosphorylation of tau and induces conformational changes in Aβ that hinder its clearance.[148,149] ApoE is a cholesterol transporter that competes with Aβ for efflux by LRP1 from the interstitium into the circulation;[150] competition for shared receptors is the primary mechanism by which ApoE mediates Aβ clearance.[151] The strongest genetic risk factor for AD is *APOE*ε4*[152] (*APOE*ε4*>*APOE*ε3*>*APOE*ε2*[151]), which codes for an ApoE isoform that is less efficient at mediating Aβ clearance than are the other ApoE isoforms.[153]

Third, ApoE4 is also associated with lower antioxidant activity than other ApoE isoforms,[154,155] and it mediates BBB breakdown through a proinflammatory pathway involving cyclophilin A in pericytes.[156] These findings are in line with evidence suggesting that increased oxidative stress[157] and loss of vascular integrity contribute to ageing[158] and AD,[159] as demonstrated by accelerated breakdown of the BBB and the neurovascular unit.

Interstitial fluid bulk-flow clearance

ISF bulk-flow clearance removes ISF—which contains eAβ—from the interstitium via ISF bulk flow into the CSF sink and perivascular space.[16,44] Here, we will discuss perivascular clearance of Aβ specifically via the perivascular drainage and glymphatic pathways.

Perivascular drainage—Aβ is cleared along perivascular drainage pathways.[83] In both AD[44,160] and CAA[44] (commonly associated with AD[84]), perivascular drainage of Aβ is impaired. Known factors affecting perivascular drainage of Aβ include *APOE*ε4*, deposition of immune complexes, arterial age, and—possibly—arterial pulsation (Table 1). The presence of ApoE4 is associated with reduced perivascular drainage of Aβ,[161] which in turn is linked to deposition of immune complexes.[162] Perivascular drainage of Aβ fails as arteries age;[163] this failure is associated not only with loss of homeostasis[164] and elevated levels of soluble Aβ in the brain, but also with accumulation of Aβ in arterial walls (as seen

in CAA), which increases the risk of intracerebral lobar haemorrhages.[165] One of the main complications following immunization against Aβ is the solubilization of Aβ from plaques and entrapment in perivascular drainage pathways, which worsens CAA.[162,166] It is possible that arterial pulsation drives perivascular drainage of ISF solutes,[88,167] and that morphological changes associated with age-related arteriosclerosis result in failure of perivascular drainage.[168] Of note, a high-fat prenatal maternal diet has recently been reported to result in a failure of Aβ clearance along cerebrovascular basement membranes. This failure was exacerbated if the high-fat diet had been lifelong, suggesting a role for epigenetic changes and diet in AD pathogenesis.[169,170]

Glymphatic clearance—Recent mouse studies suggest that the AQP4-dependent glymphatic pathway is an important clearance system for driving the removal of soluble Aβ from the interstitium. In mice, Aβ is cleared along perivascular pathways, and Aβ clearance was reduced by 55–65% in *Aqp4* knockout mice compared with wild-type mice.[16,171] Furthermore, glymphatic clearance was reduced by 40% in aged relative to young mice,[17] suggesting that the glymphatic pathway is impaired with age, which, as mentioned above, is the primary risk factor for LOAD.

Potential factors affecting glymphatic ISF bulk flow include molecular size, arterial pulsation, AQP4 expression and localization, and sleep (Table 1). Following subarachnoid injection, larger tracer molecules are slower to enter the parenchyma than are smaller tracers, and soluble perivascular Aβ can cross the 20 nm astrocytic endfeet clefts.[16]

Arterial pulsation is critical for perivascular circulation and transport of CSF into the interstitium.[79,172,173] Recirculating Aβ-rich CSF within the periarterial space, might be taken up by vascular smooth muscle cells, particularly in the presence of glymphatic stasis (caused by reduced arterial pulsation) that could facilitate protein misfolding and aggregation.[81,174–176] This is one mechanism by which Aβ might accumulate in the periarterial space, as seen in CAA, and the resulting Aβ accumulation might block perivascular pathways, further reducing glymphatic clearance.[87]

In *Aqp4* knockout mice, interstitial clearance is reduced by about 70%, resulting in a 55–65% reduction in Aβ clearance.[16,171] In AD, AQP4 expression could be decreased, given that in cultured mouse cortical astrocytes, interstitial $A\beta_{1-42}$ reduces AQP4 expression,[177] which can lead to additional accumulation of plaque-forming $A\beta_{1-42}$.[178] In traumatic brain injury (TBI)—a risk factor for AD— reactive gliosis is increased.[178] Initially, AQP4 expression is increased in TBI, but long-lasting AQP4 mislocalization from perivascular endfeet to the astrocytic soma occurs, resulting in reduced perivascular AQP4 availability, which can reduce Aβ clearance.[66,171] Both TBI and AD are associated with perivascular inflammation,[16,66] and these changes might partly explain the link between these conditions.[13]

In mice, Aβ clearance during sleep is twice as fast as during awake periods.[179] This increase in Aβ elimination is mediated by a 60% increase in the volume of the extracellular space, which might be modulated by a change in astrocyte cell volume in response to change in adrenergic signalling, as would be expected during sleep.[179–182] This expansion of the

extracellular space was caused by sleep itself rather than circadian rhythms, as it not only occurred during normal sleep, but could also be induced with anaesthesia.[17,179] It should be noted, however, that circadian rhythm disturbances have been reported in patients with AD,[183] and might affect clearance through a different mechanism involving increased oxidative stress caused by decreased expression of circadian clock genes, which are involved in protection from oxidative damage.[184,185]

The recent study describing the glymphatic system demonstrated that accelerated ISF-to-CSF bulk flow was partly responsible for the increase in total Aβ clearance during sleep, representing about 40% of total clearance, which can be calculated from the clearance rate constant data.[179] The remaining 60% is probably attributable to accelerated BBB transport of Aβ, because during these transport clearance measurements, the degradation of AB was minimal, which is in line with previous reports.[178,179] This finding might result from the glymphatic system flushing Aβ toward the BBB during sleep. Thus, sleep could indirectly increase BBB clearance of Aβ through increased glymphatic bulk flow, but it might also directly increase clearance through the BBB via various mechanisms, such as molecular changes (for example, upregulated LRP1), as seen with AD-protective physical and cognitive activity in mice.[187–189] These findings might partly explain why sleep impairment increases the risk of AD.[33,35]

Cerebrospinal fluid absorption clearance

Aβ in the circulating CSF can be absorbed either through the arachnoid villi[196] and BCSFB[136] into the circulation, or through the perivascular[16,44] and perineural spaces[191]—and possibly the meningeal lymphatics[18]—into the lymphatic system.

CSF absorption clearance of Aβ by the circulatory and lymphatic systems depends on CSF production, BCSFB integrity and transporters, arachnoid villi resistance, and lymphatic absorption of the CSF (Table 1). In ageing and AD, these factors are impaired in a number of ways. First, in ageing, and particularly in AD, CSF production by the choroid plexus is reduced, as shown by decreased water secretion into the ventricles via AQP1 water channels.[192] In AD, the choroid plexus undergoes many structural changes, such as calcification, fibrosis and Aβ deposition, all of which can obstruct CSF production.[193]

Second, these structural changes affect BCSFB integrity, thereby reducing Aβ clearance. Many of the Aβ transporters expressed at the BBB, including LRP1, LRP2, ABCB1 and RAGE, are also found at the BCSFB.[194] LRP1 is likely to have an important role in ventricular Aβ clearance at the BCSFB, given that the overall clearance rate of Aβ from the CSF is fivefold faster than the rate observed via CSF flow through the arachnoid villi.[195] However, the age-related change in expression of many BCSFB transporters follows an opposite pattern to that observed at the BBB for Aβ, such that there is increased efflux and decreased influx transporter expression, which is suggested to be a result of the BCSFB compensating for age-dependent BBB transporter defects.[136]

Third, CSF outflow resistance at the arachnoid villi is increased in AD.[196] This increased resistance is mechanistically similar to normal pressure hydrocephalus,[196] and has been proposed to result from amyloid deposition and fibrosis at the arachnoid villi,[197] resulting in

decreased CSF bulk outflow and, thus, decreased CSF Aβ absorption into the blood. Although no evidence has yet been obtained that CSF Aβ levels initially increase in LOAD, reduced CSF turnover would be expected to result in heavily Aβ-laden recirculating CSF, subsequently resulting in reduced concentration as Aβ is deposited in plaques,[111] in the vasculature as CAA,[175] and in the meninges, thereby increasing outflow resistance at the arachnoid villi.[98]

Last, lymphatic absorption of CSF decreases with age[98]—the primary risk factor for LOAD. In EOAD, by contrast, overproduction of Aβ might result in increased absorption of Aβ by the lymphatic system, as demonstrated in a transgenic mouse model of AD, in which increasing Aβ levels in cervical and axillary lymph nodes mirrored increased Aβ levels in the brain.[198]

Clearance of tau

Tau—a splicing variant of the microtubule- associated protein tau (MAPT)—is an intracellular neuronal protein that stabilizes axons.[199] Intracellular tau (i-tau) can undergo two transformations that are relevant to its clearance: modification and release. Tau modification is regulated by phosphorylation. i-Tau can undergo nondegradative cleavage by proteolytic enzymes, such as aminopeptidases, thrombin, HTRA1, calpain and caspases.[135] Rather than degrading tau, these enzymes produce proteolytic fragments, which can ultimately be degraded; however, these fragments have an increased propensity to form aggregates, resulting in reduced clearance. In AD, i-tau is hyperphosphorylated, which induces the formation of insoluble NFTs that cannot readily be cleared, and can also be neurotoxic.[200] Neuronal activation (namely, presynaptic glutamate release),[201] neuronal death and increased i-tau concentration or aggregation[202] trigger the release of i-tau into the extracellular space, leading to elevated CSF tau levels.

Tau clearance is less well understood than Aβ clearance, but also seems to be less complex. Transporters that specifically transport tau through the BBB have not been identified, which suggests that tau does not undergo clearance through the BBB, except after brain injury, when BBB permeability is temporarily increased.[203] Instead, tau is thought to be cleared from the brain primarily by degradation, ISF bulk flow, and CSF absorption clearance (Table 2). Recent studies using passive immunization with anti-tau oligomer antibodies have shown that like Aβ, pathological tau can be cleared from the brain by a peripheral sink mechanism, indicating that enhancement of tau clearance might be a therapeutic strategy in AD.[204]

Degradation clearance

Tau is mainly cleared through intracellular degradation by lysosomes via the autophagy–lysosome pathway, and by proteasomes via the ubiquitin– proteasome pathway.[202] AD-related dysfunction of these pathways has been suggested to result in the accumulation of soluble i-tau.[135] i-Tau can also be degraded by proteases (such as caspases) in response to apoptosis- inducing stressors, and by calpain in response to elevated intracellular calcium concentrations. Phosphorylation of tau by protein kinase A increases its resistance to degradation by calpain; thus, AD-associated hyperphosphorylation of tau has been suggested

to impair tau turnover and result in tau accumulation in the form of NFTs.[205] Following release of i-tau into the extracellular space—a process that could result from neuronal death or stimulation[202]—e-tau can be internalized by other neurons via endocytosis, leading to prion-like spreading of tau pathology.[206] In addition, soluble e-tau might bind to muscarinic type 1 and type 3 receptors, thereby increasing intracellular calcium levels, which might facilitate further release of i-tau.[202] Tau released into the extracellular space is highly stable:[207] its CSF half-life is 12–14 h,[208] compared with about 2 h for Aβ.[209]

Interstitial fluid bulk-flow clearance

If e-tau is not cleared by endocytosis, it might be cleared via the glymphatic system.[210] Following TBI, glymphatic clearance of ISF solutes was impaired by about 60% in wild-type mice, and to an even greater extent in *Aqp4* knockout mice that displayed NFTs, neuroinflammatory reactive gliosis, and neurodegeneration.[210] These findings support the link between TBI and tau aggregation, with resulting neurodegeneration similar to that seen in AD and chronic traumatic encephalopathy.[210,211] Recirculation of CSF poses an additional challenge to tau clearance: cells closest to the periarterial boundary might internalize tau from the tau-laden recirculating CSF within the periarterial space.[175]

Cerebrospinal fluid absorption clearance

As for any soluble substance in circulating CSF, tau can be absorbed either into the circulatory system from the arachnoid villi and BCSFB, or from the lymphatic system through the perivascular and perineural spaces. Meningeal lymphatic vessels[18] provide another possible route, although their specific contribution to tau elimination has not been tested.

Conclusions

Removal of proteins from the brain occurs via various overlapping clearance systems: enzymatic degradation and cellular uptake, transport across the BBB and BCSFB, ISF bulk flow, and absorption of CSF into the circulatory and lymphatic systems (Table 1). The majority of eAβ is cleared across the BBB, with a minority being cleared by ISF bulk flow (Figure 2 and Table 2).[14,15] However, the recently discovered glymphatic pathway (Figure 1) also seems to be an important contributor to eAβ clearance, because it can flush Aβ towards the perivascular space, thereby mediating clearance through the BBB (Figure 3) or re-entry into the capillary basement membranes.[16,17] The recently discovered meningeal lymphatic vessels might provide another clearance route,[18] but their role in Aβ and tau clearance has not yet been assessed. In contrast with Aβ, tau does not seem to undergo receptor-mediated BBB clearance; thus, the elimination of tau seems less complex than that of Aβ. However, the mechanisms involved in the clearance of tau from the brain are not completely understood, and further research into tau trafficking could help us better understand its role in AD.

Recent evidence for Aβ accumulation in LOAD points to reduced clearance, as opposed to overproduction, as the main culprit, but which specific clearance systems are defective is unclear.[10,11] The evidence for clearance failure in AD comes from the use of stable isotope

labelling kinetics,[212] which enable measurement of both Aβ production and clearance in humans. However, this technique does not provide information on the specific clearance systems themselves but, rather, provides a composite of production and clearance measures for Aβ. This technological progress has introduced an urgent need for detailed information on the specific Aβ clearance defects in patients with AD. Likewise, a technique to measure overall tau clearance and specific tau clearance defects in AD is needed, because such defects might be expected in light of the reduced CSF production and arachnoid villi defects that are observed in ageing individuals and patients with AD.

Because Aβ deposition can be increased in presymptomatic individuals years or even decades before the hallmark symptoms of AD manifest, an understanding of Aβ clearance might eventually provide strategies to restore clearance mechanisms, so as to eliminate excess Aβ deposits and delay or possibly even prevent disease onset. Whether the observed clearance defect in AD is a cause or a consequence of pathology, or merely coincidental, remains unknown. Regardless, Aβ clearance defects are a consistent finding in patients with AD, and might provide a useful biomarker and indicator of reversible clinical pathology.

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Key points

- Accumulation of neurotoxic forms of amyloid-β (Aβ) and tau proteins is the pathological hallmark of Alzheimer disease (AD)
- Excess deposition of Aβ results from an imbalance between its production and clearance; in both early-onset and late-onset forms of AD, Aβ clearance seems already impaired at the prodromal stage
- Aβ is removed from the brain by various overlapping and interacting clearance systems: degradation, blood-brain barrier (BBB) transport, interstitial fluid (ISF) bulk flow, and cerebrospinal fluid (CSF) absorption into the circulatory and peripheral lymphatic systems
- Although most extracellular Aβ undergoes BBB clearance, the recently discovered glymphatic pathway seems to be important for Aβ clearance
- Specific BBB transporters for tau have not been identified, suggesting that clearance of tau is less complex than that of Aβ, and mainly relies on degradation, ISF bulk flow, and CSF absorption
- Precise understanding of the mechanisms of clearance dysfunction in AD is paramount to develop strategies to reduce excess deposition of neuroxic protein and to halt the related pathological changes

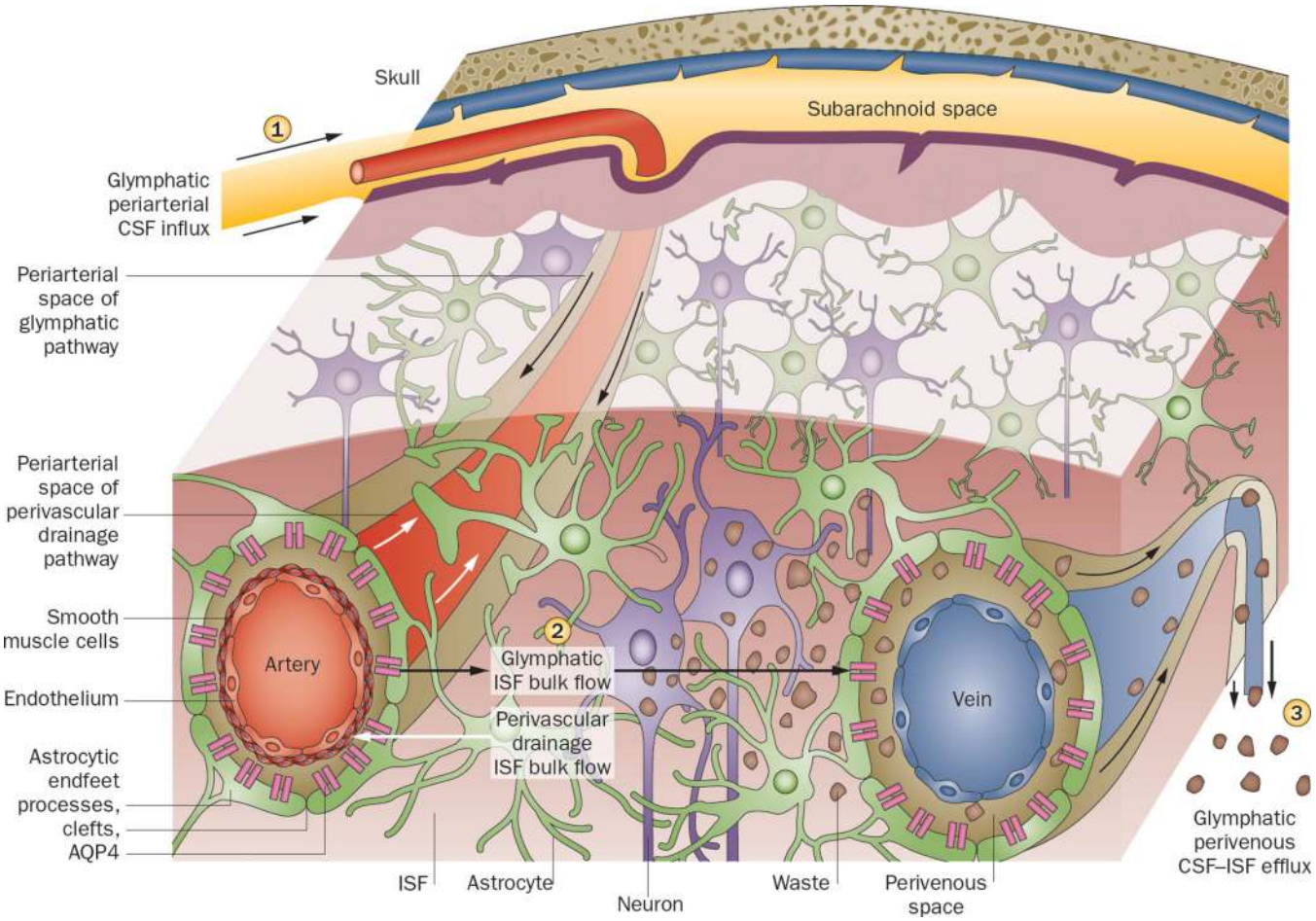

Figure 1.

Perivascular clearance comprises perivascular drainage and glymphatic pathways. The perivascular drainage Nature Reviews | Neurology pathway (white arrows) moves waste into the periarterial space (located along smooth muscle cells and the capillary basement membrane) and towards the subarachnoid space in the direction opposite to blood flow. The glymphatic pathway (black arrows) clears waste from the ISF through the brain parenchyma, and comprises three functional components. (1) CSF influx, unidirectionally with blood flow, into the periarterial space (between the basement membrane of smooth muscle cells and pia mater), where the water component of CSF crosses astrocytic AQP4 channels to enter the brain parenchyma. CSF solutes can be cleared with astroglial transporters or channels, or can pass through the astrocytic endfeet clefts. (2) CSF–ISF exchange within the brain parenchyma. (3) CSF–ISF movement into the perivenous space of deep-draining veins. Effluxed waste can then recirculate with the CSF, or eventually be absorbed into the lymphatic system. Arrows indicate direction of flow. Abbreviations: AQP4, aquaporin-4; CSF, cerebrospinal fluid; ISF, interstitial fluid. Permission obtained from Cell Press © Nedergaard, M. *Science* **340**, 1529–1530 (2013).

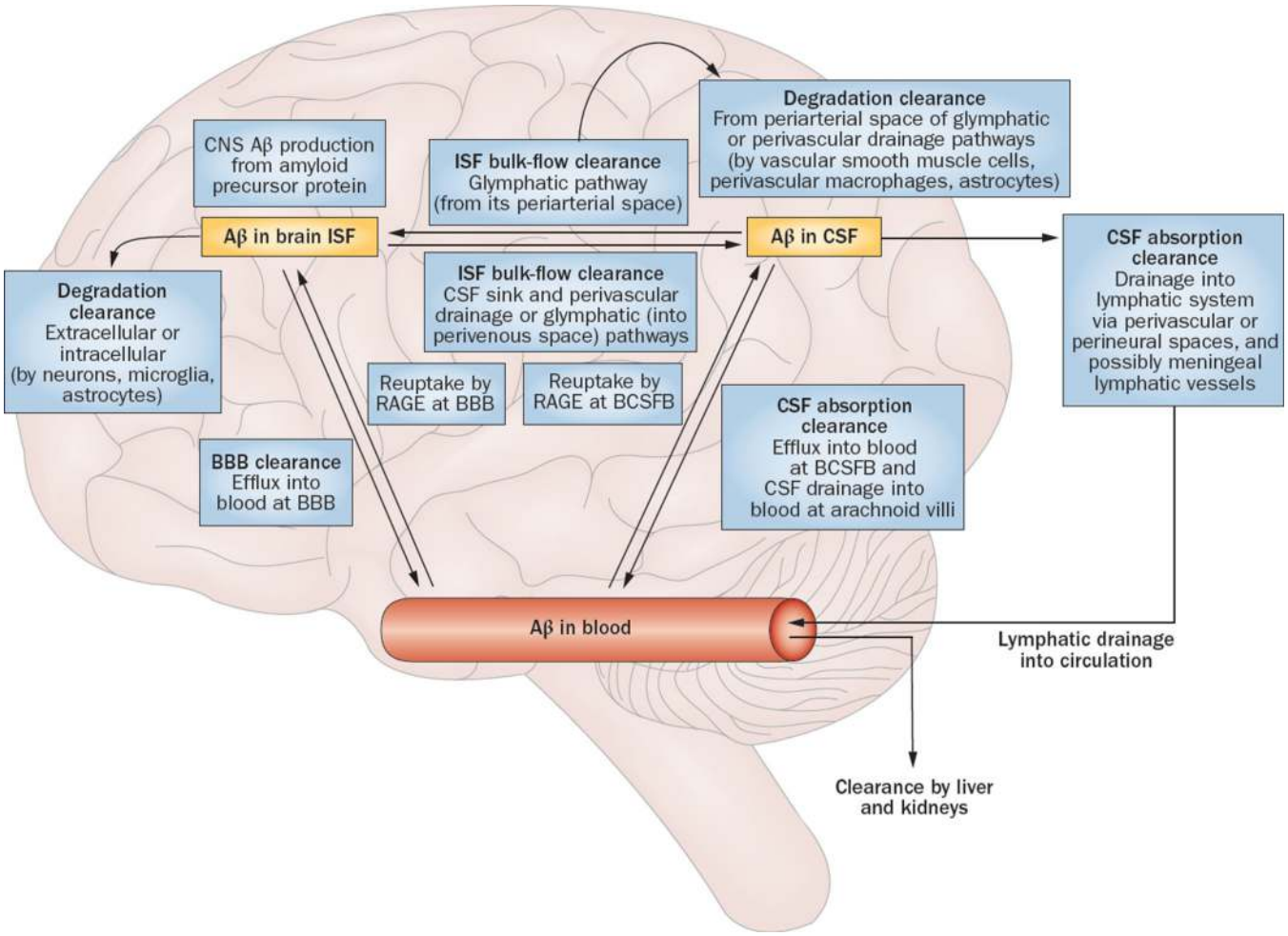

Figure 2.

Aβ clearance systems. Soluble Aβ can be removed from the brain by various clearance systems. Degradation clearance via extracellular and intracellular degradation pathways can involve either cellular uptake from the interstitium by neurons, microglia, and astrocytes, or uptake from the perivascular space by smooth muscle cells, perivascular macrophages, and astrocytes. BBB clearance involves Aβ efflux into the blood. ISF bulk flow clearance can occur into the CSF sink (ventricles and subarachnoid space), via perivascular drainage pathway, or via glymphatic pathway. CSF absorption clearance involves absorption either into the circulatory system from the arachnoid villi and BCSFB, or into the lymphatic system from the perivascular and perineural spaces—and possibly through meningeal lymphatic vessels. Abbreviations: Aβ, amyloid-β; BBB, blood–brain barrier; BCSFB, blood–CSF barrier; CSF, cerebrospinal fluid; ISF, interstitial fluid; RAGE, advanced glycosylation end productspecific receptor. Adapted with permission from Nature Publishing Group © Erickson, M. A. & Banks, W. A. *J. Cerebr. Blood Flow & Metabol.* **33**, 1500–1513 (2013).

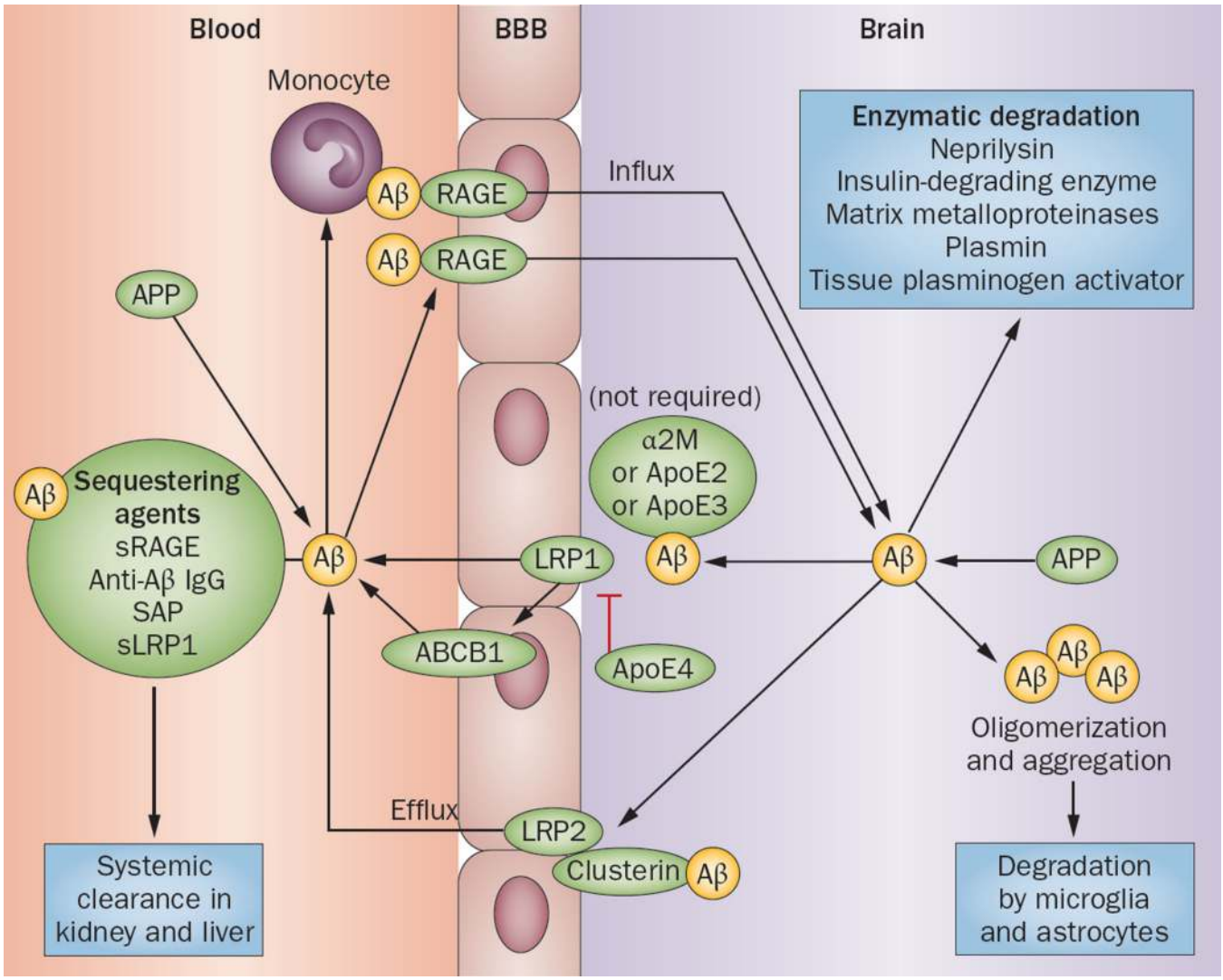

Figure 3.

Aβ efflux and influx through the BBB. Aβ can enter the brain via RAGE as a free plasma-derived peptide, or can be transported by monocytes. Sequestering agents (soluble transporters that chaperone Aβ for systemic degradation) can prevent Aβ entry from the circulation into the brain. Aβ is eliminated from the brain enzymatically or by transportation through the BBB. LRP1 mediates efflux of unbound Aβ and Aβ bound to ApoE2, ApoE3 or α2M from the brain parenchyma into the blood with the help of ABCB1; ApoE4 inhibits this transport process. Aβ bound to clusterin is transported through the BBB by LRP2. Abbreviations: α2M, α2-macroglobulin; Aβ, amyloid-β; ABCB1, multidrug resistance protein 1 (also known as P-glycoprotein 1); ApoE, apolipoprotein E; BBB, blood–brain barrier; LRP, LDL receptor-related protein; RAGE, advanced glycosylation end product-specific receptor; SAP, serum amyloid P; sLRP1, soluble LRP1; sRAGE, soluble form of RAGE. Permission obtained from Nature Publishing Group © Zlokovic, B. V. *et al Nat. Rev. Neurosci.* **12**, 723–738 (2011).

Table 1

Clearance systems in the brain

Clearance system	Source	Destination	Factors affecting clearance system	Clearance pathways
Blood–brain barrier clearance[61]	ISF	Blood	Transporter expression and activity Ligand affinity and competition Vascular integrity	Efflux transporters and mediators Influx transporters and mediators
Degradation clearance[56]				
Intracellular	ICS	Degradation	Enzyme expression and activity Ligand affinity and competition Initiation of intracellular degradation pathways	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases
Extracellular	ISF	Degradation or cellular uptake	Enzyme expression and activity Ligand affinity and competition Activation of cellular uptake	Proteases Glial phagocytes
ISF bulk fow clearance[86]				
CSF sink	ISF	CSF sink (subarachnoid space, ventricles)	Intrinsic ISF fow rate	ISF efflux into CSF sink
Perivascular drainage	ISF	Periarterial space to peripheral lymph	*APOE**ε4* Immune complex deposition Arterial age Arterial pulsation (hypothetical)	ISF efflux into basement membrane of capillary and arterial walls
Perivascular glymphatic	ISF	Perivenous space to peripheral lymph or ventricles	Molecular size Arterial pulsation *AQP4* expression and localization Sleep	CSF influx into periarterial space CSF–ISF exchange within interstitium CSF–ISF efflux along perivenous space
CSF absorption clearance[18,98]				
Circulatory	CSF	Blood	CSF production BCSFB transporters Arachnoid villi resistance	Arachnoid villi integrity and BCSFB efflux and influx ransporters and mediators
Lymphatic	CSF	Peripheral lymph	Lymphatic absorption of CSF	Perivascular space Perineural space
Meningeal lymphatic vessels	CSF	Lymph	Unknown	Subarachnoid CSF into meningeal lymphatic vessels

Abbreviations: *APOE*ε4*, apolipoprotein E ε4 allele; *AQP4*, aquaporin-4; BCSFB, blood–CSF barrier; CSF, cerebrospinal fluid; ICS, intracellular space; ISF, interstitial fluid.

Table 2

Clearance of Aβ and tau from the brain

Clearance system	Aβ[9,16,44,109,125]	Tau[135,210]
Blood–brain barrier clearance	Majority of eAβ clearance LRP1 efflux ABCB1 efflux ApoE-mediated efflux α2M–mediated efflux LRP2-mediated efflux RAGE influx	Unknown
Degradation clearance		
Intracellular	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases
Extracellular	Proteases Glial phagocytosis	Unknown
ISF bulk flow clearance		
CSF sink	Contributes to eAβ clearance	Unknown
Perivascular drainage	Contribution % to eAβ clearance unknown	Unknown
Perivascular glymphatic	Contributes to eAβ clearance (55–65%) Likely to facilitate blood–brain barrier clearance	Might contribute to clearance of non-endocytosed tau
CSF absorption clearance		
Circulatory	Arachnoid villi Blood–CSF barrier transporters (e.g. LRP1 efflux)	Arachnoid villi
Lymphatic	CSF lymphatic absorption	Unknown

Abbreviations: α2M, α2-macroglobulin; Aβ, amyloid- β; ABCB1, multidrug resistance protein 1 (also known as P-glycoprotein 1); ApoE, apolipoprotein E; CSF, cerebrospinal fluid; e-tau, extracellular tau; eAβ, extracellular Aβ; ISF, interstitial fluid; LRP, LDL receptor-related protein; RAGE, advanced glycosylation end product-specific receptor.